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Klinisk och subklinisk mastit hos mjölkkor i Kampala, Uganda

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ABSTRACT

Dairy farming in Uganda provides a source of food, employment and income. Previous studies have revealed high frequencies of bovine mastitis, a costly disease for the dairy farmer. The aims of this study were to investigate the bacteriological panorama in milk from udder quarters with clinical (CM) and subclinical mastitis (SCM) and to determine the antimicrobial susceptibility in staphylococcal isolates. Further, we intended to establish the prevalence of subclinical mastitis and to investigate some environmental factors and animal properties that might influence the frequency of mastitis. For CM, farmers made contact with the members of this study when recognizing an animal with CM. Cows were clinically examined, all quarters were examined by California Mastitis Test (CMT) and milk samples for bacteriological culturing were collected from all quarters positive for CM. A total of 24 milk samples from were collected from 18 animals. For SCM, cows were examined by CMT during afternoon milking and the diagnose of SCM was based on a CMT value ≥ 3 in quarter milk and the prevalence at cow level was determined based on the presence of SCM in at least one udder quarter. Due to practical reasons bacteriological examination of SCM cases was performed only on milk samples with a CMT ≥ 4 (n=166) which were collected from 78 animals. Bacteriological analyses were done locally at Makerere University, Kampala, Uganda and at the Swedish Veterinary Institute (SVA), Uppsala, Sweden. Antimicrobial susceptibility was tested in staphylococcal isolates. Chi^2 test and multi-variable analysis was executed to determine what factors influence the frequency of CM and SCM with a CMT ≥ 4 (SCM-CMT ≥ 4). Of the animals with CM, 22 % were affected in > 1 udder quarter. Concurrent SCM in ≥ 1 quarter was found in 83 % of the animals. At quarter level, 33.5 % of quarters were positive for CM and 47 % of quarters had SCM. The most common pathogen found in CM was coagulase negative staphylococci (CNS) (29 %), followed by *Escherichia coli* (12.5 %). Of the *Staphylococcus (S.) aureus* and CNS isolates from CM, 100 % were positive for β-lactamase production. A higher frequency of CM cases was seen in smaller herds, in open grazing systems and in animals with a parity > 1. Subclinical mastitis in ≥ 1 quarter was found in 90 % of the animals that were screened during afternoon milking. At quarter level, the prevalence of SCM was 63 %. The most common pathogen isolated from subclinical mastitis was CNS (12.0 %) followed by *Streptococcus agalactiae* (8.4 %). Of the *S. aureus* and CNS isolates 6/7 (86 %) and 16/20 (80 %) were positive for β-lactamase production. A higher frequency of SCM-CMT ≥ 4 cases was seen in zero- and confined grazing systems and in animals with a parity > 1. Zero-grazing systems were correlated to animals in poor hygienic conditions and were more common in smaller herds. Coagulase negative staphylococci, coliforms and *S. aureus* were more common in CM than in SCM-CMT ≥ 4 cases while streptococci were more common among the SCM-CMT ≥ 4 cases. In conclusion, the most common agent found in CM and SCM-CMT ≥ 4 was CNS and a high prevalence of SCM was revealed. The majority of staphylococci were positive for β-lactamase production but there was no evidence of methicillin resistant *S. aureus*. Parity, grazing system and herd size were factors that significantly influenced the frequency of mastitis. It was evident that the milking hygiene procedures were generally poor and probably a contributing factor to the poor udder health. Also, the easy access of pharmaceuticals constitutes a risk in the development of antimicrobial resistance among bacteria.

SAMMANFATTNING

Att producera mjölk i Uganda ger tillgång till föda, sysselsättning och inkomst. I tidigare studier från Uganda och andra u-länder har man funnit hög prevalens av mastit, en mycket kostsam sjukdom för mjölkproducenter. Målet med studien var att få en bättre bild av mastitläget i Uganda genom att undersöka vilka patogener som fanns i mjölk från juverdelar med klinisk och subklinisk mastit, samt att undersöka känslighet mot antibiotika hos stafylokocker. Vi ville också fastslå prevalensen av subklinisk mastit och undersöka några individ- och miljöfaktorers påverkan på mastitfrekvensen. Mjölkproducenter hörde av sig till oss när de upptäckte ett fall av klinisk mastit. Klinisk undersökning av djuret utfördes och alla spenar blev undersökta med Cailfornia Mastitis Test (CMT). Mjölkprov för bakteriologisk undersökning samlades från alla spenar med klinisk mastit och totalt 24 prover samlades från 18 djur. Beträffande subklinisk mastit besöktes gårdar under eftermiddagsmjölkningen och CMT-undersökning utfördes på mjölkade kor. Diagnosen subklinisk mastit baserades på CMT ≥ 3 på juverdelsnivå och prevalensen på konivå baserades på subklinisk mastit i minst en juverdel. Av praktiska skäl togs mjölkprover för bakteriologisk odling från alla juverdelar med CMT ≥ 4 och 166 prover samlades från 78 djur. Bakteriologiska analyser utfördes lokalt på Makerere University, Kampala, Uganda och på Statens veterinärmedicinska anstalt (SVA), Uppsala, Sverige. Stafylokocker undersöktes med avseende på antibiotikaresistens. Chi^2-test och multivariabelanalyser utfördes för att undersöka vilka faktorer som påverkade frekvensen av klinisk mastit och subklinisk mastit med CMT ≥ 4. Av de djur som identifierades med klinisk mastit hade 22 % klinisk mastit i mer än en juverdel. Hos 83 % av djuren fanns samtidig subklinisk mastit i minst en juverdel. På juverdelsnivå var 33,5 % av juverdelarna positiva för klinisk mastit och 47 % av juverdelarna hade subklinisk mastit. Den vanligaste patogenen var koagulasnegativa stafylokocker (KNS) följt av *Escherichia coli* (12,5 %). Av alla *Staphylococcus (S.) aureus*- och KNS- isolat från kor med klinisk mastit var 100 % β-laktamaspositiva. Frekvensen av klinisk mastit var högre i mindre besättningar, i öppna betessytem och hos djur med kalvningsnummer > 1. Subklinisk mastit i minst en juverdel fanns hos 90 % av djuren och prevalensen på juverdelsnivå var 63 % hos djuren som undersökts med CMT under eftermiddagsmjölkning. Hos de subkliniska mastiterna var KNS (12,0 %) den vanligaste patogenen följd av *Streptococcus agalactiae* (8,4 %). Av *S. aureus*- och KNS- isolaten var 80 % positiva för β- laktamasproduktion. Fler fall av SCM-CMT ≥ 4 sågs i betesfria system och i begränsade betessystem. De betesfria systemen var kopplade till sämre hygienstatus hos djuren och mindre besättningar. Vid jämförelse av kliniska fall av mastit och fall med SCM-CMT ≥ 4 var KNS, koliformer och *S. aureus* vanligare hos kliniska fall medan streptokocker var vanligare hos fall med SCM-CMT ≥ 4. För att sammanfatta var KNS den vanligaste patogenen hos både kliniska mastiter och från fall med SCM-CMT ≥ 4. De flesta stafylokockerna var resistenta mot penicillin men inga meticillinresistenta *S. aureus* påvisades. Kalvningsnummer, besättningsstorlek och betessystem var de faktorer som påverkade mastitförekomsten. Det var tydligt att det fanns brister i hygienrutinerna vid mjölkning, något som troligen bidragit till dålig juverhälsa. Fri tillgång till läkemedel var också en observerad riskfaktor för utvecklingen av resistenta bakterier.

TABLE OF CONTENT

LIST OF ABBREVIATIONS

CM-clinical mastitis

CMT-California Mastitis Test

CNS-coagulase negative staphylococci

CFU- colony-forming units

DDA-Dairy Development Authority

E.-Escherichia

EADD-East African Dairy Development

EUCAST-European Committee on Antimicrobial Susceptibility Testing

ILRI-International Livestock Research Institute

MAAIF-Ministry of Agriculture, Animal Industry and Fisheries

MALDI-TOF-Matrix Assisted Laser Desorption Ionization-Time of Flight mass spectrometry

MIC-Minimal Inhibitory Concentration

MRSA-methicillin resistant *Staphylococcus aureus*

OIE-World Organization for Animal Health

S.-Staphylococcus

SCC-somatic cell count

SCM-subclinical mastitis

SLU-Swedish University of Agricultural Sciences

Spp.-species

Str.-Streptococcus

SVA-Swedish Veterinary Institute

SVARM-Swedish Veterinary Antimicrobial Resistance Monitoring

INTRODUCTION

Urban and peri-urban dairy farming in Uganda

About one third (1.7 million) of the households in Uganda keep cattle to provide income, nutrition and employment. A report made by the Ministry of Agriculture, Animal Industry and Fisheries (MAAIF) in Uganda in 2008, estimated the number of cattle in Uganda to be 11.4 million, of which 93.6 % were indigenous breeds. 5.6 % were dairy cattle of exotic or cross breed and only 0.8 % were of exotic or cross breed beef cattle (Dairy Development Authority, DDA, 2010/2011).

Dairy farming in Uganda is mainly taking place in small-holder farms (DDA, 2010/2011), and the urban and peri-urban farming systems in Kampala are a consequence of prior instabilities in rural areas that forced farmers to move to the capital, bringing their animals with them. These farming systems are now decreasing in number as the city and its population are expanding. This expansion of the population has also forced new people with little experience into the farming business (Kanyima, B., pers. comm., 2012).

Actors influencing the dairy industry

Development and regulation of the dairy sector in Uganda are executed by the DDA, partly independent under the Ministry of Agriculture, Animal Industry and Fisheries (MAAIF). Some areas of work that the DDA executes include the organization of dairy farmers, promotion and safety of dairy products and provision of milk collecting equipment (DDA, 2010/2011).

An organized dairy market was first established in 1967. The sector was however negatively affected by political instability and civil war during 1971-1986. After 1986, the sector was rehabilitated and in 1993 the economy in Uganda was liberalized. The government promoted a free dairy industry market and was no longer involved in the milk production industry (DDA, 2010/2011).

In 2010, 1.5 billion liters of milk were produced in Uganda, an increase of 3 % compared to the year prior. Approximately 30 % of the milk is consumed by the producer and 70 % is sold in markets. Only 10-20 % of the marketed milk is being processed into different dairy products, the rest being sold on the informal market as raw milk. Also the import of dairy products to Uganda is decreasing and there is a coincidental increase of the exportation to regional markets. The consumption of milk per capita in Uganda has increased over the last decade and is about 50 litres per year (DDA, 2010/2011).

