Swedish University of Agricultural Sciences
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Prevalence of Subclinical Mastitis in Dairy Farms in Urban and Peri-urban Areas of Kampala, Uganda

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ABSTRACT

It is widely recognized that subclinical mastitis (SCM) is an extensive problem in the dairy industry worldwide, causing large production losses. It is of particular concern in developing countries, where the prevalence generally is higher and the economic implications greater. Earlier research has found prevalence of 25.2-55.2 % of SCM at cow level in some African developing countries. However, there are no published results from Uganda, despite the importance of the agricultural sector and dairy industry in the country. The aim of this study was to investigate the prevalence of SCM in dairy cattle in the urban and peri-urban areas of Kampala and furthermore to gain information about pathogens, antibiotic resistance patterns and possibilities of prevention. The study was conducted as a field study in 18 small-scale dairy farms. All cows at the farms were examined, and cows with signs of clinical mastitis (CM) were excluded. Cows (n=195) were tested with California Mastitis Test (CMT) and udder quarters with CMT score ≥3 were milk sampled for bacteriological analysis. To allow further sub-analysis of the results, stage of lactation, parity, milk production, production type, udder hygiene and cow breed were recorded. The effects of significant factors from a first ÷2-test analysis were further analyzed in a multivariate analysis using logistic models. Results indicate that 86.2% (n=168) of the tested cows had SCM in one or more quarters. The most common bacteriological outcome was infection with coagulase negative staphylococci (CNS) (54.7%), followed by negative growth (24.9%) and streptococci (16.2%). All susceptibility-tested streptococci (n=34) were sensitive to penicillin. Of the tested staphylococci, six out of nine CNS and four out of eight *Staphylococcus aureus* were positive for penicillinase production. Factors with significant impact on the prevalence of SCM at cow level included stage of lactation, where the prevalence increased with lactation days; parity, where multiparous cows had higher prevalence than primiparous cows; and production type, where zero grazing cows had increased prevalence compared to grazing cows. Thus, the results suggest that the prevalence of SCM in Uganda might be substantially higher than reported in previous studies and in comparable developing countries. The bacteriological pattern resembles other reports from comparable countries, but is not identical. This implies that there is a large need of improvements in terms of hygiene and management in order to reduce the prevalence of SCM. Also, further research is needed to follow up such interventions, to better map out the prevalence of SCM on national level and to identify the properties of well-functioning herds, in order to use them as role models for success given the prevailing conditions.

SAMMANFATTNING

Det är allmänt känt att subklinisk mastit orsakar omfattande problem för mejeriindustrin världen över i form av betydande produktionsbortfall. Problemen är än mer bekymmersamma i utvecklingsländer, där prevalensen subklinisk mastit generellt är högre och de ekonomiska konsekvenserna större. Studier från vissa afrikanska u-länder har visat att förekomsten subklinisk mastit ligger på mellan 25,2-55,2% på konivå. Det finns dock få publicerade resultat från Uganda, trots att jordbrukssektorn och mejerinäringen i landet är mycket viktiga näringar. Syftet med denna studie var att undersöka prevalensen subklinisk mastit hos mjölkkor i stads- och stadsnära områden i Kampala samt att samla information om orsakande patogener, antibiotikaresistensmönster och möjligheter att förebygga sjukdomen. Studien genomfördes som en fältstudie på 18 småskaliga mjölkgårdar. Alla kor på gårdarna undersöktes, och kor med tecken på klinisk mastit exkluderades. Korna (n=195) testades med California Mastit Test (CMT) och från juverfjärdedelar med CMT-poäng $\geq$ 3 togs ett mjölkprov för bakteriologisk analys. För att möjliggöra ytterligare analyser av resultaten samlades information in även rörande kornas laktationsstadium, laktationsnummer, mjölkproduktion, produktionsform, juverhygien och ras. Resultaten visar att 86,2% (n=168) av de testade korna hade subklinisk mastit i en eller flera juverfjärdedelar. Det vanligaste bakteriologiska resultatet var infektion med koagulasnegativa stafylokocker (54,7%), följt av negativ växt (24,9%) och streptokocker (16,2%). Samtliga resistensundersökta streptokocker (n = 34) var känsliga för penicillin. Av de testade stafylokockerna var sex av nio koagulasnegativa stafylokocker och fyra av åtta *Staphylococcus aureus* positiva för penicillinasproduktion. Faktorer med betydande inverkan för prevalensen subklinisk mastit på konivå var laktationsstadium; där prevalensen ökade med antal dagar från kalvning, laktationsnummer; där flerkalvare hade högre prevalens än förstakalvare och produktionsform; där kor som inte fick gå på bete hade en ökad förekomst jämfört med betande kor. Resultaten tyder således på att prevalensen subklinisk mastit kan vara högre i Uganda än vad som setts i tidigare studier och påtagligt högre än i andra utvecklingsländer. Det bakteriologiska mönstret liknar det i andra studier från jämförbara länder, men är inte identiskt. Detta visar på att det finns stora förbättringsbehov, framför allt vad gäller hygien och skötsel för att på sikt kunna minska förekomsten av subklinisk mastit. Dessutom är ytterligare forskning nödvändig för att följa upp införda förbättringsåtgärder, bättre kartlägga förekomsten av subklinisk mastit på nationell nivå och för att identifiera egenskaper på väl fungerande mjölkgårdar för att sedan använda dessa som förebilder under rådande förhållanden.

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BACKGROUND

Livestock Revolution

Recently, several reports and organizations (FAO, 2011 A) indicate that the production of meat and milk in the developing world has doubled in recent decades, as a result of increasing demands. This so-called "livestock revolution" provides income, employment and high-quality nutrition, and the livestock are important to the food security of millions of people and the trend is expected to continue. It has also been concluded that in great parts of the developing world, including developing countries of Africa, milk products consist a very important energy source for many people, and can contribute to a substantial part of the total energy intake. However, infectious diseases, as mastitis, represent serious potential constraints to further development of smallholder production in developing countries and have been described as a factor that can drive rural smallholders into chronic poverty.

The Dairy Livestock Situation in Uganda

As an example of the aforementioned "livestock revolution", the agriculture sector is the most important sector of Uganda today, in terms of capacity utilization rate (FAO, 2011 A). The country has a population of almost 34 million people, and out of these almost 74% works in the agricultural sector. Despite the fact that Uganda is, in vast part, a very fertile country, about 21% of Ugandans suffers from undernourishment (FAO, 2011 A). This underlines the need of increased agricultural production in the country, in which milk production plays a key role (FAO, 2011 C).

The milk production was the 4th largest food and agricultural commodity in Uganda 2009, ranked by value (FAO, 2011 B). The total value was 252 465 000 US$ (applicable International commodity prices were used). For comparison, the value of Swedish milk industry, where milk production is the most important commodity, was 923 389 000 US$ during the same year. In Uganda, each cow produces around 350 kg milk/year, compared to Swedish cows, which produce around 8 300 kg/year (FAO, 2005; Swedish Dairy Association, 2011). The total dairy cow population of Uganda was almost 800 000 livestock in 2010, compared to the Swedish population of 2010, which was barely 350 000 dairy cows in total (Kanyima, 2012; Swedish Dairy Association, 2011).

Besides mastitis, one can discuss several possible reasons for the comparatively poor milk yield in Uganda. More than 90 % of the cattle population in Uganda is owned by small hold farmers and due to poverty, lack of land and transportation limits, especially in urban and peri-urban environments, the farmers can face great difficulties in terms of sufficient food supply for their cattle (FAO, 2005; SLU, 2011). Lack of proper and sufficient feed is in turn a main reason of limited milk production. Another possible factor that can cause production losses in the Ugandan dairy industry is heat stress. André *et al.* (2010) estimated in a Dutch study, where cows were held above a critical temperature (17.8°C) during a period for more than 5.5 days, that heat stress contributes to a milk loss of 31.4 ± 12.2 kg/cow and year. Finally, another reason of lower milk production might be the lack of knowledge concerning

correct calving interval and the non-existing use of dry period, which was commonly observed.

Economical Impact of Mastitis

Bovine mastitis, in either its clinical or subclinical form, is the most widespread bovine production disease. It can cause serious economic losses for dairy farmers worldwide and several different studies point out that subclinical mastitis (SCM) is more economically important than clinical mastitis (CM). (Godkin, *et al.*, 1990; Kader, *et al.*, 2003; Joshi & Gokhale, 2006; Seegers, *et al.*, 2003; Singh & Sing, 1994). This is explained by the fact that SCM is more difficult to diagnose and therefore usually persists longer in the dairy herds, causing production losses. As any infectious disease, SCM can have many different consequences, of which four were pointed out in FAO's (Food and Agriculture Organization of the United Nations) World Livestock 2011 – Livestock in food security report: 1) reduced herd population by death or culling; 2) reduced production and income; 3) creating market shocks when demand falls and supply contracts in response; and 4) disrupting international trade in livestock products. These effects can have impacts at macro and micro levels, i.e. concerning both the trade of a whole country, as well as the affected smallholder.

