Sveriges lantbruksuniversitet
Fakulteten för veterinärmedicin och husdjursvetenskap
Institutionen för kliniska vetenskaper

Prevalence of subclinical mastitis and udder pathogens in small holder dairy farms in Mapepe, Batoka and Choma areas in Zambia

Lisa Eriksson

Uppsala

2013

Examensarbete inom veterinärprogrammet

ISSN 1652-8697
Examensarbete 2013:64

SLU
Sveriges lantbruksuniversitet

Prevalence of subclinical mastitis and udder pathogens in small holder dairy farms in Mapepe, Batoka and Choma areas in Zambia

Prevalens av subklinisk mastit och juverpatogener hos mjölkkor i småskaligt lantbruk i Mapepe, Batoka och Choma i Zambia

Lisa Eriksson

Handledare: *Karin Östensson, Institutionen för kliniska vetenskaper*

Biträdande handledare: Ylva Persson, Enheten för djurhälsa och antibiotikafrågor, SVA

Examinator: Karin Persson Waller, *Institutionen för kliniska vetenskaper samt Enheten för djurhälsa och antibiotikafrågor, SVA*

Examensarbete inom veterinärprogrammet, Uppsala 2013
Fakulteten för veterinärmedicin och husdjursvetenskap
Institutionen för kliniska vetenskaper
Kurskod: EX0736, Nivå A2E, 30hp

Key words: subclinical mastitis, somatic cell count, bacteriology, Zambia
Nyckelord: subklinisk mastit, celltal, bakteriologi, Zambia

Online publication of this work: http://epsilon.slu.se
ISSN 1652-8697
Examensarbete 2013:64

ABSTRACT

Subclinical mastitis (SCM) is a well-known problem in the dairy sector, where it causes severe economic losses mainly due to reduced milk production. This is a problem not only in the western world but also in developing countries. Surveys from different developing countries have shown a SCM prevalence of 52.4 – 88.6 % at cow level and 26.7 – 63.2 % at quarter-level. To combat mastitis is important to optimize the milk production of the cow. The aim of this study was to investigate the prevalence of SCM and udder pathogens on small scale dairy farms in three different areas in southern Zambia, and to obtain information about the distribution of different pathogens and penicillin resistance among staphylococcal species. A field study was conducted including 26 farms. From these farms udder bulk milk samples and quarter milk samples were taken from 111 cows without signs of clinical mastitis (CM). All milk samples were analyzed for somatic cell count (SCC) using the DeLaval cell counter. Bacteriological culturing was done on all quarter samples (n=432). Staphylococcal strains were tested for production of β-lactamase to evaluate the penicillin resistance. Based on SCC > 200 x 10^3/ml, the prevalence of SCM in the studied cow population was 48.7 % at cow level and 26.9 % at quarter level. The prevalence of intramammary infection (IMI) on cow and quarter level was 41.4 % and 31.5 % respectively. The most common udder pathogens were Coagulase Negative Staphylococci (CNS), followed by *Staphylococcus (S.) aureus*, accounting for 60.0 % and 30.4 % of the isolated bacteria, respectively. Of the staphylococcal isolates tested for penicillin resistance, 75.9 % of the *S. aureus* and 34.5 % of the CNS were positive for production of β-lactamase. According to the results, the prevalence of SCM in these areas of southern Zambia is modest compared to reports from other developing countries, both at cow and quarter level, but high compared to countries with a more developed dairy sector. The bacteriological findings are similar to results from other comparable countries, with the exception of the frequency of streptococcal isolates which was low; all together they represented 1.6 %, more than half of which emanated from the same farm. *Streptococcus (Strept.) agalactiae*, a strict udder bound and contagious pathogen, was only found on one farm which implies that it is not commonly occurring and measures ought to be taken to prevent this bacterium from becoming a growing mastitis problem. Although the prevalence of SCM in this study was not notably high it still indicates that there is a potential for improvement of the udder health to increase the milk production in Zambia. Increasing the level of milk production contributes in turn to better availability of milk which is a wholesome and therefore valuable food item as well as increased income from the sale of milk for the small scale dairy farmer. Achieving improved udder health requires organized control programs with advisory service to the farmers about mastitis prevention as well as resources to implement necessary interventions. Regular surveys monitoring the effects of such programs are needed for success in a long term perspective.