International Livestock Research Institute (ILRI) is providing help and solutions through research for farmers suffering from poverty in developing countries all over the world. The East African Dairy Development (EADD) project is one program led by ILRI where more effective and profitable dairy farming is being implemented in Kenya, Uganda and Rwanda (ILRI, 2012).

Charity organizations such as Heifer International are also influencing the market by providing farm animals that are given to people as charity to reduce poverty and hunger (Heifer International, 2012).

Bovine mastitis

Mastitis refers to an inflammation of the mammary tissue and is a common disease in dairy cattle. It is also one of the conditions that is most expensive for the dairy industry (Kossaibati & Esslemont, 2007). Mastitis is often caused by infection with different microorganisms, most commonly bacteria. Mastitis can affect one or more of the udder quarters and the disease can be divided into different categories. Clinical mastitis (CM) may for example vary from to severe according to symptoms seen in the animal. In subclinical mastitis (SCM), there are no visible changes in the animal´s milk, udder or general condition. Instead, diagnosis is made by laboratory analysis of the milk. Another way to categorize mastitis is taking into consideration duration of the disease, referring to mastitis as acute or chronic.

In a study from 2011 that was performed in the Kampala area in Uganda, the prevalence of subclinical mastitis was 86.2 % (Abrahmsén *et al*., 2012) and in a study performed in 2008 in the area around Jinja in Uganda, the prevalence of subclinical mastitis was 37.2 % (Byarugaba *et al*,. 2008). In Sweden it was estimated that incidence of clinical mastitis was 13 per 100 completed or interrupted lactations, based on reported treatments by practicing veterinarians in 2010/2011. Approximately 25.8 % of Swedish dairy cows have subclinical mastitis during a year based on a somatic cell count > 199 000/ml (Svensk Mjölk, 2010/2011).

Impact of mastitis

The impact of mastitis is mainly economical and the most evident costs are reduced milk yield, veterinary costs and the disposal of milk (Kossaibati & Esslemont, 2007). Examples of more indirect costs are reduced fertility, increased work load for the farmer and reduced quality of milk that aggravate the making of cheese and yogurt (Lavon *et al*., 2012; Kossaibati & Esslemont, 2007; Rogers & Mitchell, 1994). Mastitis may also cause severe illness and suffering and may lead to increased costs if the animal is culled or dies from the disease (Nielsen, 2009). Also, mastitis milk contains bacteria that might be a threat to food safety if the milk is not pasteurized before consumption.

Somatic cell count

The somatic cells found in milk are of immunologic origin and are body cells that enter from the animal´s bloodstream (for review, see Sandholm, 1995). An elevated somatic cell count (SCC) is almost always caused by mastitis and SCC can therefore be used as an indicator of udder health (Harmon, 1994). However, the SCC might also be elevated to some extent for other reasons, for example immediately after calving and at the end of lactation. Otherwise, the SCC remains constantly low during lactation in a healthy cow (for review, see Sandholm, 1995). Elevated SCC´s are correlated to reduced productivity and milk quality (for review,

see Kehrli & Shuster, 1994; Rogers & Mitchell, 1994) and may also indicate poor animal welfare (Broom, 2006). At mid-lactation, the SCC is normally < 100 000/ml and in a healthy udder quarter the somatic cells are mainly of macrophage origin. In milk from a mastitic udder quarter, 90 % of the somatic cells are of neutrophil origin (for review, see Sordillo *et al*., 1997). There are many methods available for assessing the SCC, both directly counting (e g Fossomatic™, DeLaval Cellcounter) and indirectly estimation (e g California Mastitis Test, Coulter Counter). With elevated SCC there is an increased risk that the quarter is harbouring an infection. The Swedish Dairy Association recommends bacteriological culturing of milk from animals with a SCC ≥ 150 000/ml (Svensk Mjölk, 2010).

California Mastitis Test

California Mastitis Test (CMT) is a method that indirectly evaluates the approximate concentration of somatic cells in milk (Schalm *et al*., 1971). A plastic vessel with four shallow wells is used for collecting approximately 2 ml of milk from each udder quarter. By adding a reagent that reacts with the DNA of somatic cells present in milk, different degrees of gel formation occur that correlates to the SCC in the milk when the container is tilted and rotated (for review, see Sandholm, 1995). According to the system used in the Nordic countries, scoring is made from 1-5 (for review, see Saloniemi, 1995), see Table 1 for interpretation of CMT-reaction.

Table1. Interpretation of CMT-reaction according to the Nordic scoring system. Modified from Schalm et al. (1971)

CMT-score	Interpretation	Visual reaction	Corresponding SCC/ml
1	Negative	No thickening or gel formation, fluid stays homogenous	0-200 000
2	Trace	Mild thickening of fluid when vessel is tilted	150 000-500 000
3	Weakly positive	Clear thickening of fluid when vessel is tilted	400 000-1 500 000
4	Positive	Clear thickening of fluid with a tendency of gel formation that disappears when vessel is not rotated	800 000-5 000 000
5	Strongly positive	Clear thickening and gel formation that remains when vessel is not rotated	> 5 000 000

Common agents in bovine mastitis

Staphylococcus (S.) aureus, coagulase negative staphylococci (CNS), *Streptococcus (Str.) dysgalactiae*, *Str. agalactiae* and *Str. uberis* are common causes of both clinical and subclinical mastitis (for review, see Pyörälä, 1995). Coliform bacteria such as *Escherichia coli* and *Klebsiella* spp. most commonly cause clinical mastitis and seldom give rise to subclinical cases (for review, see Hogan & Smith, 2003). *Staphylococcus aureus* and *Str.*

agalactiae are referred to as contagious udder pathogens as they are bound to the bovine udder or the cow and are mainly transmitted from cow to cow. Good milking hygiene is one important factor in order not to spread these organisms within a herd (for review, see Pyörälä, 1995). The coliform bacteria are called environmental pathogens as their main source of transmission is from the surroundings of the animal and are best managed by good environmental practices (for review, see Hogan & Smith, 2003) Coagulase negative staphylococci, *Str. uberis* and *Str. dysgalactiae* are considered to be both contagious and environmental pathogens (for review, see Taponen & Pyörälä, 2006; Todhunter *et al*., 1995; for review, see Pyörälä, 1995).

In Sweden, clinical mastitis is commonly caused by *S. aureus* and streptococci (Ericsson *et al*., 2009), and in subclinical mastitis *S. aureus*, CNS and streptococci are the most common pathogens found (Persson *et al*., 2011). In more limited studies in Uganda, the most common cause of clinical mastitis was *Klebsiella* spp. (Tweyongyere & Nambasa Kasirye, 1998), and the most common cause of subclinical mastitis identified was CNS followed by streptococci (Abrahmsén *et al*., 2012).

Staphylococcus aureus

Staphylococcus aureus and other staphylococci are part of the microflora of the udder skin and may colonize skin abrasions and also the teat canal. *Staphylococcus aureus* possesses many virulent factors. For example, it adheres to the fat compartment in milk, making it able to reach the mammary tissue quickly, where it remains and often causes chronic infections. Prognosis is poor if fibrotic foci develop in the mammary tissue. The production of α-hemolysin is a destructive factor causing gangrenous and potentially fatal mastitis, but usually the disease is less dramatic. The disease may become acute again, if a chronic infection is established (for review, see Taponen & Pyörälä, 2006; Pyörälä, 1995).

Coagulase negative staphylococci

Coagulase negative staphylococci are a group of pathogens that usually cause subclinical mastitis or mild cases of clinical mastitis (Taponen *et al*., 2006). The grade of tissue damage is often mild. It may cause infections that lead to isolation of the pathogen over a long period of time in the same animal. Persisting mastitis caused by CNS may cause a decrease in milk production. Mastitis is common in heifers prior to parturition (for review, see Pyörälä, 1995).

Streptococci

Streptococcal mastitis causes a superficial infection as the pathogen stays in the milk ducts. *Streptococcus agalactiae* is an obligatory udder pathogen and is highly contagious. It spreads mainly during milking. Mastitis is often chronic or recurrent but may also be acute, with mild to moderate symptoms (for review, see Pyörälä, 1995). In Sweden, *Str. agalactiae* has been a rather rare cause of mastitis but the prevalence has increased in larger herds using automatic milking systems in Sweden (AMS) (Persson Waller & Landin, 2012). *Streptococcus dysgalactiae* is less contagious than *Str. agalactiae* and mastitis is often acute and occurs in early lactation. Symptoms are often more serious than for other streptococci. *Streptococcus*

uberis is found everywhere in the cow´s surroundings and mastitis often occurs in early lactation and in the end of the dry period (fore review, see Pyörälä, 1995). Symptoms are commonly moderate to severe (Milner *et al*., 1997).

Coliforms

Coliforms (e g *E. coli* and *Klebsiella* spp.) belong to the colonic flora and infect the udder via fecal contamination of the cow´s surroundings. They do not survive long inside the udder. Coliform mastitis is common during the puerperal period and symptoms are often acute to peracute as a consequence of endotoxin production (for review, see Sandholm & Pyörälä, 1995).

Antimicrobial resistance patterns of udder pathogens

In both human and veterinary medicine antimicrobial resistance in bacteria is a problem on the rise. It is stated by the World Organization for Animal Health (OIE) that the human, animal and plant sectors must take responsibility for reducing the development of resistant pathogens. Prudent use of antimicrobial therapies and monitoring of antimicrobial susceptibility of bacterial flora in animals are two examples of recommendations to achieve this goal (OIE, 2013).

In Sweden, mastitis accounts for the majority of antibiotics used in dairy cattle. Systemic treatment with penicillin is the most common choice of treatment of mastitis (SVS, 2011). In Sweden strict policies concerning the use of antimicrobial therapy in veterinary medicine are applied and a stable or declining use of antimicrobial drugs in animals is seen. For example, it is recommended that antimicrobial therapy is based on results from bacteriological culturing. Also, medical treatments in animals are commonly preceded by a visit from a practicing veterinarian and all antimicrobial drugs are by prescription. The pharmacies also keep and deliver data concerning sales of medical products (SVARM, 2011). In Uganda, the farmers can easily treat their own animals as pharmaceutical products are available over the counter. Byarugaba´s report from 2004 revealed a high frequency of bacteria resistant for a majority of antimicrobial substances when analyzing pathogens seen in bovine mastitis in Uganda. The resistance patterns for most of the examined substances also showed an increasing trend over a 10-year period (1991-2000) (Byarugaba, 2004). Trends of resistance patterns for specific substances are shown in Table 2.