Other studies also reveal that direct and indirect consequences of SCM, like reduced milk yield, reduced sustainability due to changes in milk composition and discarded milk due to antibiotic treatment, brings serious economical deficits for the farmers (Godkin, *et al.*, 1990). According to Houben (1995). The three major income losses are caused by reduced milk production, premature culling and cost of treatment. These factors account for 78, 14 and 8% of the total loss, respectively (Schepers & Dijkhuizen, 1991). Another significant economic loss is due to extra work for the farmer. The same authors also found that SCM is responsible for more than 90% of the total loss in milk production. Nielsen (2009) investigated the economic impact of mastitis in Swedish dairy industry and established that mastitis costs Swedish milk producers 192 million SEK (27 million US$) yearly, and that CM on average cost 2 800 SEK (400 US$) and SCM on average 600 SEK (86 US$) per cow and occasion. Among other conclusions, Nielsen also pointed out that the biggest economic losses are due to reduced production.

Hence, the prevalence, diagnosis and treatment of SCM are very important fields both in terms of veterinary medicine and economy of developing countries.

Mastitis

Mastitis is an inflammation of the mammary tissue, usually caused by bacteria (Sandholm & Korhonen, 1995) entering the teat canal. The inflammation of the udder gland results in classical inflammatory symptoms, such as redness, swelling, heat, pain and losses of udder function, which in turn result in decreased milk production (Sandholm, 1995b). Also, the composition of the milk is changed as a result of the inflammation (Korhonen & Kaartinen, 1995). These changes are physical, chemical and microbiological. Somatic cell count (SCC)

describes the concentration of body cells, mainly leukocytes present in the milk, which increases in an inflammation.

Mastitis is classified into two forms, based on symptoms: CM and SCM. It can also be divided either into an acute or a chronic form, based on the time course of disease. The former categorization is important in order to decide the right way of treatment and prevention. (Sandholm, 1995a, b). The definition for CM is *visible symptoms* (general: fever and debilitation; local: udder redness, swelling, heat and pain, and milk cloth or other macroscopic milk transformations). These symptoms are graded according to severity (mild, moderate or severe). CM is therefore, because of the visible symptoms, often uncomplicated to diagnose. On the contrary, detection of SCM is a more demanding process, since the definition of SCM is mastitis *without clinical/visible symptoms*. To diagnose SCM, one therefore has to use laboratory methods (Sandholm, 1995a).

Because of the difficulty to diagnose SCM without laboratory tests, it is not uncommon that it remains concealed in the udder and persists in the herd for a substantial time.

Common Pathogens Causing Subclinical Mastitis

The most common cause of mastitis is a bacterial infection. The bacteria can be classified into two different groups, based on origin: udder bound/contagious and environmental bound.

The most common pathogens causing mastitis are the genera staphylococci (usually *Staphylococcus* (*S.*) *aureus* and coagulase negative staphylococci (CNS)) and streptococci (usually *Streptococcus* (*Str.*) *uberis*, *Str. dysgalactiae* and *Str. agalactiae*) (Pyörälä, 1995). Persson *et al.* (2011) also pointed out that together; these genera are responsible for over 90% of the SCM in the Nordic countries.

Staphylococci

Bacteria from the CNS genera are important mastitis pathogens worldwide, so also in Uganda, where Byarugaba *et al.* in a study from 2008 isolated CNS in 30.5% of the CMT-positive quarters, looking at both CM and SCM. In Sweden, CNS is the second most common subclinical mastitis pathogen, found in 16.0% of all cases (Persson *et al.*, 2011). According to Pyörälä (1995), the most common isolates in the Nordic countries are *S. hyicus*, *S. simulans* and *S. epidermidis*, but the distribution of these species may be different in other parts of the world. Thorberg (2008) showed that the most commonly isolated CNS species in Swedish SCM were *S. epidermidis*, *S. simulans*, *S. chomogenes*, *S. xylosus* and *S. haemolyticus.*

Coagulase negative staphylococci is a heterogeneous group of bacteria which can be both contagious and environmental. However, most CNS are part of the cows' normal micro flora and hence a lowered resistance of some kind is necessary for infection to occur (Pyörälä, 1995). Coagulase negative staphylococci bacteria in general are considered to be less virulent than e. g. *S. aureus* (but there are also a few CNS that can be more virulent than *S. aureus*),

causing a milder, often subclinical mastitis (Pyörälä, 1995). The tissue damage is limited; hence the prognosis is fairly good.

Most CNS mastitis can be prevented through good milking procedures, accurate management measures and god overall hygiene (SVA, 2011a).

Staphylococcus aureus is the fourth most common subclinical mastitis pathogen in Uganda, found in 11.9% of all cases (both CM and SCM), whereas it was the most common finding in a Swedish study from 2011, where it was isolated in 19.0% of the samples (Byarugaba *et al.*, 2008; Persson *et al.*, 2011). *S. aureus* is a part of the cow's normal micro flora and occurs on skin and mucous membranes of the nose and throat. In herds with problem with *S. aureus* mastitis, the bacteria can also be detected in wounds and on the point of hock. It is considered to be one of the most problematic mastitis pathogens due to its' strong virulence factors and the fact that most of the *S. aureus* are cow bound (Pyörälä, 1995). The most destructive virulence factor is the α-haemolysis, which causes a gangrenous, often fatal, mastitis. The *S. aureus* mastitis symptoms range from SCM without clinical symptoms to severe CM with high fever, violent udder swelling and milk changes (SVA, 2011b). The infection is easily spread within the herd and cows get infected primarily during the milking process. Despite the fact that *S. aureus* often is sensible to penicillin (figures mentioned below), the antibiotic treatment is in many case unsuccessful due to the bacteria's skills of hiding deep in the udder tissue – thus, the mastitis becomes chronic (Pyörälä, 1995). Nevertheless, the treatment recommendations in Sweden state that acute CM should be treated with benzylpenicillin as soon as possible, whereas SCM might be treated during the dry period (SVS, 2011). Cows with chronic infection or with penicillin resistant bacteria should be eliminated to avoid new infections.

Like CNS mastitis, *S. aureus* mastitis are prevented through good milking procedures including strict milking order and grouping according to udder health, accurate management measures and good overall hygiene, particularly associated with milking (SVA, 2011b). It is best to totally avoid introduction of new animals into a healthy herd, but if that is not possible it is paramount to examine the udder of newly bought cows bacteriologically to avoid introduction of *S. aureus* infected cows.

In a SCM susceptibility study from 2011, Persson *et al.* reported that ß-lactamase production was found in 35% of the SCM caused by CNS, and in 4% of the SCM caused by *S. aureus.* Mastitis caused by CNS is generally easy to treat since the pathogens are not very invasive. Still, as a group the bacteria are refractory, since only two thirds of the cases were caused by pathogens sensitive to penicillin (Persson, 2011 SVA, 2011a). SCM caused by CNS can be locally treated with antibiotics during the dry period. However, an upcoming concern is the increasing prevalence of ß-lactamase-producing CNS. In a study conducted on South African SCM cows, Swartz *et al.* (1984) concluded equal figures of CNS resistant to penicillin G (29%) and much higher figures for *S. aureus* (33%).

Streptococci

In their Ugandan study from 2008, Byarugaba *et al.* found 2.0% streptococcal isolates (in both CM and SCM). *Streptococcus dysgalactiae* and *Str. uberis* are more common mastitis pathogens in Sweden, responsible for 9.0% and 8.0% of the SCM cases, respectively (Persson *et al.*, 2011). In broader perspective, the amount of both *Str. dysgalactiae* and *Str. uberis* mastitis varies in different parts of the world (Pellhagen & Persson Waller, 2006; SVA, 2011d). *Streptococcus agalactiae* mastitis is a substantial problem in many European and developing countries, but rare in Sweden where it contributes to less than 2% of the samples (Persson *et al.*, 2011). Sjögren (2009) found that 37% of the cows in a study conducted on 117 cows on 20 farms in southern Vietnam were infected with *Str. agalactiae*, which was the most frequent pathogen in that SCM study.

Both *Str. agalactiae* and *Str. dysgalactiae* are strict udder pathogens and contagious. *Str. uberis* is both an environmental and (sometimes) cow-bound bacteria that usually is not found on a healthy udder (Pyörälä, 1995; SVA, 2011c, d, e). Since infection with *Str. agalactiae* particularly transmits via milking equipment, it is highly contagious, and if spread in a herd the morbidity can be up to 60%. This makes *Str. agalactiae* mastitis highly pathogenic and very important to combat. *Str. dysgalactiae*, on the other hand, is not as contagious and the morbidity in an infected herd usually remains low. All streptococcal mastitis cases can be acute or chronic, clinical or subclinical and the symptoms can range from mild to very severe. Inflammatory changes in the udder, together with visible or invisible milk changes and an elevated somatic cell count (SCC), are not unusual.