SAMMANFATTNING

Subklinisk mastit (SKM) är ett välkänt problem inom mjölkproduktionen som orsakar stora ekonomiska förluster, främst på grund av minskad mjölkproduktion. SKM är inte bara ett problem i västvärlden utan också i utvecklingsländer. Studier från olika u-länder har visat en prevalens av SCM på 52,4 - 88,6 % på konivå och 26,7 - 63,2 % på juverdelsnivå. Mastitbekämpning är ett viktigt led i att försöka optimera mjölkproduktionen genom minskade förluster, men trots detta har inga studier avseende prevalensen av SKM i Zambia gjorts. Syftet med denna studie var att undersöka prevalensen av SKM hos kor i småskalig mjölkproduktion i tre olika områden i södra Zambia samt förekomsten av juverpatogener och penicillinresistens hos stafylokockerna. En fältstudie utfördes på 26 gårdar. Prover togs på individuell samlingsmjölk från hela juvret samt från varje juverdel separat från 111 kor utan tecken på klinisk mastit. Analys av mjölkens celltal utfördes på samtliga prover med DeLaval's cellräknare. Bakteriologisk odling gjordes på alla juverdelsprover (n=432). Stafylokockstammar som isolerades testades för produktion av β-laktamas för att utvärdera eventuell penicillinresistens. Baserat på SCC > 200 x 10^3/ml hade 48,7 % av de inkluderade korna SKM och på juverdelsnivå var prevalensen 26,9 %. Förekomsten av intramammära infektioner på ko- och juverdelsnivå var 41,4 % respektive 31,5 %. Vanligast förekommande patogener var koagulasnegativa stafylokocker (KNS), följt av *Staphylococcus (S.) aureus*, vilka utgjorde 60,0 % respektive 30,4 % av bakterieisolaten. Av de stafylokockisolat som testades för penicillinresistens var 34,5 % av KNS och 75,9 % av *S. aureus* positiva för produktion av β-laktamas. Resultaten visar att prevalensen av SKM både på ko- och juverdelsnivå i Zambia är ganska modest jämfört med andra utvecklingsländer men den är hög om man jämför med länder med en mer utvecklad mjölkproduktion. De bakteriologiska fynden liknar resultaten från andra jämförbara länder, med undantag för den lägre frekvensen av streptokockisolat som tillsammans endast representerade 1,6 % varav mer än hälften kom från samma gård. *Streptococcus (Strept.) agalactiae*, en strikt juverbunden och mycket smittsam patogen, isolerades endast från en gård vilket tyder på att den inte är så spridd. Det är positivt och ger goda förutsättningar för att förhindra att denna bakterie blir ett växande mastitproblem. Även om prevalensen av SKM som observerades i denna studie inte var anmärkningsvärt hög tyder det ändå på att det finns en god potential till förbättring av juverhälsan för att åstadkomma en förbättrad mjölkproduktion i Zambia. En större volym producerad mjölk bidrar till större tillgänglighet av mjölk, som är ett nutritionellt fullvärdigt och därför värdefullt livsmedel, samt högre inkomster från försäljning av mjölk för de småskaliga mjölkbönderna. För att åstadkomma ett bättre juverhälsoläge behövs organiserade kontrollprogram med rådgivning till bönderna om mastitförebyggande åtgärder samt resurser för att genomföra nödvändiga förbättringar. Regelbundet återkommande uppföljningar för att övervaka effekterna är nödvändiga för att lyckas i ett långsiktigt perspektiv.

ABBREVIATIONS

CFU	Colony forming unit
CM	Clinical mastitis
CMT	California Mastitis Test
CNS	Coagulase negative staphylococci
DCC	DeLaval cell counter
FAO	Food and Agriculture Organization of the United Nations
GART	Golden Valley Agricultural Research Trust
IMI	Intramammary infection
MCC	Milk collection centre
NGO	Non-governmental organization
SCC	Somatic cell count
SCM	Subclinical mastitis
SVA	National Veterinary Institute, Sweden
PHO	Potassium hydroxide

CONTENTS

BACKGROUND

Zambia is a developing country in southern Africa, with an area of approximately 750,000 km^2 and a population of 13.5 million people. It has eight neighbouring countries, but no coastal line. The most important exports are copper and cobalt (Utrikespolitiska institutet, 2011).

Agriculture and dairy farming in Zambia

Almost 65 % of Zambia's population lives in rural areas and their main source of livelihood is agriculture. Still, agriculture accounts for slightly less than 20 % of Zambia's GDP (Utrikespolitiska institutet, 2011). The livestock sector has a great potential for expansion; the density of cattle is one of the lowest in the region. The 20.3 million hectares of grazing area in Zambia supports about 3 million cattle, while Kenya, with about the same amount of grazing area as Zambia, has a cattle population of 13.4 million (World Bank, 2011).