Table 2. Antimicrobial resistance of mastitis pathogens in Uganda in the periods of 1991-1995 and 1996-2000. Source: Veterinary Microbiology Laboratory, Makerere University, Kampala. Modified from Byarugaba (2004)

Antimicrobial substance	**1991–1995**		**1996–2000**	
	No. of isolates tested	**Percentage resistance**	**No. of isolates tested**	**Percentage resistance**
Penicillin	32	93.7	43	83.5
Ampicillin	160	78.8	137	85.2
Erythromycin	135	76.3	140	76.8
Tetracycline	114	73.7	121	76.5
Streptomycin	129	69.7	82	76.8
Cloxacillin	93	61.0	27	87.6
Chloramphenicol	125	29.6	139	38.1
Kanamycin	162	14.2	152	13.2
Gentamycin	163	7.4	142	3.4

Staphylococci may be resistant to β-lactam antibiotics by two mechanisms (SVA, 2012a). The most common mechanism is production of β-lactamase that degrades penicillins and aminopenicillins. The other mechanism is a structural change of the penicillin binding protein that disables any β-lactam antibiotic (penicillins, aminopenicillins and cephalosporins) from attaching to the bacterium. This is referred to as methicillin resistance, and *S. aureus* that possess this mechanism is called MRSA (methicillin resistant *Staphylococcus aureus*). In a study performed in the Jinja area in Uganda, resistance to penicillin among staphylococci was 86.8 % (Byarugaba *et al*., 2008). In a study on clinical cases of mastitis in Sweden, the figures were 1.9 % for *S. aureus* and 12.5 % for CNS (Bengtsson *et al*., 2009), and for subclinical mastitis the corresponding figures were 4 % and 35 % (Persson *et al*., 2011).

OBJECTIVES

The aims of this study were to investigate the bacteriological panorama in milk from udder quarters with clinical and subclinical mastitis and to determine the antimicrobial susceptibility in staphylococcal isolates. Further, we intended to establish the prevalence of subclinical mastitis and to investigate some environmental factors and animal properties that might be correlated to the frequency of mastitis.

MATERIALS AND METHODS

General information

Collection of data

A protocol (Appendix 1) was used during field work for recording data concerning complete herd size (including calves and young stock), number of lactating cows (only counting the animals being milked at the time of the visit) and grazing system. Information about parity, stage of lactation (days) and peak daily milk production (L/day) from examined animals was recorded when accessible. The majority of data was collected from hand written records kept on farms. Hygiene status was identified as either "good" (macroscopically clean) or "poor" (macroscopically dirty) and was visually evaluated in the majority of studied animals.

During field work, notes were also made about milking hygiene procedures, milking technique, type of feed and choice of treatment for clinical cases of mastitis. Photos were taken on each farm.

Herds

The study was conducted on 17 dairy farms in and around Kampala, the capital of Uganda, during the period of September to November 2012. This period was during the second seasonal rain period in Uganda (Holden, 2012). The farms visited were chosen by local supervisor Dr Benon Kanyima, veterinarian and PhD-student at the College of Veterinary Sciences, Animal resources and Biosecurity, Makerere University, Kampala. The complete herd size (including calves and young stock) varied between 2-474 animals (mean value 64.4, median value 23) and the number of lactating animals (only counting the animals being milked at the time of the visit) varied between 1-67 (mean value 19.5, median value 12) on the visited farms. The majority of animals were crosses of Holstein/Friesian breed. Jersey, Guernsey, German and local breeds were also encountered, but less frequently.

Milking procedure

All 17 farms had hired personnel that milked the cows manually (by so called "strip milking"). Sixteen farms milked their animals two times daily, in the morning and in the afternoon. One farm milked their animals three times daily. On all farms the udders were cleaned with water. The water used was collected during rains and was seldom heated before cleaning the udders. Most commonly the milker kept the water in a small cup and wiped off the udder with his wet hand. The same cup was used for all animals. If a towel was used for cleaning and drying the udder, it was not changed between animals. No solution for dipping teats was used after milking. A majority of the milking personnel used a salve to lubricate the teats and facilitate milking.

Grazing system

Three different types of grazing systems were included in this study; zero-grazing (Figure 1), open grazing (Figure 2) and confined grazing (Figure 3) systems. Distribution of grazing systems in this study is shown in Table 3 and locations are shown in Figure 4.

Table 3. Distribution of farms and animals according to grazing system

Grazing system	No of farms (%)	No of animals (%)
Zero-grazing	5/17 (29)	21/138 (15)
Open grazing	8/17 (47)	60/138 (43.5)
Confined grazing	4/17 (24)	57/138 (41.5)

Figure 1. Zero-grazing system. Photo: Sandra Björk

Figure 2. Open grazing system. Photo: Sandra Björk

Figure 3. Confined grazing system. Photo: Sandra Björk

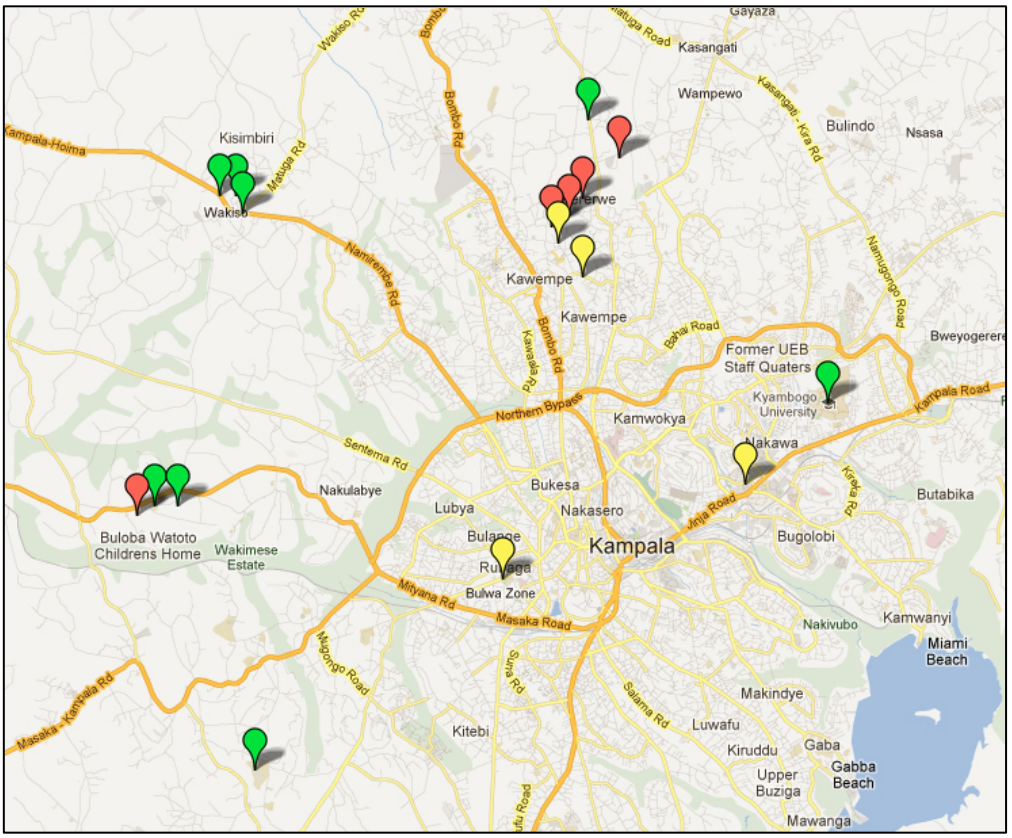

Figure 4. Map of Kampala showing the distribution of visited farms.
=zero-grazing unit, = open grazing unit, =confined grazing unit.

Feeding

Aside from pasture, animals were commonly fed from peelings of bananas or potatoes, maize and/or fresh elephant grass. Some farms also used a concentrate ("dairy meal") that was either commercial or prepared manually, and silage from maize or elephant grass in addition to mentioned feeds and pasture.

Clinical mastitis

Study population

Prior to the study, farm owners were contacted and instructed to contact the local supervisor when an animal with any of the previously mentioned signs of clinical mastitis was recognized on the farm. Farm owners were also instructed not to treat the animal with any substances prior to sampling. Fourteen farms were visited during the study and complete herd size (including calves and young stock) varied between 4-474 animals (mean value 74, median value 25). The number of lactating cows (only counting the animals being milked at the time of the visit) varied between 2-67 (mean value 21.7, median value 16). See Table 4 for details on visited farms. Farms were visited at any time during the day, but as soon as possible after farm owners had contacted the local supervisor. Some of the cases were identified during the study about animals with a subclinical mastitis, which is described later, and were therefore visited during afternoon milking.

Table 4. Information on complete herd size and number of lactating cows on visited farms, "-" refers to missing data

Farm no.	Total no. of animals	No. of lactating cows	Farm no.	Total no. of animals	No. of lactating cows
1	16	12	8	474	67
2	4	2	9	79	28
3	22	10	10	36	20
4	19	-	11	27	9
5	99	41	12	-	3
6	11	-	13	23	8
7	74	20	14	-	40

Clinical examination

Evaluation of general condition including measurement of rectal temperature and palpation of the udder was executed on all animals. Clinical findings and CMT-score were recorded in a protocol (Appendix 1). If there were any signs of clot formation, discoloration, alterations in viscosity, aberrant smell or blood in the milk from any of the udder quarters, the animal was identified as a clinical case of mastitis. The same applied to any abnormal findings in the animal´s udder such as increased firmness, swelling, soreness and redness. All clinical findings, also including alterations in general condition such as fever (rectal temperature > 39°C), depression or decreased appetite, were noted. At quarter level, alterations in milk or

udder were required for a quarter to be classified as positive for clinical mastitis. Animals that had received treatment before sampling were included in the study as all information concerning treatments was considered very uncertain due to readily accessible pharmaceutical products.

Collection of milk samples for bacteriological examination

Milk from all quarters of the affected animals was examined for visual abnormalities and was then examined with CMT. Approximately 5 ml of milk were collected aseptically from all quarters with signs of clinical mastitis. Milk samples were kept cool during transportation using ice packs and were stored refrigerated (4°C) for a maximum of 12 hours before culturing. A total of 24 milk samples were collected from 18 animals. All but two animals were examined and sampled by the author. Two animals were examined and sampled by the local supervisor, and these animals were not examined by CMT.

Subclinical mastitis

Study population

Cases of subclinical mastitis based on CMT were investigated. Thirteen farms were visited during the afternoon milking. Complete herd size (including calves and young stock) varied between 4-99 animals (mean value 32, median value 19) and number of lactating cows (only counting the animals being milked at the time of the visit) varied between 1-41 (mean value 15, median value 11). The intention was to examine all four quarters of all lactating animals by CMT on the visited farms. However, due to practical problems, between 40-100 % (mean value 83 %, median value 99 %) of the lactating animals turned out to be examined. The total number of examined animals was 120. See Table 5 for specific details from visited farms. If an animal showed signs of clinical mastitis it was included in the study of clinical cases described above. A CMT ≥ 3 according to the scoring system used by the Nordic countries was considered as subclinical mastitis.