Benzylpenicillin is the drug of choice for treatment of both acute clinical and subclinical streptococcal infections, since *Str. agalactiae, Str. dysgalactiae* and *Str. uberis* almost always are proved sensitive (Bengtsson *et al.*, 2009; Persson *et al.*, 2011). All streptococci in Swartz' *et al.* (1984) South-African study were susceptible to penicillin. On the other hand, chronic cases do not respond as good when treated with penicillin. Chronic cases of *Str. agalactiae* mastitis should therefore be culled to avoid further spread of the infection.

Subclinical mastitis caused by Str. agalactiae and Str. dysgalactiae is primarily prevented through good milking procedures/milking technique and prevention of teat injuries (SVA, 2011c, d). Further preventions to minimize the transmission of bacteria spreading in a Str. agalactiae-infected herd are strict grouping, milk order, accurate management measures, good overall hygiene and screening of new animals (Landin *et al.*, SVA, 2011c). Good overall hygiene is the most important prevention in *Str. uberis* infected herds.

Coliforms

Coliforms, i.e. Ehrlichia (E.) coli and Klebsiella spp., are natural inhabitants of the bovine intestinal flora (Sandholm & Prörälä, 1995). Klebsiella spp. can also be ubiquitous. It is spread through faeces, contaminates the environment, including the cow udder, and can thereby cause mastitis. Both E. coli and Klebsiella spp. give rise to predominantly acute, clinical mastitis and seldom causes SCM (SVA, 2011f, g). In Swedish herds, E. coli and Klebsiella spp. are found in 2.9% and less than 1.0% of SCM, respectively (Persson et al.,

2011), while Byarugaba et al. (2008) isolated coliforms in 14.4% of the CMT-positive quarters, looking at both CM and SCM.

Somatic Cell Count

One can always find some somatic cells, even in milk from a healthy udder (Saloniemi, 1995). These cells are mostly inflammatory cells, like macrophages, neutrophilic granulocytes and lymphocytes and are called somatic cells (i.e. body cells) to differentiate them from e.g. bacterial cells (Andersson, *et al.*, 2011). In a healthy udder, the milk cells mainly consist of macrophages, lymphocytes and epithelial cells. Epithelial cells in milk are eliminated cells from the inner parts of the udder and are part of the natural, ongoing renewal of the body cells. When the udder tissue is exposed to an infection, the levels of neutrophilic granulocytes will increase as an effect of the rapid recruitment of inflammatory cells to the site of inflammation (Andersson, *et al*., 2011; Sandholm, M., 1995b). This raise of the somatic cells in the milk can be counted, using different tests, and the result can be used as an indicator for udder health at cow level and prevalence of SCM at herd level (Andersson, *et al*., 2011). In the early inflammation, up to 95% of the total somatic cell count (SCC) can consist of neutrophilic granulocytes. Later in the inflammatory process, there will be an increase in lymphoid T-cells with an antigen-restricted function (Sandholm, M., 1995b).

There are two main methods to evaluate the levels of somatic cells in the milk: indirect tests, such as California Mastitis Test (CMT), and direct tests such as De Laval cell counter (DCC) or Fossomatic, which give an exact SCC (Saloniemi, 1995). The CMT reagent is added to the milk sample and reacts with the DNA in the cell nuclei, which increases the viscosity of the mixture. This increase in viscosity is then measured and graded. CMT is mostly used at quarter level as an indirect test and does not count the exact somatic cell number. On the other hand, it is a quick and cheap "cow-side" test. The measurement is subjectively made by the investigating person, but several studies reveal that a skilled person reliably can categorize a quarter as healthy or inflamed (Joshi & Gokhale, 2006; Saloniemi, 1995). Direct measurement methods can be used at both udder quarter-, cow- and herd level. In the direct tests, a machine makes the measurement by optically counting every single cell, producing an objective result. Somatic cell count is a useful tool in udder health programs, but at herd level, the result has to be interpreted bearing in mind the different factors that might affect it. For instance, as the size of the herd increase, milk from a single cow will influence less on the total SCC result.

The most important factor increasing the SCC in the milk of a single cow is an infection caused by bacteria (Andersson, *et al*., 2011). However, other factors can also affect the SCC directly: noninfectious mastitis; and time of the day. There are also factors that make the cow more sensitive to infection and therefore indirectly affect the SCC: lactation stages; age/parity of the cow; breed; temperature and season; stress; and care factors. This must always be taken into consideration, as well as the daily milk yield since it also affects the SCC – cows with a low milk production can, due to a concentration effect, naturally have an increased SCC.

Generally, a healthy udder is considered to have less than 100 000 cells/ml and a healthy quarter less than 50 000 cells/ml, which is somewhat less than the cut-off level for a negative CMT ("CMT 1", corresponding to ≤200 000 cells/ml) (Andersson, *et al*., 2011; Brolund, 1985; Forsbäck, *et al.*, 2009; NMC, 2001; Saloniemi, 1995). At herd level, EU regulations stipulate that the SCC should not exceed 400 000 cells/ml (as an average value over a three month period, with at least one milk sample per month) (DIREKTIV 92/46/EEG). Considering acceptable bulk tank SCC levels, the cut-off limit somewhat differs between different regulations. In New Zeeland and the EU, the regulations stipulate less than 400 000 cells/ml, while in Canada the limit is less than 500 000 cells/ml and in USA less than 750 000 cells/ml.

There are several effects of a high SCC (Andersson, *et al*., 2011). Many developed countries have some kind of payment systems/scheme for the milk price with regard to the quality of the milk, i.e. the SCC. In Sweden and Norway, the difference in price between milk with 200 000 and 300 000 cells/ml ranged from 4.6 öre (0.01 US$) to 34 öre (0.05 US$) between different price models used by different dairy plants. Other cost effects of a high SCC are the same as those earlier mentioned for CM and SCM: primarily discarded milk and reduced production but also cost of treatment and extra work. In West European conditions, looking only at production losses, the income is reduced with 8 SEK (1.15 US$) per 1000 cells/ml rise per cow and year (Nielsen, 2008).

According to Hogan (2005), there might be potential food safety risks indirectly associated with high SCC, such as ingestion of potentially pathogenic microorganisms (especially if the milk is not pasteurized), bacterial toxins and/or antibiotic residues. The lower quality and the diminished sustainability of milk with a high SCC both constitute a potential health risk and also affect the possibility of producing other dairy products, e.g. cheese and yoghurt (Andersson *et al.*, 2011)

The most important way to reduce high SCC levels is to work with preventive udder health in order to reduce the prevalence of SCM and CM in the herd (Andersson, *et al*., 2011). Interventions such as improved overall hygiene, especially milking hygiene, identification of cows with high SCC in order to separate them from healthy cows (grouping), introduction of milk order (i.e. milking of the high-cells cows after the low-cell cows), practice of good dry period routines and dry period treatment and spot out CM in order to give them a early and adequate treatment.

The Prevalence of SCM in Developing Countries

According to previous Ugandan studies, the prevalence of SCM in the country is substantial (Okello-Uma & Gibson, 1976; Nakavuma *et al.*, 1994; Byarugaba *et al.*, 1998 & Kintu *et al.*, 2000 in Byarugaba e*t al.*, 2008). For example, Byarugaba *et al.* (2008) reported an overall cow level prevalence of SCM at 37.2%, in the Jinja province in Uganda. Studies of prevalence and incidence of dairy cow SCM performed in other developing countries in the region and in other parts of the world also show a considerable prevalence of SCM. For example, Bitew, *et al.* (2010) conducted a prevalence study on Ethiopian Holstein crossbreed cows and local breeds that showed an overall SCM prevalence of 25.2% at cow level and

12.3% at quarter's level. This study also revealed what other reports also show that SCM had a greater, overall negative impact than CM. It was also shown that Holstein crossbreed cows were more sensitive to SCM compared to local breeds. Another Ethiopian study, suggesting a linkage between SCM and reduced milk production in affected udder quarters on crossbreed dairy cows in Ethiopia, showed that the prevalence of SCM were 52.3% at cow level and 32.4% at quarter level (Mungube, *et al.*, 2005). This study also presented a significantly (p<0.05) higher prevalence of SCM in small-scale farms compared to large-scale farms, and also in urban dairy farms related to other production systems. A prevalence study carried out in the peri-urban area of Hamdallaye, Niger, by Harouna, *et al.* (2009), showed that the prevalence of SCM varied from 27.1 to 55.2% (p<0.05) between dry and rainy seasons. Mdegela, *et al.* (2009) presented similar figures in a prevalence study of both CM and SCM on Tanzanian small holder cows. They estimated that the prevalence was 51.6% at cow level and 30.0% at quarter level.

In India, an incidence study in improved and periurban dairy farms indicated an overall incidence rate of SCM of 46.0% in Holstein crossbreed cows (Joshi & Gokhale, 2006). Another prevalence study from the neighboring country Bangladesh conducted by Rabbani & Samad, (2010) presented a prevalence in Holstein crossbreed cows of 43.75% SCM and for local-bred (Red Chittagong) cows 45.0% SCM.

Studies conducted on dairy cows in developing countries in South America reveals similar results. Gianneechini, *et al.* (2002) presented that 52.4% of the cows and 26.4% of the quarters were diagnosed with SCM in their study from the West Littoral region in Uruguay. Other studies show similar results of SCM at quarter level (Brown, *et al.*, 1998); for Bolivia 19% (Edwards, *et al.*, 1982), Guyana 23% (Motie Ramudit & Mohabir, 1985, Mauritius 26% (Rangasamy, *et al.*, 1983), Colombia 26% (Martinez, 1988) and for Jamaica 56% (Zingeser, *et al.*, 1991).

One can presume that the above-mentioned figures stand in stark contrast to the situation in Western countries. There are no reliable figures of the SCM incidence in Sweden, but for comparison one can instead compare the bulk tank somatic cell count (BTSCC), which is a good marker for the SCM situation at herd level (Persson, *et al.*, 2011). In a report from the Swedish Dairy Association (2011), Andersson *et al*. pointed out that in a herd with a BTSCC of around 200 000 cells/ml, approximately 15% of the cows were infected with SCM. Consequently, if the BTSCC was around 700 000 cells/ml, approximately two thirds of the cows suffered from SCM. In 2009/10, the average delivered BTSCC from Swedish herds to the dairy factories, was 211 000 cells/ml (arithmetic) (The Swedish Dairy Association, 2010). Similar figures, ranging from approximately 130 000-240 000 cells/ml (BMSCC geometric means), are presented for the rest of the Nordic countries (NMSM, 2009). Hallén-Sandgren (2000) also saw similar results in her study of Swedish and Finnish herds, which had an average SCC of 180 000 cells/ml and 130 000 cells/ml, respectively. Altogether, these results suggest that the prevalence of SCM in developing countries is higher compared to Sweden and other Nordic countries.

Antibiotic Resistance

As already pointed out, antibiotics therapy is an important tool in the control of mastitis. Therefore, it is of great importance to minimize the risk of further selection and spread of antibiotic resistance among bacteria, where antibiotic usage of course is a contributing factor. Hence, antibiotic treatment of SCM is recommended only during drying off. During lactation, antibiotic treatment is strictly not recommended according to Swedish policy (SVS, 2011). For mastitis, as for most other illnesses, resistance is not due to mutations, but rather due to spread of resistant bacteria and resistance genes – yet another reason to keep the prevalence of SCM and CM low.

AIMS OF THE STUDY

The main purpose of this study was to investigate the prevalence of SCM in dairy cattle in the urban and peri-urban areas of Kampala, based on California Mastitis Test (CMT), and furthermore to gain information about pathogens, antibiotic resistance patterns and . The study was a part of a larger study – "Influence of Reproductive and Udder Health Management on Productivity of Dairy Cows around Lake Victoria and Lake Kyoga Crescents in Uganda" performed by PhD student Dr. Benon Kanyima (MSc) at Makerere University.

MATERIAL AND METHODS

Study Area

The study was conducted in the urban and peri-urban areas around Kampala, the capital of Uganda. The area around Kampala is situated just north of the equator, meaning that the climate is tropical with two rainy seasons per year: "the great rains" from March to May and "the small rains" in October and November (Bewer, 2009). The mean annual rainfall of the area ranges between 200-700 mm/month, the temperatures averaging around 26°C during the day and around 15°C during the night and the altitude is around 1000 meters above sea level. The study was conducted in October, i.e. during "the small rains".

Study Animals

The study populations were lactating cows with no signs of clinical mastitis (temperature ≤39.5ºC, no sign of sickness, no inflammatory signs of the udder and visible normal milk) in 18 smallholder farms. All cows were hand milked. The herd size of these farms ranged from 1-34 cows with an average number of 10.4 cows per farm. A total of 195 lactating cows at different lactation stages, parity and level of milk production were included in the study. The included cows consisted of 101 Holstein-Friesian cows, 70 Holstein-Friesian/local crossbreed cows, 18 Jersey/Guernsey cows, three Holstein-Friesian/Jersey/Guernsey crossbreed cows, two Jersey/Guernsey/Local crossbreed cows and one local breed cow.

Study Design

The farms were visited between October 10th and October 28th 2011. The farms were each visited once and the cows were examined at cow and quarter level to expel clinical mastitis.

Cows with symptoms of clinical mastitis were excluded from the study, whereas cows without any sign of clinical mastitis were tested with CMT to reveal subclinical mastitis prevalence. Cows with a CMT score ≥3 in any quarter were considered positive for SCM, whereby milk samples were collected from each affected quarter to divulge bacterial presence and indentify the pathogens. To allow further analysis of the results, stage of lactation, parity, milk production (according to information from the farmer), grazing system, udder hygiene and cow breed were also recorded. The different analyses were in turn individually divided into a number of subgroups (Table 1).

Table 1. Division of sub-analyses

ANALYSES																			
Stage of lactation			**Parity**						**Milk production**[1]			**Grazing system**		**Udder hygiene**		**Breed**	
< 60 days	60 – 120 days	> 120 days	First division				Second division		< 7 Liter	7-15 Liter	> 15 Liter	Grazing	Zero-grazing	Good[2]	Poor[3]	Holstein-Frisian (HF)	Other breeds[4]
			Primiparous	2 calves	3 calves	≥ 4 calves	Primiparous	Multiparous									

[1]The group selection derivate from the average mean milk production (according to information from the farmer) of all cows included in the study, the value was 11.3 L (median = 10.5 L, SD = ±5.1 L), [2]clean udder and teats, [3]dirty udder and/or teats, [4]Holstein-Frisian/local crossbreed cows (HFx), Jersey/Guernsey cows (J/G), Holstein-Frisian/Jersey/Guernsey crossbreed cows (HFJx/HFGx), Jersey/Guernsey/Local crossbreed cows (Jx/Gx) and Local breed cow (L).

CMT Screening

The test was carried out according to the method described by Mellenberger & Roth (2000). Approximately 2 ml of milk was sampled during ongoing milking from each udder quarter into each of the four shallow cups in the CMT paddle. To acquire the right amount of milk in the cups, a CMT paddle with marked lines for 2 ml was used. The same volume of CMT reagent was thereafter added to each cup, where a marked syringe was used to obtain the right level of CMT reagent in each cup. To mix the contents, the paddle was then rotated with a

circular motion in the horizontal plane for not more than 10 seconds, after which the result was controlled. The CMT paddle was rinsed with water after each test.

The test result was scored from 1-5 according to the Scandinavian scoring system, where 1 is negative result (no gel formation), 2 is traceable (possible infection) and 3 or above indicates a positive result, where 5 has the most gel formation (Saloniemi, 1995).

Milk Sample Collection

All quarters with CMT ≥3 were milk sampled during ongoing milking, for further bacterial examination. The sampling was carried out according to the method recommended by The National Mastitis Council (NMC) (NMC, 2004). The teats were cleaned with 70% alcohol, starting with the teats furthest away from the collector. After a short moment, to give the teats time to dry, the milk was collected in pre-marked tubes, starting with the teats nearest the collector. When opened, the open end of the test tube was then held facing downwards at all times. The first streams of milk were not used. During the sampling, the tube was held in an angel of approximately 45 degrees. After collection, the milk samples were placed in an ice box and later, when returning to the laboratory not more than a few hours later, put into a fridge. The continued laboratory work of culturing the milk samples was processed not more than 24 hours after the milk sampling.

Bacterial Examination

The milk samples were delivered to the microbiology laboratory at the Faculty of Veterinary Medicine at Makerere University, Kampala, where the bacterial analyses were performed. The bacterial examinations followed standard procedures used by the accredited mastitis laboratory at the Swedish National Veterinary Institute (SVA).

Ten µl of each milk sample were spread on blood agar plates (5% bovine blood), using an expedient plastic loop. The plates were then put in to aerobic incubators at 37° C for 16-24 hours, where plates with dubious growth were allowed an extra 24 hours before final examination. To be classified as positive bacterial growth, at least one colony-forming unit (CFU) was needed for *S. aureus* and *Str. agalactiae*, and at least three CFUs for the other genera.

The bacteria on each plate were then analyzed and categorized according to colony morphology and á-, â- or double hemolysis. Depending on genera, the cultures were then categorized based on potassium hydroxide (PHO) test reaction, catalase test reaction, P-test reaction and coagulase test reaction. To determine the amount of bacterial growing, the amount of colonies in each sample was quantified and then put into three categories: mild growth (<10 colonies), moderate growth (10-50 colonies) and severe growth (>50 colonies).