Table 5. Information on complete herd size, number of lactating cows and number of examined lactating cows on visited farms, "-" refers to missing data

Farm no.	Total no. of animals	No. of lactating cows	No. of examined lactating cows (%)
1	16	12	7/12 (58)
2	4	2	2/2 (100)
3	22	10	9/10 (90)
4	19	-	4/-
5	99	41	40/41 (98)
6	11	-	8/-
7	68	12	5/12 (42)
8	74	20	20/20 (100)
9	5	-	3/-
10	9	1	1/1 (100)
11	27	9	9/9 (100)
12	-	3	3/3 (100)
13	-	40	16/40 (40)

Collection of milk samples for bacteriological examination

Only milk with a CMT ≥ 4 was subjected to bacteriological examination in this study. After cleaning of the udder by the milking personnel, a minimum of 10-15 streaks of milk were allowed to be milked before examination with CMT. Mid-stream milk from all quarters of the examined animals was checked with CMT. Of the 480 examined quarters, 465 were evaluated with CMT as 3 % of the quarters were identified as blind and not further examined. Milk was sampled for bacteriological examination in the same way as for clinical cases. The total number of milk samples was 166 and they were collected from 78 animals. All clinical examinations and collection of samples were executed by the author.

Bacteriological analyses of milk samples

Bacteriological examination

The following analyses were executed locally at the reproduction laboratory at the College of Veterinary Sciences, Animal resources and Biosecurity, Makerere University, Kampala.

Samples were examined for bacterial growth by collecting 10 µl of the milk with a sterile loop and spreading it on 5 % bovine blood agar plates with aesculine. Plates were evaluated 24 hours and 48 hours after aerobic incubation in 37°C. A preliminary diagnosis of bacteria was based on bacterial colony appearance and growth patterns (SVA, 2012b; National Mastitis Council, 1999; Quinn *et al*., 1994). Growth of ≥ 3 colony-forming units (CFUs) were considered as positive growth, except for *S. aureus* and *Str. agalactie*, where ≥ 1 CFU were considered positive. Growth patterns were described as mild (< 10 CFUs), moderate (10-50

CFUs) or severe (> 50 CFUs). A growth of ≥ 2 different microorganisms was classified as mixed flora, if there was no growth of major udder pathogen (*S. aureus* or streptococci species associated with mastitis).

The potassium hydroxide test was made to distinguish Gram positive and Gram negative bacteria and the catalase test (hydroperoxide) was used to separate streptococci from staphylococci. Milk from samples with a positive growth of colonies with a Gram negative appearance were put on MacConkey agar (selective media for Gram negative bacteria) and then incubated aerobically for 24 hours in 37°C. After evaluation, purple colonies were tested for production of β-D-glucuronidase by using SELMA® P-test (National Veterinary Institute (SVA), Uppsala, Sweden) to distinguish *Klebsiella* spp from *E. coli*. Colonies without purple color were categorized as coliform bacteria.

All streptococci, staphylococci and uncertain isolates were transported to Sweden for further analyses. Colony material was collected with a sterile 1 µl loop and inoculated in cryo tubes containing blood agar base no 2, oxoid CM0271 and 5 % horse serum. The tubes were then kept refrigerated (4°C) during storage and kept cool using ice packs during transportation.

Further analyses and typing of bacteria

As previously mentioned, all staphylococci, streptococci and uncertain isolates were transported to Sweden for further investigation. The following analyses were executed according to routine methods by personnel at the accredited mastitis laboratory at the National Veterinary Institute (SVA) in Uppsala.

Staphylococcal isolates were analyzed using the reversed CAMP-reaction test and the coagulase test. The streptococci were analyzed using the CAMP-reaction test and were then typed into species by using 12 different biochemical reactions ("SVA-strept", National Veterinary Institute (SVA), Uppsala, Sweden); hippurate, aesculine, salicine, sorbitol, mannitol, raffinose, lactose, saccharose, inuline, trehalose, starch and glycerine. Mass spectrometry analysis with MALDI-TOF (Matrix Assisted Laser Desorption Ionization-Time of Flight mass spectrometry) was performed on CAMP-positive streptococci that could not be typed into species by biochemical tests and on CNS isolates.

Other analyses than the above mentioned included Gram coloring, oxidase test and indol test.

Staphylococci that were not *S. aureus* or CNS were classified as "staph other" and coliform bacteria that could not be classified as *E. coli* or *Klebsiella* spp were classified as "coliforms". When cultures could not be typed into species they were referred to as "other".

Examination of antimicrobial susceptibility

All *S. aureus* and CNS isolates were examined for β-lactamase production, using the penicillinase test ("clover leaf method").

All *S. aureus* isolates that were positive for β-lactamase production were also tested for antimicrobial susceptibility by determination of minimum inhibitory concentration (MIC) using a microdilution method. Antimicrobial substances that were analyzed were penicillin (Pc), tetracycline (Tc), cephalothin (Ct), oxacillin (Ox), gentamicin (Gm), erythromycin (Em), kanamycin (Km), chloramphenicol (Cm), ciprofloxacin (Ci), clindamycin (Cl) and trimethoprim (Trim). Testing was performed by the use of VetMIC™ panels (National Veterinary Institute (SVA), Uppsala, Sweden) and cation adjusted Mueller-Hinton broth. For testing of oxacillin susceptibility, 2 % NaCl was added to the broth. A quality control strain (*S. aureus* ATCC 29213) was tested in parallel with the isolates. Isolates were classified as susceptible or resistant based on cut off values presented by European Committee on Antimicrobial Susceptibility Testing (EUCAST) (EUCAST, 2010).

Statistical analyses

General information

Descriptive statistics were made to visualize the grouping of animals according to individual and herd management factors (Table 6). Chi^2 (χ^2) test was performed to discover correlations between factors. Factors that were used in statistical analyses were grazing system (GS), complete herd size including calves and young stock (HS), parity (Par), stage of lactation (Lac), peak milk production (MP), hygiene status (Hyg), and mastitis status (Stat). Subdivisions of factors are shown in table 6.

Due to practical reasons, all analyzed correlations were made on clinical mastitis and on subclinical mastitis with a CMT $\geq$ 4 (SCM-CMT $\geq$ 4). Chi^2 test was also performed to compare bacteriological results between clinical and SCM-CMT $\geq$ 4 cases of mastitis. Grouping of pathogens were made to even out distribution of observations in the different categories. All staphylococci except *S. aureus* were put into one group (“Staph”), all streptococci (“Strept”) were put into one group as were all negative and mixed growths (“Neg/MF”) and coliform bacteria (“Coliform”). Correction of the analysis was necessary due to low numbers of observations in certain categories.

Table 6. Subdivision of individual and herd management factors used in statistical models

Factor	Subdivision	Factor	Subdivision
GS	Zero-grazing	MP	≤ 20 L
	Open grazing		> 20 L
	Confined grazing		
HS	≤ 20 animals	Hyg	Good
	> 20 ≤ 50 animals		Poor
	> 50 ≤ 90 animals		
	> 90 animals		
Par	1	Stat	CMT ≤ 3
	2-3		SCM-CMT ≥ 4
	>3		CM
Lac	≤ 120 days		
	> 120 days		

Clinical mastitis

Descriptive statistics were used to present bacteriological and antimicrobial susceptibility results and other data about the clinical cases.

Correlations between factors (mentioned above) and clinical mastitis were investigated by χ^2 test.

Subclinical mastitis

Descriptive statistics were used to present prevalence, distribution of CMT-cores and blind quarters as well as other data concerning subclinical mastitis. Descriptive statistics were also used to present bacteriological and antimicrobial susceptibility results from the SCM-CMT ≥ 4 cases.

Correlations between factors (mentioned above) and frequency of subclinical mastitis with a CMT ≥ 4 (SCM-CMT ≥ 4) were investigated by χ^2 test. Factors that were found to significantly influence the frequency of SCM-CMT ≥ 4 after performed χ^2 test were analyzed by multi-variable analysis to further investigate their impact on the frequency of SCM-CMT ≥ 4. Factors were put together in logistic models to reveal more possible correlations between them that were not obvious after χ^2 test. Analyses were made to identify the most important factors that influence the frequency of SCM-CMT ≥ 4.

Sources of error

Factors concerning the laboratory equipment used in Uganda were the main cause of problems during this study. Some of the agar plates froze during transportation and fluids from the frozen agar contaminated a large number of the remaining plates. The contamination was revealed after a few days when bacterial growth was found on several plates. It was not

possible to predict which plates were contaminated, resulting in the use of plates with questionable quality while waiting for a new batch to arrive. There were also issues with keeping an even temperature in the refrigerator where the plates were being stored, resulting in the freezing of more dishes. New plates were ordered from Sweden but these unfortunately got lost in luggage handling and were regained over 24 hours after expected arrival. These plates were however in good condition at arrival, but it cannot be excluded that these plates were affected negatively by the improper handling.

The weather conditions during the field work made aseptic sampling difficult on one farm, as heavy rain made the environment very moist and caused water to splash when hitting the ground.

The selection of study animals was made exclusively by the local supervisor and were therefore not randomized (convenience sampling).

RESULTS

General information concerning individual and herd management factors

Collected information concerning individual and herd management factors from all 138 studied animals was processed and put into Table 7. Most individuals had a CMT ≥ 3 in at least one quarter and their general hygiene status was good. A majority of the studied animals were in a late stage of lactation (> 120 days) and had delivered 2-3 calves.

Table 7. Distribution of studied animals (n=138) according to individual and herd management factors. "Total" indicates the total number of animals where current data was accessible

Factor	Subdivision	No. of examined animals (%)
Grazing system	Zero-grazing	21 (15)
	Open grazing	60 (43.5)
	Confined grazing	57 (41.5)
Total:		138 (100)
Herd size (total no. of animals)	≤ 20 animals	29 (24)
	> 20 ≤ 50 animals	21 (18)
	> 50 ≤ 90 animals	27 (23)
	> 90 animals	42 (35)
Total:		119 (100)
Parity	1	29 (25)
	2-3	72 (62)
	> 3	15 (13)
Total:		116 (100)
Stage of lactation	≤ 120 days	45 (42)
	> 120 days	61 (58)
Total:		106 (100)
Peak milk production	< 10 L	12 (10.5)
	10-20 L	66 (58)
	> 20 L	36 (31.5)
Total:		114 (100)
Hygiene status	Good	108 (79)
	Poor	28 (21)
Total:		136 (100)
Mastitis status	CMT ≤ 2	12 (9)
	CMT ≥ 3	108 (78)
	CM	18 (13)
Total:		138 (100)

Open grazing and confined grazing systems were most commonly found in larger herds ($p < 0.0001$), and more individuals with a high peak milk production and in a late stage of lactation were found in confined grazing systems ($p=0.0038$ and $p=0.0495$). There was a strong correlation found between poor hygiene status and zero-grazing systems ($p < 0.0001$).

In larger herds there were more cows at a late stage of lactation ($p=0.0047$) and a higher proportion of animals with a high peak milk production ($p < 0.0001$). Poor hygiene status was mainly seen in smaller herds ($p < 0.0001$).

The peak milk production increased with parity ($p < 0.0001$) and was lower in animals with a poor hygienic status ($p=0.0339$).

Clinical mastitis

Eighteen clinical cases of mastitis were included and all animals had visible abnormalities in milk. Thirteen (72 %) of the animals had palpable changes in the udder and 8 (44 %) of the animals had fever, which was the only alteration in general condition found in this study.

Of these cases, 14/18 (78 %) animals had clinical mastitis in one quarter and 4/18 (22 %) animals had clinical mastitis in more than one quarter and 15/18 animals (83 %) had concurrent subclinical mastitis with a CMT ≥ 3 in at least one quarter. One animal had a blind quarter. At quarter level, 24/72 (33.5 %) of examined quarters were classified as positive for clinical mastitis, 34/72 (47 %) quarters had subclinical mastitis with a CMT ≥ 3 and 1/72 (1 %) quarters was classified as blind. Distribution at animal and quarter level according to properties is summarized in Table 8.

Table 8. Information on blind quarters and no. of quarters affected by CM and SCM at cow and quarter level

Properties of clinical cases	**No. of animals (%)**	**No. of quarters (%)**
Blind quarters per cow		
0	17/18 (94)	71/72 (99)
≥ 1	1/18 (6)	1/72 (1)
Total:	18/18 (100)	72/72 (100)
No. of affected quarters (CM) per cow		
1	14/18 (78)	14/72 (19.5)
2	2/18 (11)	4/72 (5.5)
3	2/18 (11)	6/72 (8.5)
4	0/18 (0)	0/72 (0)
Total:	18/18 (100)	24/72 (33.5)
No. of affected quarters (CMT ≥ 3)		
0	1/18 (6)	7/ 72 (9.7)
≥ 1	15/18 (83)	34/72 (47)
not examined	2/18 (11)	7/72 (10)
Total:	18/18 (100)	48/72 (66.5)

Bacteriological results

The most common pathogen found was CNS (29 %), followed by mixed flora (25 %) and *E. coli* (12.5 %). When excluding mixed flora and negative growth, the proportion of CNS was 7/16 (44 %) followed by *E. coli* (3/16, 19 %). Distribution of pathogens is presented in Figure 5.

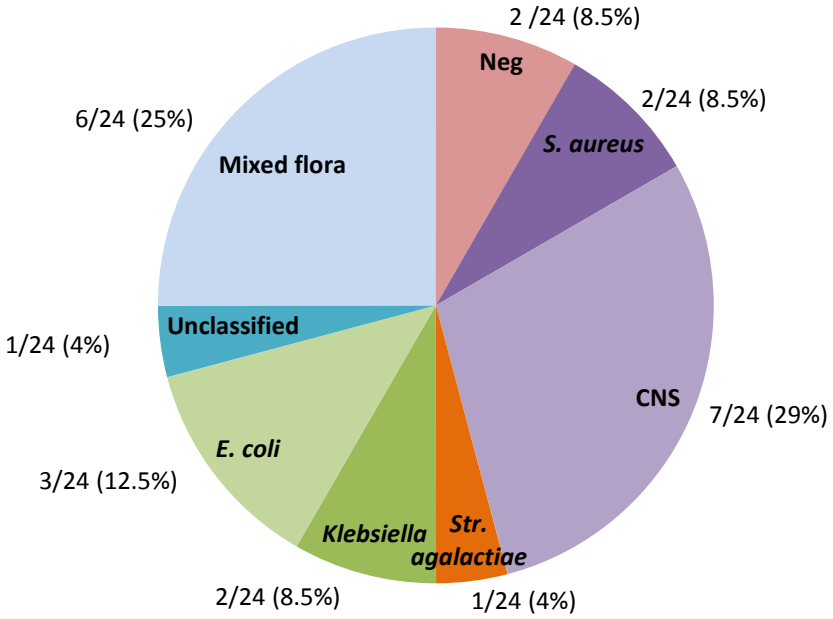

Figure 5. Distribution of pathogens from quarters with clinical mastitis (n=24). When cultures could not be typed into species they were referred to as "unclassified".

Typing of coagulase negative staphylococci from clinical mastitis

Two species of CNS was found, *S. epidermidis* (86 %) and *S. warneri* (14 %) (Figure 6).

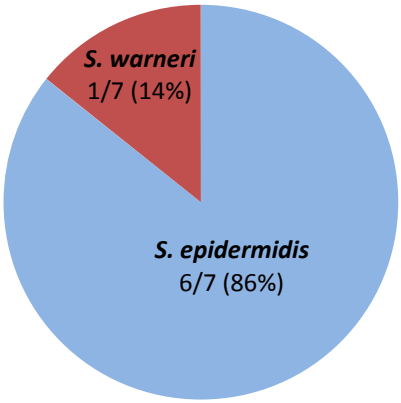

Figure 6. Distribution of CNS species from quarters with clinical mastitis caused by CNS.

Antimicrobial resistance patterns of staphylococci from clinical mastitis

Beta-lactamase production

Positive growth of *S. aureus* or CNS was found in 9 of the 24 samples. These isolates were examined for β-lactamase production. Of the *S. aureus* and CNS isolates 2/2 (100 %) and 7/7 (100 %) were positive for β-lactamase production (pc+) respectively.

Antimicrobial susceptibility based on MIC-values

Resistance patterns for the two *S. aureus* isolates positive for β-lactamase production are presented in Table 9. Both isolates had a MIC-value above the cut off value for resistance for trimethoprim and penicillin (EUCAST, 2010). All other substances that were tested had a MIC-value that were below or equal to the set cut off value for resistance for both isolates.

Table 9. Presentation of MIC-values of the two S.aureus isolates positive for β-lactamase production for each tested antimicrobial substance

Antimicrobial (cut-off value for resistance)	Pc (>0.12)	Ct (>1)	Ox (>2)	Em (>1)	Cm (>16)	Cl (>0.25)	Tc (>1)	Gm (>2)	Km (>8)	Ci (>1)	Trim (>2)
Isolate											
1	>4	0.5	2	0.5	8	≤0,25	≤0,5	1	4	1	4
2	>4	0.25	1	0.5	8	≤0,25	≤0,5	≤0,5	4	0.25	4

Treatment of clinical cases of mastitis

Three of the clinical cases had received antibiotic therapy prior to sampling. All animals had a positive bacterial growth despite treatment; One *S. aureus* pc+, one CNS pc+ and one pathogen that could not be typed into species.

The most commonly used treatment for clinical mastitis was intramammary suspensions including a combination of procaine penicillin, streptomycin sulphate, neomycin sulphate and prednisolon (Multiject IMM, Norbrook, Great Britain). Intramammary suspensions containing trimethoprim and sulphadiazin was occasionally used (Duofast, Norbrook, Great Britain). Intramammary therapies were often combined with systemic treatment with penicillin, streptomycin and oxytocin. No other measures such as treatment with NSAID´s, frequent milking or separation of ill animals were implemented.

Effect of individual and herd management factors on clinical mastitis

The number of individuals with clinical mastitis was higher in open grazing systems ($p=0.0302$). Clinical cases of mastitis were more commonly found among individuals with a parity > 1 ($p=0.0140$) and in herds with ≤ 50 animals ($p=0.0007$). Figure 7 summarize the distribution of animals for factors parity and herd size.

There were no significant correlations to peak milk production, stage of lactation or hygiene status found in this group.

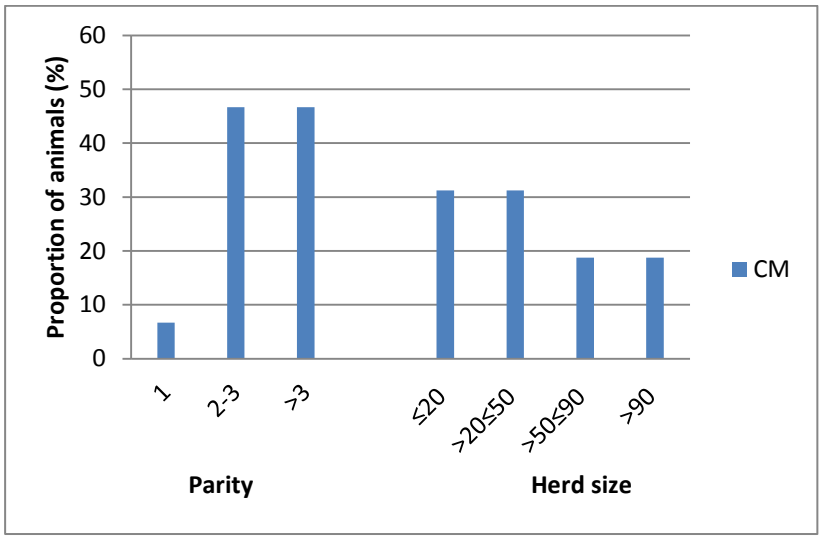

Figure 7. Distribution of cows with clinical mastitis according to parity and herd size

Subclinical mastitis

California Mastitis Test

Of 480 quarters, 465 were evaluated with CMT. The proportion of blind quarters was 15/480 (3 %). Distribution of CMT and blind quarter is presented in Figure 8.

Subclinical mastitis based on a CMT ≥ 3 in at least one quarter was found in 108/120 (90 %) animals. It was most common to have a CMT ≥ 3 in all four quarters (36.6 %) and the majority (72.5 %) of animals was positive in more than one quarter. Number of affected quarters in all examined animals is presented in Table 10. At least one blind quarter were found in 9/120 (7.5 %) animals.

Table 10. Distribution of animals (n=120) with different number of udder quarters affected with subclinical mastitis (CMT ≥ 3)

No. of quarters per cow affected with subclinical mastitis (CMT ≥ 3)	**No. of animals (%) n=120**
0	12 (10)
1	21 (17.5)
2	23 (19.2)
3	20 (16.7)
4	44 (36.6)
Total	120 (100)

At quarter level, the prevalence of subclinical mastitis (CMT ≥ 3) was 303/480 (63 %) and a CMT-score of 3 was the most common finding (28.5 %).