Colonies with typical morphology for staphylococci, double (á and â) hemolysis, negative PHO test and a positive catalase test reaction, were categorized as *S. aureus*. These colonies were also sensitivity tested for penicillinase production using the Cefinase test.

Colonies with typical appearance for staphylococci, but without or just á-hemolysis, negative PHO test and positive catalase test reaction, were categorized as coagulase negative staphylococci (CNS). Uncertain colonies that looked like staphylococci species were tested for coagulase formation to differentiate between *S. aureus* and CNS. A few samples judged to be either *S. aureus* or CNS were isolated and stored in agar tubes in aerobic incubator for 24 hours and then stored in fridge before being brought to SVA for further analysis and final typing using reversed CAMP-test and coagulase test (Quinn *et al.*, 1994; Klastrup & Schmidt Madsen, 1974).

Colonies with typical manifestation for streptococci, negative PHO test and negative catalase test reaction, were categorized as streptococci species. These samples were isolated and stored in agar tubes in aerobic incubator for 24-76 hours, depending on growth, and then stored in fridge before being brought to SVA for further analysis: typing using CAMP test and 12 biochemical reactions (hippurate, aesculine, salicine, sorbitol, mannitol, raffinose, lactose, saccharose, inuline, trehalose, starch and glycerine) and also antibiotic sensitivity test (Quinn *et al.*, 1994). These tests were carried out to distinguish among *Str. agalactiae*, *Str. dysgalactiae*, *Str. uberis* and other *Str.* Spp.

Colonies with typical Gram negative appearance and positive PHO test were further tested with P-test to distinguish between *Ehrlichia coli* (*E. coli*) and *Klebsiella* spp., the two most common Gram negative bacteria. The test detects the enzyme â-glucuronidase, which among Gram negative bacteria is produced almost exclusively by *E. coli*, of which about 95% are positive.

If the plate had a growth of three or more diverse bacterial agents, it was classified as mixed growing.

Susceptibility Testing

Staphylococcal isolates brought to Sweden were tested at SVA for penicillinase production using the penicillinase test (sensititre method) to distinguish if they were sensitive to benzylpenicillin or not (Franklin & Wierup, 1982).

Isolates of *Str. agalactiae*, *Str. dysgalactiae* and *Str. uberis* brought to Sweden were tested at SVA for antimicrobial susceptibility by determination of minimum inhibitory concentration (MIC), using a micro dilution method. Testing was performed according to recommendations from the Clinical and Laboratory Standards Institute using VetMIC™ panels (National Veterinary Institute, Uppsala, Sweden) and cation adjusted Mueller-Hinton broth (Becton Dickinson, Cockeysville, USA) (Clinical and Laboratory Standards Institute, 2007). The streptococci isolates were classified as susceptible or resistant based on species-specific epidemiological cut-off values for each type of antibiotic, issued by European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Statistical Analyses

Analyses of the frequency of positive cows (at least one quarter with CMT ≥ 3) were first performed. The results were hereafter first analyzed by $\div^2$–test for each individual factor (stage of lactation, parity, milk production, production system, udder hygiene and cow breed). The effects of close to significant factors from the first $\div^2$-analysis were further analyzed together in a multivariable analysis while using logistic models (PROC LOGISTIC, SAS version 9.2).

Further analyses of the number of quarters affected (with CMT ≥ 3) in "positive cows" (n=161 cows), were performed to show intensity of positiveness. The number of quarter affected was analyzed by general linear models (ANDVA, PROC GLM, SAS version 9.2). All factors and interactions between two factors were initially introduced in the model. Non-significant interactions were then progressively removed to get the final model, which contained effects of stage of lactation after calving, parity (primiparous vs. multiparous), amount of milk production (three classes), production system, hygiene, breed and interaction between hygiene and breed. When the effect of a given factor was significant, estimates given by the model were subsequently compared by Scheffe's test (protected test for multiple comparisons).

The relationship between the frequencies of cows having different numbers of pathogens (one single type of pathogen, two pathogens, three pathogens and four or more pathogens), type of pathogen/s (n=168) and the numbers of quarters affected were studied by ÷2–analysis (SAS version 9.2). The relationship between CMT score (3, 4 or 5) of each quarter (n=421) and present pathogen was also studied using the same model.

Potential Sources of Error

In this study, we did not perform sensitivity test (Cefinase) on the CNS in the laboratory in Uganda. However, some samples of CNS were brought to SVA for further analysis and these strains were tested for penicillinase production. This might have distorted the prevalence of penicillinase-producing CNS.

Some of the streptococci and staphylococci samples brought to Sweden for further typing and sensitivity tests unfortunately got damaged during the transportation. Several containers, carrying the different samples, were affected. At the laboratory at SVA, further analyses were carried out only on all the samples that for certain could be separated from each other. Yet, the loss of samples might have affected the prevalence figures of different streptococci subspecies.

RESULTS

Descriptive & Statistic Data

Overall prevalence of SCM

The results of the CMT screening indicate that 86.2% (n=195) of the tested cows had SCM in one or more quarters. At quarter level, the prevalence of SCM was 55.4% (n=760).

Distribution of CMT figures

The CMT figures were distributed as follows: CMT score 1 18.0% (n=137), CMT 2 26.6% (n=202), CMT 3 30.8% (n=234), CMT 4 20.7% (n=157) and CMT 5 3.9% (n=30).

Prevalence of SCM at different stages of lactation

The prevalence of SCM was analyzed on basis of stage of lactation. The results were 80.6%, 75.0% and 89.9% at cow level and 45.1%, 46.4% and 60.1% at quarter level, for <60 days, 60-120 days and >120 days, respectively (table 2). The figures from the cow level results in the first $\div^2$–test, in which the individual factors/marker of the frequency of positive cows (at least one quarter with CMT $\geq$ 3) were analyzed, showed that there is a close-to significant positive correlation for SCM and stage of lactation (*p<0.06*). When these results then were analyzed in the second, multivariable $\div^2$–test, in which correlation for the effects of other factors/markers was analyzed, it resulted in a significant (*p<0.02*) difference between the cows, where cows with less than 60 days elapsed from last calving had a lower prevalence of SCM than cows with more than 120 days from last calving. The number of quarters with CMT score ≥3/cow in each subgroup of stage of lactation (figure 1) points in the same direction: cows with <60 days had 1.86 affected quarters/cow and cows with >120 days had 2.59 affected quarters/cow, which is close to significant (*p<0.06*).

Prevalence of SCM on the basis of parity

The prevalence of SCM was also analyzed on basis of parity. The results were 79.2% and 88.4% at cow level and 47.9% and 57.8% at quarter level, for primiparous and multiparous cows, respectively (table 2). When the results were analyzed in the second, multivariable $\div^2$–test, it resulted in a strong significance (*p<0.02*), where primiparous cows had a lower prevalence of SCM than multiparous cows.

Prevalence of SCM on the basis of milk production

The prevalence of SCM was analyzed on basis of milk production. The results were 80.0%, 88.1% and 81.5% at cow level and 58.8%, 54.4% and 57.4% on quarter level, for <7 liters, 7-15 liters and >15 liters, respectively (table 2). When analyzing the figures from the cow level results in the first $\div^2$–test, no significant correlation (*p=0.42*) between milk production and SCM was found.

Prevalence of SCM on the basis of breed

The prevalence of SCM was analyzed on basis of cow breed. The results were 87.1% and 85.1% at cow level and 59.3% and 51.1% on quarter level, for HF and other breeds,

respectively (table 2). When analyzing the figures from the cow level results in the first $\div^2$–test, no significance (*p=0.68*) between SCM and cow breed was found.

Prevalence of SCM on basis of udder hygiene

The prevalence of SCM was also analyzed on basis of udder hygiene. The results were 81.8% and 87.9% at cow level and 48.8% and 57.9% on quarter level, for good and poor udder hygiene, respectively (table 2). When analyzing the figures from the cow level results in the first $\div^2$–test, there is a tendency of significant correlation between SCM and udder hygiene (*p=0.27*), where cows with good hygiene had a lower prevalence. Looking further at the number of quarters with CMT ≥3/cow, analyzing the udder hygiene of just HF cows in each subgroup (figure 2), HF with good hygiene had 1.63 quarters/cow and cows with poor hygiene had 2.72 quarters/cow. This is also a significant correlation (*p<0.003*). There were no such significant correlation for the group other breeds.