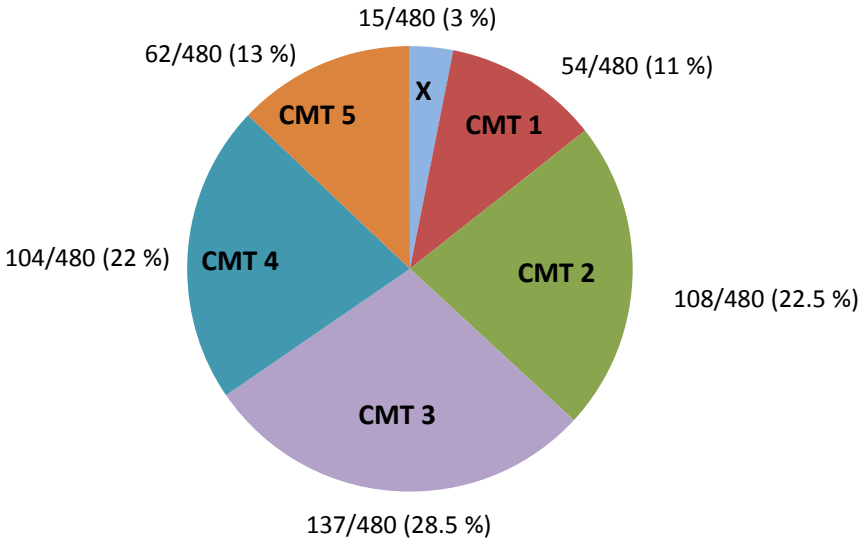

Figure 8. Frequency of blind quarters and CMT-scores and at quarter level. X=blind quarter.

Bacteriological results

Milk samples were cultured from all quarters with a CMT ≥ 4 (n=166). Among all samples, the most common finding was CNS (12.0 %) followed by *Str. agalactiae* (8.5 %). When mixed flora and negative growth were excluded, the figures were 31.7 % and 22.2 % respectively. The results are presented in Figure 9.

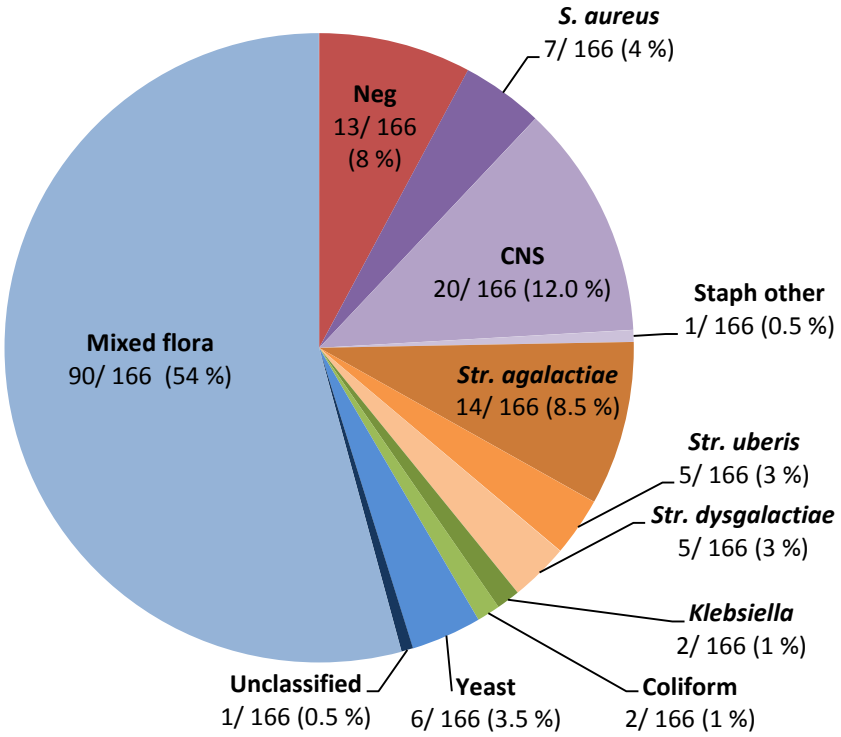

Figure 9. Results of bacteriological examination of milk samples with a CMT ≥ 4 (n=166). When cultures could not be typed into species they were referred to as "unclassified".

Typing of coagulase negative staphylococci from subclinical mastitis

Two species of CNS was found, *S. epidermidis* (85 %) and *S. haemolyticus* (15 %) (Figure 10).

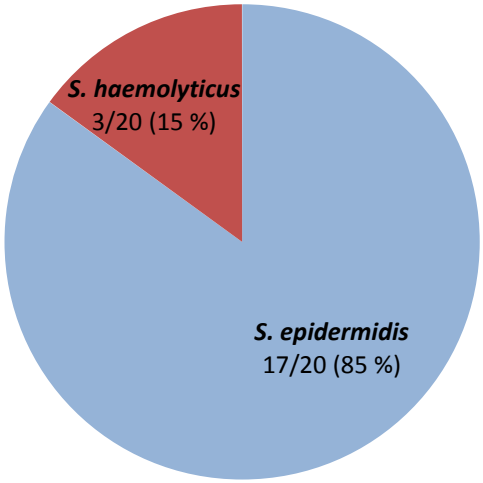

Figure 10. Distribution of CNS species from quarters with CMT ≥ 4.

Antimicrobial resistance patterns of staphylococci from subclinical mastitis

Beta-lactamase production

Positive growth of *S. aureus* or CNS was found in 27 of the 166 samples. These isolates were examined for β-lactamase production. Of the *S. aureus* and CNS isolates 6/7 (86 %) and 16/20 (80 %) were positive for β-lactamase production respectively (Figure 11).

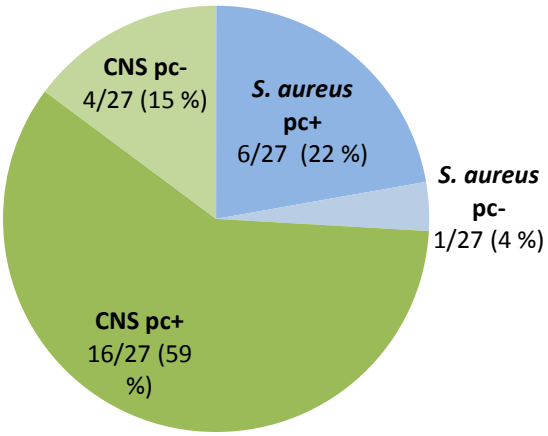

Figure 11. Distribution of β-lactamase production in S. aureus and CNS isolates (n=27). Pc+=β-lactamase positive, Pc-=β-lactamase negative.

Antimicrobial susceptibility based on MIC-values

Resistance patterns for the six *S. aureus* isolates positive for β-lactamase production are presented in Table 11. All isolates had a MIC-value above the cut off value for resistance for penicillin and 3/6 (50 %) isolates were above the cut off value for resistance for trimethoprim. One isolate was above the cut off value for resistance for each oxacillin and chloramphenicol and two isolates were above the cut off value for resistance for tetracycline. All other substances that were tested had a MIC-value below or equal the set cut off value for resistance.

Table 11. Presentation of MIC-values for the six S.aureus isolates positive for β-lactamase production for each tested antimicrobial substance

Antimicrobial (cut-off value for resistance)	Pc (>0.12)	Ct (>1)	Ox (>2)	Em (>1)	Cm (>16)	Cl (>0.25)	Tc (>1)	Gm (>2)	Km (>8)	Ci (>1)	Trim (>2)
Isolate											
1	4	0.25	4	1	16	≤0.25	≤0.5	≤0.5	2	0.5	2
2	>4	0.5	2	1	8	≤0.25	≤0.5	≤0.5	4	1	4
3	4	0.5	2	0.5	8	≤0.25	16	≤0.5	1	0.25	2
4	>4	0.5	1	0.5	8	≤0.25	≤0.5	≤0.5	2	0.25	4
5	>4	0.25	1	0.5	8	≤0.25	≤0.5	≤0.5	2	0.5	4
6	4	0.5	2	0.5	8	≤0.25	16	≤0.5	2	0.12	2

Effect of individual and herd management factors on subclinical mastitis with a CMT ≥ 4

There was a higher percentage of cows with SCM-CMT ≥ 4 in zero- and confined grazing systems and a lower percentage in open grazing systems. (p=0.0169). In herds with ≤ 50 animals and in large herds (> 90 animals) there was a higher number of individuals with SCM-CMT ≥ 4 when compared to herds with > 50 ≤ 90 animals (p=0.0002). There were fewer individuals with a SCM-CMT ≥ 4 among cows that only calved once compared to cows with a higher parity (p=0.0134).

There were no effects of hygiene status, stage of lactation or peak milk production on the frequency of SCM-CMT ≥ 4 found.

Summary of results is presented in Figure 12.

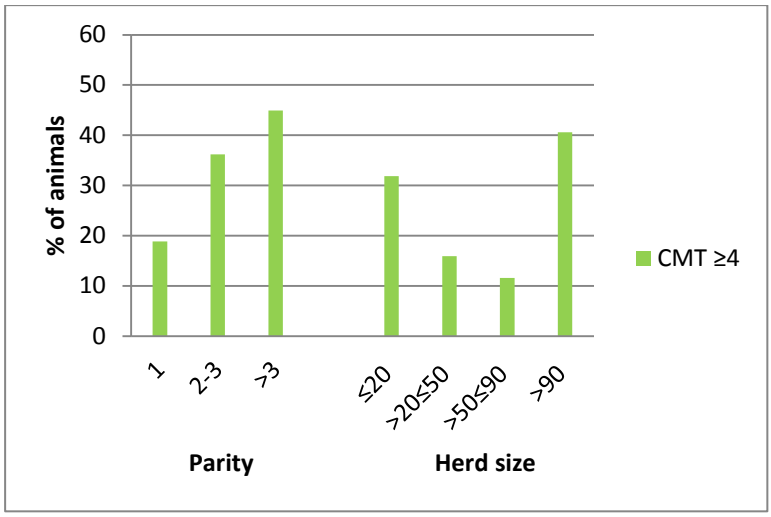

Figure 12. Distribution of cows with at least one quarter affected with SCM (CMT ≥ 4) according to parity and herd size.

After multi-variable analyses of the factors found to significantly affect the frequency of SCM-CMT ≥ 4 cases after χ^2 test, grazing system and parity still had a significant correlation to SCM-CMT ≥ 4 frequency when analyzed together (p=0.0372 and p=0.0261 respectively). When analyzing stage of lactation, maximum milk production and parity together, only parity still showed a significant correlation to SCM-CMT ≥ 4 (p=0.0378). When parity was put together with herd size, parity had only a tendency to correlate to frequency of SCM-CMT ≥ 4 cases (p=0.0740) while herd size had a significant effect on SCM-CMT ≥ 4 frequency (p=0.0044). Table 12 summarizes results from χ^2 test and multi-variable analysis, see previous section for description of correlations. Since herd size and grazing system were strongly linked, either one of them together with parity were the only two factors that significantly influenced the SCM-CMT ≥ 4 frequency.