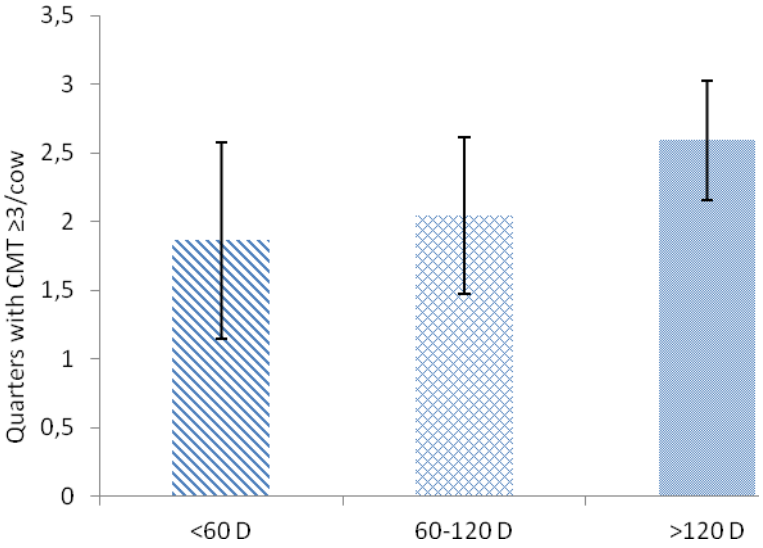
Prevalence of SCM on the basis of grazing system

The last factor analyzed was SCM on basis of production system. Results were 95.7% and 83.8% at cow level and 75.5% and 53.8% at quarter level, for zero-grazing and grazing, respectively (table 2). When analyzing the figures from the cow level result in the first $\div^2$–test, a tendency of significant correlation (*p=0.13*) between SCM and cow production system, where grazing cows had a lower prevalence.

Table 2. Factors affecting SCM and their relation to prevalence at cow and quarter level

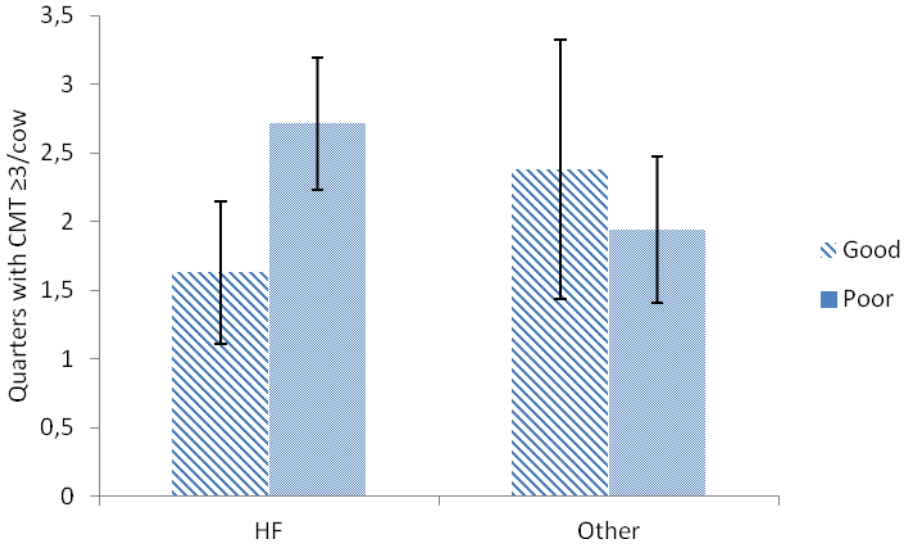
Factors	**Type**	**No. of cows tested**	**No. of CMT ≥3 (% of cows)**	**No. of udder quarters tested**	**No. of CMT ≥3 (% of udder quarters)**
Stage of lactation	<60 D	31	25 (80.6)	122	55 (45.1)
	60-120 D	36	27 (75.0)	138	64 (46.4)
	>120 D	119	107 (89.9)	464	282 (60.1)
	Total:	186	159 (85.5)	714	401 (56.2)
Parity	1	48	38 (79.2)	190	91 (47.9)
	2	37	32 (86.5)	144	64 (44.4)
	3	43	38 (88.4)	169	95 (56.2)
	≥4	66	59 (89.4)	253	168 (66.4)
	Total:	194	167 (86.1)	756	418 (55.3)
Parity	Primiparous	48	38(79.2)	190	91 (47.9)
	Multiparous	146	129(88.4)	566	327(57.8)
	Total:	194	167(86.1)	756	418(55.3)
Production	>15 L	27	22 (81.5)	108	62 (57.4)
	7-15 L	143	126 (88.1)	553	301 (54.4)
	<7 L	25	20 (80.0)	99	58 (58.6)
	Total:	195	168 (86.2)	760	421 (55.4)
Breed	HF	101	88 (87.1)	396	235 (59.3)
	Other breeds	94	80 (85.1)	364	186 (51.1)
	Total:	195	168 (86.2)	760	421 (55.4)
Udder hygiene	Good	55	45 (81.8)	211	103 (48.8)
	Poor	140	123 (87.9)	549	318 (57.9)
	Total:	195	168 (86.2)	760	421 (55.4)
Grazing system	Zero-grazing	23	22 (95.7)	83	61 (73.5)
	Grazing	142	119 (83.8)	560	301 (53.8)
	Total:	165	141 (85.5)	643	362 (56.3)

Figure 1. Quarters with CMT ≥3/cow in the subgroups of stage of lactation



The columns describe the mean number of quarters with CMT ≥3/cow of all cows in each subgroup of stage of lactation with error bars showing ±2 standard derivations.

Figure 2. Quarters with CMT ≥3/cow in the subgroups of hygiene correlated to breed



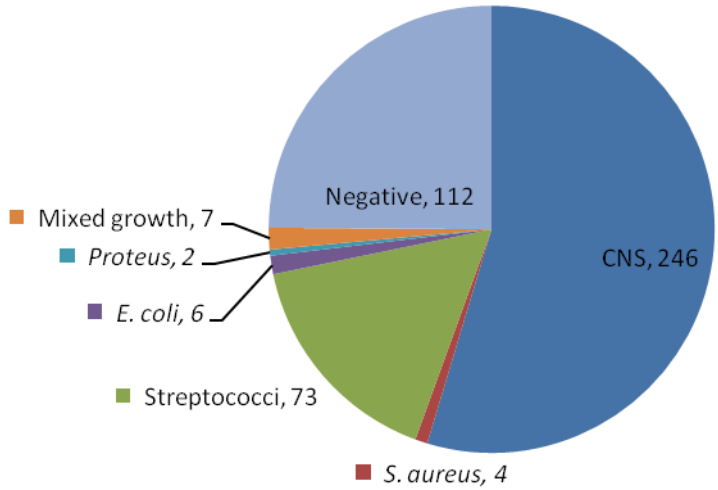
*The columns describe the mean number of quarters with CMT ≥3/cow of all cows in each subgroup of udder hygiene, separating Holstein-Friesian and other breeds, with error bars showing ±2 standard derivations. Note the significance (*p*<0.003) between good and poor udder hygiene in Holstein-Friesian cows.*

Distribution of Udder Pathogens

The most common bacteriological outcome (Figure 3) was infection with coagulase negative staphylococci (54.7%), followed by negative growth (24.9%), streptococci (16.2%), mixed growth (1.6%), *E. coli* (1.3%) and *S. aureus* (0.9%). Of the 73 strains of streptococci, 48 were

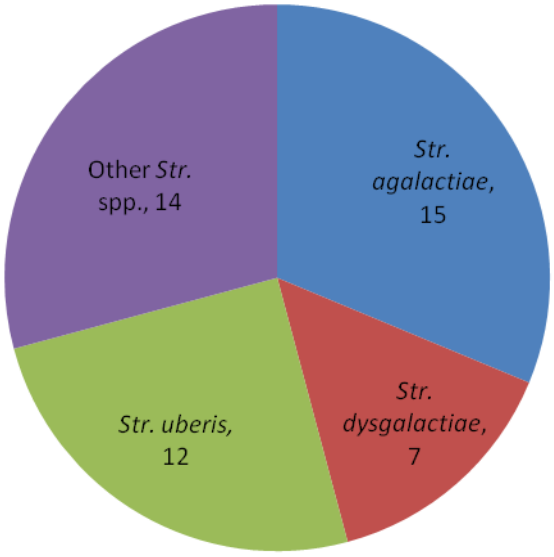
subtyped. Out of this, the distribution was 15 *Str. agalactiae*, 12 *Str. dysgalactiae*, 7 *Str. uberis* and 14 other streptococci species (figure 4).

Figure 3. Distribution of growth at quarter level (n=450)



The figures after each pathogen represent the actual number of positive cultures.

Figure 4. Distribution of streptococci.



The figures after each pathogen represent the actual number of positive cultures.

Antimicrobial Susceptibility Testing

The results from the streptococci susceptibility test are showed in Table 4. Final concentrations of antibiotics ranged from ≤0.03 to 64 mg/l. 100% of the tested streptococci (n=34) were sensitive to penicillin (table 4). Of the tested staphylococci, six out of nine CNS were positive for penicillinase production in the penicillinase test carried out at SVA and four out of four *S. aureus* were tested positive. Of the four Cefinase-tested *S. aureus* tested in Uganda, all four were negative for penicillinase production.