Table 12. Results after single χ^2 test and multi-variable analysis on factors effect on SCM-CMT ≥ 4 frequency. Factors included showed no internal correlations after single χ^2 test. P-values are shown, NS=not significant

Factors	**Results after single χ^2 test**	**Results after multi-variable analysis**
GS	p=0.0169	p=0.0372
Par	p=0.0134	p=0.0261
HS	p=0.0002	p=0.0044
Par	p=0.0134	p=0.0740 (tendency)
Lac	NS	NS
MP	NS	NS
Par	p=0.0134	p=0.0378

Differences in bacteriological results between cases of clinical and subclinical mastitis

Correlations between bacteriological results and mastitis status were investigated on the clinical mastitis cases and the SCM-CMT ≥ 4 cases (see Table 13). Overall distribution of pathogens was different in the two groups. Findings of staphylococci other than *S. aureus* were markedly higher in the clinical mastitis group than in the SCM-CMT ≥ 4 group (31.0 % and 12.7 % respectively). Coliform bacteria and *S. aureus* were also more common in this group (11.9 % and 7.1 % compared to 2.4 % and 4.2 %). Streptococci were more common in the SCM-CMT ≥ 4 group (14.5 % compared to 2.4 %). Level of significance was $p < 0.01$.

Table 13. Frequencies of pathogens found in all sampled quarters from clinical and SCM-CMT ≥ 4 cases of mastitis. "Staph"= all staphylococci except S. aureus, "Strept"= all streptococci , "Coliform"= all coliforms, "Neg/MF"= negative and mixed growth

Pathogen	***S. aureus***	**Staph**	**Strept**	**Coliform**	**Unclassified**	**Neg/MF**	**Total**
Type of mastitis							
SCM	7/166 (4.2 %)	21/166 (12.7 %)	24/166 (14.5 %)	4/166 (2.4 %)	7/166 (4.2 %)	103/166 (62.0 %)	166
Clinical mastitis	3/42 (7.1 %)	13/42 (31.0 %)	1/42 (2.4 %)	5/42 (11.9 %)	2/42 (4.8 %)	18/ 42 (42.9 %)	42
Total	10	34	25	9	9	121	208

DISCUSSION

General information

Correlations between individual and herd management factors

There were obvious connections between a small herd size, zero-grazing systems and poor hygiene status. The zero-grazing systems provide a solution that enables small-scale dairy farming in the backyard of urban homes. As the name suggests, there was no availability of pasture in these systems and the animals were often restricted to a confined mud paddock, only a few square meters wide. This is a reasonable explanation to why there were more animals in a poor hygienic condition in these systems compared to those that have access to pasture, a tendency that has been shown in Abrahmséns *et al.´s* study from 2012. Grazing system, herd size and parity were factors that significantly influenced the frequency of mastitis in our study, both for clinical and SCM-CMT ≥ 4 cases of mastitis. In larger herds there were more animals in a late stage of lactation (< 120 days), maybe indicating a weaker monitoring of individuals in larger herds.

The correlations found to milk production will not be discussed as they reflect only the peak milk production during lactation and do not provide good information about an animal´s milk production as a whole.

Blind quarters

The frequency of cows with at least one blind quarter was found to be 10/138 (7.2 %) when including all animals in this study. At quarter level the prevalence was 16/552 (2.9 %). In Northern Ethiopia, the prevalence at quarter level was 1.1 % (Haftu *et al*., 2012). The figures in Uganda are quite equal when compared to Sweden where it is estimated that 5-15 % of the cows have at least one blind quarter (Landin, H., pers. comm., 2013). No indication of strategies concerning drying off individual quarters was seen in Uganda and blind quarters may be a consequence of previous cases of mastitis, e g coliform mastitis (Sandholm & Pyörälä, 1995).

Clinical mastitis

Bacteriological findings in clinical mastitis

The most common pathogen found in clinical cases of mastitis was found to be CNS followed by coliform bacteria. It was somewhat surprising to find CNS as the most common pathogen. This is not in line with a study from the same area, where *Klebsiella* spp. was found as the most common cause of clinical mastitis. (Tweyongyere & Nambasa Kasirye, 1998). In a study from Northern Ethiopia, *E. coli* was the most common agent in clinical mastitis and the prevalence of clinical mastitis at cow level was 3.6 % (Haftu *et al*., 2012). These studies reflect that the most common pathogens were of environmental origin, indicating poor environmental practices. *Staphylococcus aureus* has been found to be the most common cause of clinical mastitis in Sweden and Uruguay (Ericsson *et al*., 2009; Gianneechini *et al*., 2002), indicating that contagious pathogens play an important role in the disease. Staphylococcal species derive mainly from the skin flora of the cow. They often thrive in skin abrasions and might enter the teat cistern because of poor milking hygiene procedures and faulty milking technique (for review, see Honkanen-Buzalski & Pyörälä, 1995). In Uganda, it was obvious that milking hygiene procedures were insufficient and this is probably the major cause of spreading contagious udder pathogens such as staphylococci within a herd. In our study, *S. epidermidis* was the most prevalent CNS in clinical mastitis, followed by *S. warneri*. In a Swedish study on different CNS, *S. epidermidis* was more common in subclinical mastitis, while *S. warneri* was an unusual finding in both clinical and subclinical mastitis, although somewhat more prevalent in clinical mastitis (Persson Waller *et al*., 2011). *Staphylococcus epidermidis* has been described as more udder-adapted than environmental (Piessens *et al*., 2011, 2012). This is supporting our observations regarding the sub-optimal milking procedures, suggesting transmission from cow to cow during milking. It cannot be excluded that faulty milking technique might also contribute to the high proportion of staphylococci found in this study. Another observation during this study concerning cleaning procedures before milking was made; prior to sampling, tissues soaked in alcohol were being used to clean the tip of the teats. Often the tissues were visibly dirty and since sampling was executed after cleaning by the milking personnel, this indicated insufficient cleaning procedures before milking. Coagulase negative staphylococci were more frequent in clinical mastits when compared to subclinical mastitis, indicating that CNS may be a more serious pathogen than previously assumed (for review, see Pyörälä, 1995). However, CNS was overall the most

common agent found in this study and the clinical cases may be a result of individual factors that may have weakened the resistance to a common agent. To find that coliforms were more common in clinical mastitis than in subclinical mastitis was expected as they are typical pathogens of clinical mastitis (for review, see Hogan & Smith, 2003). Over all, prevalence figures concerning clinical mastitis have been difficult to find, both from Uganda and from other countries. One study from Uganda presents a prevalence of 16.1 % but the selection of animals were inconsistent as nearly half of the animals were reported to the author by the farm owners (Tweyongyere & Nambasa Kasirye, 1998). The general access of pharmaceuticals makes the information concerning treatment prior to sampling uncertain and might have influenced the bacteriological results. Therefore, treated animals that came to our knowledge were still included in the study.

Antimicrobial susceptibility of staphylococci in clinical mastitis

All *S. aureus* isolates and all CNS isolates were β-lactamase positive in this study. These figures are similar to what was found in a study performed in Uganda in 2008 (Byarugaba *et al*., 2008). In a study of clinical cases of mastitis in Sweden, the corresponding figures were 1.9 % and 12.5 % respectively (Bengtsson *et al*., 2009). In Sweden, penicillin is the treatment of choice (SVS, 2011) along with other measures such as frequent milking (Svensk Mjölk, 2009). Still, the prevalence of β-lactamase positive staphylococci remains low. This is probably due to recommendations of culling of cows carrying β-lactamase positive staphylococci (SVS, 2011) combined with good biosecurity practices. All *S. aureus* isolates showed a reduced sensitivity for trimethoprim but not as low as expected for a resistant population. A direct analysis method was used that may cause higher concentrations of the inoculates and may cause an overall reduced sensitivity of the investigated isolates (Finn, M., pers. comm., 2013). There was no evidence of MRSA found in this study. I would have expected a higher frequency of antimicrobial resistance in this study based on recent studies in Uganda (Byarugaba, 2004). Also, in Northern Ethiopia a high frequency of resistance to several antimicrobial substances was found in *S. aureus* isolates (Haftu *et al*., 2012). The few isolates in this study is however making it difficult to draw any general conclusions. The excessive use of readily accessible antimicrobials in Uganda was another factor that led me to believe that I would find a higher frequency of antimicrobial resistance to several substances. Observations during field work revealed wide use of broad-spectrum antimicrobial drugs during lactation for treating mastitis. Many farmers were not members of any cooperative and records were often incomplete and mainly kept in handwritten books on the farms, making the herd treatment frequency and health situation difficult to judge.

In this study, there were three animals that had been treated with antibiotics prior to sampling (that we know of) and they all still had a positive bacterial growth. Two of the isolates were positive for β-lactamase production and this underlines the importance of choosing a correct antimicrobial treatment based on bacteriological results.

Correlations between clinical mastitis and individual and herd management factors

A higher frequency of clinical cases of mastitis was found among individuals in a higher parity, a finding consistent with studies in Ethiopia (Biffa *et al*., 2005) and Uganda (Byarugaba *et al*., 2008). As zero-grazing systems and smaller herds were linked to poor hygiene status, it not was surprising to find that clinical cases of mastitis were more common in smaller herds. The poor hygiene status of the animals indicates that the surrounding environment is not sufficiently clean and provides a risk for spreading of environmental udder pathogens (for review, see Hogan & Smith, 2003). Therefore, it was not expected to find more clinical cases in open grazing systems. However, the effect of and the factors closely linked to herd size must be cautiously interpreted as there were many animals examined from two herds that represent one whole category alone (> 90 animals). Also, the low number of clinical cases along with the selection of studied animals might have distorted the results.

Subclinical mastitis

Prevalence of subclinical mastitis

The prevalence of subclinical mastitis with a CMT ≥ 3 at cow level was 90 % in this study, a figure that is even higher than in a study from 2011 when prevalence was found to be 86.2 % (Abrahmsén *et al*., 2012), and also a drastic increase from 2008 when prevalence was found to be 37.2 % (Byarugaba *et al*., 2008). Here follow some examples of prevalences of subclinical mastitis at cow level from other developing countries; Southern Ethiopia: 23 % (Biffa *et al*., 2005) and 25.4 % (Abera *et al*., 2012), Tanzania: 75.9 % (Karimuribo *et al*., 2006) and Uruguay: 52.4 % (Gianneechini *et al*., 2002). In the studies by Aberas *et al*. and Gianneechini *et al*., the prevalence was based on diagnosis by other methods than CMT-scoring. In this study a high proportion of subclinical mastitis was found on a majority of the visited farms. The high prevalence might be an effect of the observed poor milking hygiene (Sraïri *et al*., 2009) and traumatic strip milking technique being practiced on all farms. However, it has not yet been shown that the strip milking technique is causing higher SCC´s than other hand milking techniques (Millogo *et al*., 2012). Only one farm distinguished itself by having very few cases. Observations revealed that this farm was the only one that tethered their animals and fed them directly after milking in order to keep them standing during the immediate period after milking, a protective measure that is generally recommended (Radostits *et al*., 2007). These animals were in an open grazing system and had an overall good hygiene status.

Bacteriological findings

Coagulase negative staphylococci were also the most common finding followed by *Str. agalactiae*. See section about clinical mastitis for discussion about staphylococci. Due to practical reasons, only subclinical cases with a CMT-score of ≥ 4 were sampled for bacteriological cultivation in this study, and therefore the bacteriological results do not reflect the common definition of subclinical mastitis. In other studies on subclinical mastitis from the same area, CNS has also been the most common finding (Abrahmsén *et al*., 2012; Byarugaba *et al*., 2008) but *S. aureus* has been the most commonly encountered pathogen in a number of

studies from other countries (Haftu *et al*., 2012; Persson *et al*., 2011; Karimuribo *et al*., 2006, Gianneechini *et al*., 2002). The separation between clinical and subclinical mastitis is unfortunately not clear in all of the compared articles.

Streptococci were more common in subclinical mastitis when compared to clinical mastitis, indicating a significant role in subclinical cases with high SCC. In our study, *S. epidermidis* was the most prevalent CNS in subclinical mastitis, followed by *S. haemolyticus*. In a Swedish study from 2011, *S. haemolyticus* was found equally in clinical and subclinical mastitis (Persson Waller *et al*., 2011). Please also see previous discussion about *S. epidermidis* in clinical mastitis. *Streptococcus agalactiae* was the most common streptococcus in our study, a finding consistent with a study of subclinical mastitis in Uruguay (Gianneechini *et al*., 2002). In Sweden, *Str. uberis* was found to be the most common streptococcus (Persson *et al*., 2011). The bacteriological findings in this study are pointing out milking hygiene as a major factor in subclinical mastitis in the studied area.

The series of problems concerning the laboratory equipment cannot be ignored when discussing the laboratory results. The status of the agar plates might have influenced the bacteriological results, so interpretation of these results must be made with caution. The contaminated plates are likely to have caused the large proportion of mixed flora and this might have a significant impact on the distribution of pathogens. Also, a higher frequency of mixed flora was seen in samples from the farm that was visited during a heavy rain fall. Another factor that might have distorted the bacteriological result is the uncertainty concerning treatments prior to sampling due to the access of antimicrobial substances without the requirement of a prescription.

Antimicrobial susceptibility of staphylococci in subclinical mastitis

The prevalence of β-lactamase positive isolates was 86 % of the *S. aureus* isolates and 80 % of the CNS isolates. Of the β-lactamase positive *S. aureus* isolates, there was one isolate that was resistant to oxacillin, a finding that may indicate methicillin resistance. To confirm the diagnosis MRSA, detection of the genes coding for the structural change in the penicillin-binding protein is required (SVA, 2012a). The oxacillin resistant isolate in this study was not resistant to cephalothin, an indication that the isolate was not methicillin resistant (Finn, M., pers. comm., 2013). Two isolates were resistant to tetracycline, a substance that I did not observe in mastitis treatments. It was however the treatment of choice for another commonly encountered disease, East Coast Fever, and maybe this is one explanation of the resistance patterns seen. Please see previous section about antimicrobial susceptibility of clinical cases of mastitis for discussion about penicillin and trimethoprim.

Correlations between SCM-CMT ≥ 4 mastitis and individual and herd management factors

A higher frequency of SCM-CMT ≥ 4 cases was found among individuals with higher parity, a finding consistent with a number of previous studies (Haftu *et al*., 2012; Rabbani *et al*., 2010; Byarugaba *et al*., 2008; Biffa *et al*., 2005). The stage of lactation had no significant

impact on cows with SCM-CMT ≥ 4 in this study even if there were many animals in a late stage of lactation. The opposite has been shown in several other studies (Abrahmsén *et al*., 2012; Haftu *et al*., 2012; Rabbani *et al*., 2010; Byarugaba *et al*., 2008; Biffa *et al*., 2005). One difference in this study compared to others is that only subclinical cases with a CMT-score ≥ 4 were included. The SCM-CMT ≥ 4 cases were mainly found in smaller herds except for a large proportion of cases in very large herds (> 90 animals). This deviation might be explained by the fact that only two herds represented this category. In contrast to a previous study (Byarugaba *et al*., 2008), no significant correlations between hygiene status and SCM-CMT ≥ 4 frequency were found in this study, but a poor hygiene status was clearly connected to smaller herds and zero-grazing systems.

Other observations

The study was conducted as an eight week Minor Field Study (MFS), partly funded by the Swedish International Development Cooperation Agency (Sida). The study was a continuation of Abrahmsén *et al.´*s study about subclinical mastitis from 2012 and a step in establishing a complete picture of the mastitis status in the area. Both projects were part of a PhD-project in reproduction and udder health that will generate a protective health program for Ugandan dairy herds in the future. The work of contacting and engaging farm owners in advance was more difficult than expected and would perhaps have required more time in order to gain a larger number of cases. Also, more studies would be desirable to establish a prevalence of clinical mastitis in Uganda, to further complete the picture concerning bovine mastitis.

The selection of farms in this study may be biased on the basis of visited farms having the local supervisor as a practicing veterinarian. When it comes to contacting the local supervisor concerning clinical cases of mastitis, it cannot be excluded that these farmers are the most attentive and this possibly influences their handling of animals, not making them fully representative. However, when conducting a study in a foreign country as a short term visitor one relies on the hospitality and cooperation of farmers. To have a known person that introduces you to the farm owners is almost mandatory in order to gain access to the animals, making a completely randomized selection difficult.

Other observations made during field work were that feeding of animals was not well regulated and the animals were fed mostly on organic left-overs after house hold cooking. Animals were overall in a good body and general condition but could probably increase their productivity with adjusted feeding. Of course, there are limitations concerning storage alternatives and economy, but it would be interesting to examine the available feed sources to make the best of the situation. Some cows were found to be in very late lactation stages (e g three years without producing a calf) and this affects the milk yield negatively. The Holstein breed was the most popular among farmers manly due to its black and white color, even though it is a breed that has a higher general incidence of mastitis (Radostits *et al*., 2007) and is not well adapted to tropical and subtropical climate (Dikmen *et al*., 2012). This attitude might be difficult to change, according to the local supervisor, even with information

concerning the disadvantages of the Holstein/Friesian breed (Kanyima, B., pers. comm., 2012).

The industry is hard for the authority (DDA) to supervise due to a lot of small scale business and the constant moving of farms. Also, the DDA finds that it is hard to convince people to accept an organized dairy industry with correct handling, transportation and processing when people´s opinion is that fresh farm milk is the top choice. Also many small-scale farmers make more money by selling their milk directly compared to a cooperative (DDA, pers. comm., 2012). However, it seems that DDA plays an important role in uniting the dairy farmers and developing the dairy business by mobilizing resources such as cooling plants and vehicles for transportation of milk. It is of great importance to organize the dairy business, the government and the university in order to improve the farming business and to provide safe food. As one step to improve the milking routines, the DDA has designed written instructions with pictures showing a correct milking procedure. (Figure 13).

Figure 13. Written instruction concerning milking procedures. Photo: Sandra Björk

Concerning charity organizations providing people with farm animals, this might contribute to an increased number of inexperienced farmers and it is unclear how this is affecting the spread of animal diseases.

CONCLUSIONS

The most common agent found in clinical cases of mastitis was CNS, and a higher frequency of clinical mastitis cases was seen in smaller herds, in open grazing systems and in animals with a parity > 1. A high prevalence of subclinical mastitis was revealed and the most common pathogen in subclinical mastitis was CNS. A higher frequency of SCM-CMT ≥ 4 cases was seen in zero- and confined grazing systems and in animals with a parity > 1. Coagulase negative staphylococci, coliforms and *S. aureus* were more common in clinical cases of mastitis than for SCM-CMT ≥ 4 cases and streptococci were more common in the SCM-CMT ≥ 4 group. A large proportion of the staphylococci isolates was resistant to penicillin but there was no clear evidence of methicillin resistance among *S. aureus* isolates.

Recommendations

Here follows a proposal of protective measures that could improve the general udder health and milk production:

- Improve hygienic measures during milking by;
    - proper washing of hands before milking and between animals
    - only using clean water and separate towels for cleaning the udders
    - implementing the use of teat-dip after milking
    - keeping animals from lying down immediately after milking
- Divide herd into groups according to udder status in order to establish a milking order
- Implement correct and gentle milking technique
- Apply correct treatment of mastitis based on bacteriological culturing by consulting a veterinarian
- Avoid zero-grazing systems
- Do not keep high parity cows with a poor udder health
- Improve feeding routines
- Implement lactation periods with recurrent dry periods in between
- Improve record keeping at farms and for practicing veterinarians in order to gain statistics on health status of dairy cattle in herds and on a national level

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APPENDIX 1

Protocol for dairy farms

Farm no ..... Name ..... Village ..... Date ..... Photo no .....

Herd size .....

Open grazing (og)/confined grazing (cg) zero-grazing (zg) .....

Cow no/id	Milking (m/e)	Breed (HF/JG/LB/x)	Parity	Last calving date	Stage of lactation (days)	Peak milk production (L)	Treatment (y/n)	Rectal temperature	General condition (n/mi/mo/se)	Hygiene status (g/p)	Udder (n/f/r/so/sw/x) 1 2 3 4

Milk (n/cl/co/b/x) 1 2 3 4	CMT (1-5/x) 1 2 3 4	Collected samples 1 2 3 4	Other

Milking: m=morning, e=evening **Breed:** HF= Holstein/Freisian, , JG=Jersey/Guernsey, LB=Local breed, x=crossbreed **Treatment:** y=yes/n=no, **General condition:** n=normal, mi=mild, mo=moderate, se= severe **Hygiene status:** g=good, p=poor **Udder:** n=normal, f=firm, r=red, so=sore, sw=swollen, x=blind Milk: n=normal, cl=clots, co=color, b=blood, x=blind **CMT:** 1-5, x=missing