Table 4. Resistance and distribution of MIC for Str. agalactiae (n = 15), Str. dysgalactiae (n = 7) and Str. uberis (n = 12)

			Distribution (number of isolates) of MICs[1] (mg/l)											
Substance	**Species**	**Resistance (%)**	**≤0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**>64**
Cefalothin	*S. agal.*	0		3	6	6								
	S. dysg.	-			6	7								
	S. uberis	-			9	2		1						
Clindamycin	*S. agal.*	0				15								
	S. dysg.	0				7								
	S. uberis	-				12								
Ciprofloxacin	*S. agal.*	0					3	12						
	S. dysg.	-					3	4						
	S. uberis	-					7	5						
Chloramphenicol	*S. agal.*	-							11	4				
	S. dysg.	-						1	3	3				
	S. uberis	-							2	10				
Erythromycin	*S. agal.*	0				15								
	S. dysg.	-				7								
	S. uberis	-				12								
Fusidine	*S. agal.*	0									15			
	S. dysg.	-						1	3	1	2			
	S. uberis	-									12			
Gentamicin	*S. agal.*	-						1	5	5	4			
	S. dysg.	-						2	4	1				
	S. uberis	-							2	5	5			
Kanamycin	*S. agal.*	-									3	4	8	
	S. dysg.	-									2	4	1	
	S. uberis	-									1	5	6	
Oxacillin	*S. agal.*	-			2	6	6	1						
	S. dysg.	-			7									
	S. uberis	-			4	8								
Benzylpenicillin	*S. agal.*	0	3	7	5									
	S. dysg.	0	7											
	S. uberis	-	7	4		1								

	S. agal.	100						1	1	4	9	
Tetracycline	*S. dysg.*	-					1	2	1	3		
	S. uberis	-		12								
	S. agal.	-				1	4	5	4	1		
Trimethoprim	*S. dysg.*	-			2	4	1					
	S. uberis	-				7	3	2				

[1]White fields denote range of dilutions tested for each substance. MICs above the range are given as the concentration closest to the range. MICs equal to or lower than the lowest concentration tested are given as the lowest tested concentration. Bold vertical lines indicate EUCAST epidemiological cut-off values. When no cut-off value is available isolates are not classified as susceptible or resistant.

DISCUSSION & CONCLUSIONS

The overall prevalence of SCM, 86.2% at cow level and 55.4% at quarter level, is an unexpected high prevalence. This applies especially to the prevalence at cow level, where Byarugaba e*t al.* (2008) reported an overall cow level prevalence of SCM at 37.2% in their study from the Jinja province in Uganda. However, the comparison to this study is somewhat difficult since the authors have not clearly declared their way of calculating the frequency of CM and SCM. Studies from other developing countries found prevalences ranging from 25.2 to 55.2% (Bitew, *et al.*, 2010; Gianneechini, *et al.*, 2002; Harouna, *et al.*, 2009; Joshi & Gokhale, 2006; Mdegela, *et al.*, 2009; Mungube, *et al.*, 2005; Rabbani & Samad, 2010). These studies used similar criteria to characterize a cow as SCM-positive or not. A possible explanation to the high prevalence of SCM found in this study could be that some of the risk factors contributing to this were more predominant; e. g. a high proportion of zero-grazing cows, a high proportion of poor udder hygiene and a higher proportion of cows in late parity and in late stage of lactation. This could all contribute to a higher prevalence of SCM. Another contributing factor could be the low milk production, which possibly could have increased the SCC.

The bacterial findings in this study were mainly Gram-positive bacteria. CNS was the most common (55%), followed by negative finding (25%) and streptococci (17%). Of the streptococci, *Str. agalactiae*, other streptococci, *Str. uberis* and *Str. dysgalactiae* were the findings, in falling order. This is resembling, but not identical to the findings of earlier studies from Uganda and other developing countries. Byarugaba e*t al.* (2008) presented negative growth as the most predominant finding (48.1%) in their Ugandan study, followed by CNS (30.5%), coliforms (14.4%), *S. aureus* (11.9%), streptococci (2.0%) and *Pseudomonas aeruginosa* (1.2%). Unfortunately, these results concern both CM and SCM, which makes a direct comparison somewhat difficult. Bitew *et al.* (2010), in their Ethiopian study of SCM, presented that CNS was the most common finding (56.2%), followed by streptococci (17.8%) and *S. aureus* (16.4%). Negative finding was not presented in this study, but conclusions drawn from the positive findings, negative growth was found in 28.8%. Rabbani & Samad (2010) concluded in their study from Bangladesh that *S. aureus* was the most common finding (62.2%), followed by streptococci (19.5), enterococci (8.2%) and CNS (7.4%). In a Nigerian study of SCM in dry and rainy season, Harouna *et al.* (2009) presented that negative finding

was the most predominant result (72.0% in dry season/44.8% in rainy season), followed by *S. aureus* (11.5/32.0%), CNS (12.8/3.2%) and environmental bacteria (2.8/20.0%). The same pattern is found in Swedish studies, where Persson *et al.* found (2011) prevalences of 19% and 16% for *S. aureus* and CNS, respectively. The main difference compared to my results was that the prevalence of CNS was higher, but lower for *S. aureus*. However, this was also reported by Bitew *et al.* (2010) and Byarugaba e*t al.* (2008), who also found a higher prevalence of CNS compared to *S. aureus*. Also, negative finding was not as common as in the mentioned Ugandan study.

Analyzing the antibiotic resistance pattern of this study, it seems that the tested streptococci has a higher MIC to tetracyklin and trimethoprim, but similar MIC to penicillin compared to the results of Persson *et al.* (2011) in their Swedish study. However, only *Str. dysgalactiae* and *Str. uberis* were examined in that study, making it difficult to compare the resistance pattern of *Str. agalactiae*. Comparing the penicillin resistance of CNS and *S. aureus*, the resistance seems to be higher in Uganda than in Sweden: 6/9 resistance test for CNS and 4/8 resistance tests for *S. aureus* were positive, respectively. Still, objections can be made to this comparison, since the number of tested CNS and *S. aureus* is limited in this study. Yet, the high resistance pattern of staphylococci observed in this study is cause for worry. In their Ugandan study, Byarugaba e*t al.* (2008) also showed a high penicillin resistance among staphylococci, namely 86.6%. This is a higher figure compared to the findings in my study. At the same time, the earlier-mentioned study investigated only the resistance for staphylococci in general, making a comparison of the results intricate.

One reason contributing to the high antibiotic resistance in Uganda could be that farms do not consistently cull penicillinase-postive cows. This will in turn lead to infection of new cows with resistant bacteria. Another reason to the high antibiotic resistance could be that farmers are able to obtain antibiotics over the counter without prescription. This is increasing the risk of antibiotic misuse, which could contribute to development of further resistance (Byarugaba, 2004 in Byarugaba *et al.*, 2008). Nonetheless, SCM in Uganda still seems to be treatable with penicillin during the dry period in most cases – a fact illustrated by the observation that 100% of the examined streptococci were sensitive to benzylpenicillin. However, penicillinase-positive bacteria should not be treated regardless of antibiotic preparation or resistance pattern, since the cure rates are low regardless of antimicrobial agent used (Sol *et al.*, 1997; Ziv & Storper, 1985). Instead, cows infected with such bacteria should be culled in order to avoid further spread of resistant bacteria. Speaking of this, it is also paramount to diagnose the agent prior to the selection of antibiotic treatment, in order to choose a narrow-spectrum antibiotic.

As for the explicit significance regarding stage of lactation, where cows with less than 60 days from the last calving date have a lower prevalence and a lower number of udder parts with CMT $\geq$ 3/cow than cows with more than 120 days past from last calving, is somewhat as expected. The udder is most sensitive to acute CM and SCM during the period after the calving, whereas chronic mastitis, most often subclinical, is more frequent later during the lactation. On the other hand, cows also get a natural high cell count towards the end of

lactation because of a reduced milk production (Andersson *et al.*, 2011). At the visited farms, the use of dry period was almost non-existing. Hence, some cows could be milking for a very long time, up to several years, increasing the risk of SCM. The bad routine management regarding dry period might be one explanation to the figures implying that cows in late lactation were more susceptible to SCM. Joshi & Gokhale (2006) presented similar, but not identical results in their study conducted on Indian cows, where cows in the 4^{th} to 5^{th} month of lactation were found to be more sensitive to SCM (59.5%) than cows in the 1^{st} to 3^{rd} month (42.2%). Also Byarugaba *et al.* (2008) observed that the mastitis prevalence increased with increased stage of lactation.

Looking at the figures of stage of lactation at cow level (Table 2), there are less SCM positive cows in the group 60-120 days after last calving than in the group <60 days from last calving. The reason that it is less significance for the former group in comparison to the group >120 days from last calving, which had the highest proportion of SCM positive cows, is that in the 60-120 days-group there was a greater proportion of other factors resulting in good udder health (primiparous, grazing and good udder hygiene), as compared to the group <60 days from last calving.

In terms of parity, primiparous cows had a lower prevalence and a lower number of quarters with CMT ≥ 3/cow than multiparous cows. This is as expected. Joshi & Gokhale (2006) and Byarugaba *et al.* (2008) also found that the prevalence of SCM increased with increasing parity, and Rabbani & Samad (2010) presented similar results in their Bangladeshian study. Older cows are more susceptible for SCM (Biaffa *et al.*, 2005 in Neelesh *et al.*, 2012), where the breakdown of the streak canal barrier and the udder tissue with progressing age is one of the contributing factors (Schalm *et al.*, 1971).

Also, the clear significance regarding udder hygiene was expected. HF cows with poor udder hygiene had a higher amount of CMT ≥3/cow, compared to HF with good udder hygiene. A dirty udder is more susceptible to SCM. Grazing cows on pasture, which have better conditions to maintain good udder hygiene, have a tendency of lower prevalence than zero-grazing cows. This has also been shown in an earlier Ugandan study, where poor udder hygiene, western breed and zero-grazing were particularly pointed out as contributing factors for a high prevalence of SCM (Byarugaba *et al.*, 2008).

When performing the statistical analyzes of the number of quarters affected (with CMT ≥ 3) in "positive cows" to show intensity of positiveness, it was only focused on the number of quarters per cow with CMT ≥3, and nothing else. Factors such as the CMT score (3 to 5), the amount of bacterial growth (mild, moderate or severe) or bacterial agent, were not included in this test. These parameters are all very important to include in an overall picture. Hence, these analyses render just a part of the truth, but still seem to point in the same direction as the other prevalence figures.

The main strength with this study is its' focus not only on the prevalence of SCM, but also on the gathered information of environmental and cow factors/markers that could provide

information of factors causing a high prevalence of SCM. Other strengths are the big sample size and the focus just on SCM. Swartz *et al.* (1984) pointed out that if resources to diagnose SCM are poor; there is a large risk that the problem with SCM will continue, even if the problems with CM are solved. The invisible SCM will continue to cause both big production and economical losses. Some authors have even stated that it may be impossible to completely eradicate SCM from dairy farms and stated that its occurrence can only be minimized to acceptable levels (Blood & Radostitis, 1989 in Byarugaba e*t al.*, 2008).

Yet, there are a few weaknesses in this study. First, as a cause of a misunderstanding, we did not perform sensitivity test (Cefinase test) on the CNS at the laboratory in Uganda. However, some samples of CNS were brought to SVA for further analysis and these strains were tested for penicillinase production. This might have distorted the prevalence of penicillinase-producing CNS. Other weaknesses are that some of the streptococci and staphylococci samples brought to Sweden for further typing and sensitivity tests unfortunately got damaged during the transportation. Several containers, carrying the different samples, were affected. At the laboratory at SVA, further analysis was carried out only on all the samples that for certain could be separated from each other. The loss of samples might have affected the prevalence figures of different streptococcal subspecies.

Cows with CM were excluded from the study. If they had been part of the study, they would, like healthy cows, have contributed to a lower prevalence of SCM. This might have influenced the prevalence of SCM. Still, cows with CM were relatively few compared to cows with SCM, so the influence of not including them is negligible. It had also been problematic to include these cows, since they could potentially suffer from both CM and SCM in different quarters. In summation, the potential errors have not been that serious and not of a greater source of errors.

The statistics of the prevalence of SCM in this study have mainly been focused at cow level. However, the significant results seem to be even more obvious at quarter level (table 2). Yet, considering the size of the study population, it can still be said to be big enough to presume that the high prevalence of SCM really is reflecting the truth. The reason to this high prevalence is multifaceted, but there are a few anticipatory mechanisms deserving particular elucidation. Looking at the impact of certain factors, such as zero-grazing, late stage of lactation, high parity and poor udder hygiene; all seem to increase the risk of getting SCM. Another reason to the high prevalence of SCM could be the absence of dry periods and poor dry period routines. Out of 60 questioned Ugandan small-scale dairy farms, only one respondent practiced dry cow therapy in an earlier study (Byarugaba *et al.*, 2008). The reason of these poor dry period routines could be connected to bad farming management and the lack of keeping proper farm records. This could in turn lead to that the dry period is initiated by the calf: when a cow refuses to feed the calf the farmer separates them, and thus the cow is dried. Alternatively, dry periods can be caused by repeated failure of getting the cow in gestation, which sooner or later usually lead to that the cow gets dried by herself.

Even though I did not perform any statistics on it, my own reflection is that the overall hygiene and especially the hygiene routines around milking time are the main reasons of the high prevalence. The access to clean water is a contributory factor, although how the water is used for cleaning before milking is an even bigger problem. According to my observations, most of the farmers had a substantial shortage in terms of good practice, especially at the time of milking. E. g., most of them used their bare hands (which they also used for milking) to get rid of dirt from the udder and used the same water bucket for several different cows. Nor did they use udder cleansing tissues before milking, neither teat dipping afterwards. Also, I did not observe the use of grouping the cows or milking them in a predetermined order, according to their udder status. These observations have also been made in an earlier Ugandan study, where most farmers were observed only to use a spade for cleaning the milking place after the milking, alternatively just water (Byarugaba *et al.*, 2008). The same authors also saw that most of the farmers (66.7%) did not follow any particular milking order. They also concluded that most of the farmers actually cleaned the udder in some way before milking. At the same time, most of them used the same towel for all cows, which can spread and sustain mastitis in the herd (Kassa *et al.*, 1999; Kivaria *et al.*, 2006 & Mdegela *et al.*, 2004 in Byarugaba *et al.*, 2008). Byarugaba *et al.* (2008) also found that almost none of the farmers seemed to have the knowledge of methods to control mastitis: udder washing, good hygiene, culling of chronic cases, following a predetermined milking order or teat dipping were all unusual measures. Studies show that teat dipping after milking reduces the spread of infection from cow to cow, while dry cow therapy reduces the reservoir, which in turn further reduce the teat bacterial exposure (Smith & Hogan, 1995). During the dry period, a keratin protein substance is produced to protect the streak canal (Eberthart, 1986). Hygienic milking routines are also decreasing the exposure to bacteria (Nickerson & Boddie, 1995).

SCM seems to be very common in Ugandan dairy cows. This seems in turn to be connected to the lack of knowledge; most of the farmers did not even know that SCM existed (Byarugaba *et al.*, 2008). The high prevalence is also connected to the lack of resources to work with good overall hygiene, especially milking hygiene, as most of the bacterial findings were contagious pathogens. Another factor that seem to be involved with the high prevalence is the use of zero-grazing; cows that not were held on pasture had higher prevalence of SCM than grazing cows.

The results of this study provide new information and will hopefully contribute to a possibly lower prevalence of SCM in the future. The results also suggest that it is important to work with preventive work in the farms, in order to lower the prevalence of SCM. Previous studies show that the best educational results were obtained when farmers visited each other, and together with experts discussed ways of improving their milking technique (Vaarst *et al.*, 2007 in Byarugaba *et al.*, 2008). At the same time, resources are limited, implying that focus needs to be set on easy and not so expensive interventions. Still, large improvements could probably be done with such measures, e.g.:

- Identification of cows with high SCC in order to introduce milking order, grouping and/or culling of infected cows. A relatively inexpensive method to achieve this would be to teach the farmers themselves to perform CMT in the cows.
- Improvement of the overall hygiene on the farms, especially the milking hygiene and especially for HF cows:
    - Cleaning the teats before milking with clean water and separate udder cleansing tissues.
    - Adopt teat dipping after milking.
- Improvement of the milking technique. Most of the milking is currently made by strip milking, a method leading to a mechanical irritation which in turn may lead to inflammation and reduction of both the mechanical and biological defense.
- Introduction of dry period routines.
- Proper treatment of subclinical as well as clinical mastitis.
- Keeping the cows on pasture rather than using zero-grazing systems.

Another factor of fundamental importance in animal production in general, and in dairy industry in particularly, is a good record keeping, both for the monitoring of health and production in individual cows and for monitoring of herd health and production. These records can then be used as a decision tool to develop standard operations procedures at both cow and herd level, conformed for each dairy farm. The records could also be used for "benchmarking", i.e. usage of the records as key figures in order to know how the farm compares to both previous own results and other farms', nationally and internationally. Without proper registrations, it is impossible for the veterinary advisors and the inseminators to support development in the right direction. Unfortunately, to my opinion, the record keeping at the visited dairy farms was very inadequate.

This field work is a SCM prevalence study executed on dairy cows in Uganda, providing new insight to the current situation. Hopefully, the study can contribute to the development of better routines that possibly can lower the high occurrence of SCM. However, further research is needed to investigate the prevalence of clinical mastitis in Uganda in order to obtain a more comprehensive picture of the current mastitis situation. Also, further studies of well-functioning/healthy herds are necessary in order to survey success factors and to use these farms as role models for non-functioning herds.

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