The majority of the rural population lives below the poverty line, and smallholder dairy production plays an important role in poverty reduction. It enables regular monetary earnings, which have an obvious advantage compared to accessing cash only once a season, as is the case for families only harvesting crops (Mumba *et al.*, 2011). Regular earnings make it easier to plan for the future and contribute to improving the standard of living of the rural population, for example by making it possible for children to go to school instead of being forced to work. Milk production also creates employment opportunities throughout the dairy chain.

Food and Agriculture Organization of the United Nations (FAO) recommends a consumption of about 200 litres of milk per capita and year, but the consumption in Zambia is estimated to be as low as 24 litres per person (Mumba *et al.*, 2011). Since milk is a wholesome food item, containing fat, proteins, carbohydrates, minerals and various vitamins, it plays an important role in reducing under nutrition. The need for nutritious food is especially great in Zambia since a considerable proportion (around 17 %) of the country's adult population is living with HIV/AIDS (Utrikespolitiska institutet, 2011) and poor nutrition hastens the progression of AIDS.

The average milk yield per cow per year is 300 kg in Zambia (FAO, 2005), compared with 9,210 kg per year per cow in the Swedish milk recording scheme (Swedish Dairy Association, 2012a). The beef and dairy production in Zambia is under-performing and have great difficulties competing on the regional market, due to several reasons. However, both these industries have a huge potential in contributing to more jobs and increased prosperity (World Bank, 2011).

Support of small scale dairy farming in Zambia

The estimated total milk production per year in Zambia is slightly more than 215 million litres of which approximately 115 million litres milk is produced by smallholder dairy farmers including traditional (beef) cattle keepers (Mumba *et al.*, 2011). Thus, smallholders play an important role for the national milk supply and milk production is important for the families' food security and cash income. Several NGO's are cooperating with the government of

Zambia to strengthen smallholder dairy farming, for example by providing milk collection centres which enables the farmers to sell their milk to big dairy plants even if they live in a remote area and their milk production is low. In the year 2000 the Golden Valley Agricultural Research Trust (GART) started as a joint venture between the Government and farmers´ organization, with the aim of promoting farming development. Since 2004 there is a special dairy development program for smallholders, focusing on the HIV/AIDS afflicted families, aiming at better livelihood, food security and nutrition of the families (Pandey & Muliokela, 2006).

There are programmes in GART to improve milk hygiene but, so far, very little has been done concerning the mastitis situation. This, despite the fact that mastitis is known to be commonly present, especially among the smallholder's cows and the consequences of mastitis have a direct negative impact on the amount of milk which is available for the families' consumption and sale to generate income. Moreover, mastitis stands for a significant use of antibiotics with high risk for the development of resistance. Mapping the mastitis situation is therefore important in organizations such as GART, to form a base for proper advisory service. The field survey presented in this degree thesis was performed as a pilot study in connection with GART and was conducted in parallel with a study on milking and husbandry routines performed at the same farms.

INTRODUCTION TO THE RESEARCH PROJECT

Mastitis – a disease with variable course

Mastitis is the most common disease of dairy cows. Mastitis means inflammation of the mammary gland. The cows udder consists of four mammary glands (udder quarters) that can be considered as independent units, from a mastitis point of view. As a rule, mastitis does not afflict the whole udder but rather single quarters. Inflammation is the body's response to tissue injury or a foreign substance or object, such as microorganisms. Mastitis may occur with or without infection. In general, the inflammatory reaction lingers after the infection is eliminated. (Sandholm, 1995a).

Based on the duration of the disease, mastitis is classified into several subgroups ranging from peracute to chronic. How the disease is progressing depends on the balance between the defence of the cow and characteristics of the microorganisms. In general, among cows in good general condition, the peracute form is not so common. Classification can also be based on symptoms, that is, clinical (CM) or subclinical (SCM) mastitis. The clinical form is defined as mastitis with one or more visible symptoms in the udder, such a , swelling, redness, pain, heat and/or clot formation in the milk. Mastitis may also be associated with systemic immunological reactions that might cause fever and depressed general condition. To diagnose the subclinical (silent) form, laboratory methods have to be used since there are no visible symptoms. SCM is much more prevalent than the clinical form.

Somatic cell count

The most common inflammatory indicator used to diagnose SCM is the milk somatic cell count (SCC; concentration of cells per ml of milk). Somatic cells (i.e. body cells in contrast to bacteria cells) are normally found in milk in low concentration. The somatic cells in milk

consist mainly of leukocytes. During mastitis, leukocytes (particularly neutrophils) are recruited in large numbers to the udder and milk to combat the insult, resulting in increased milk SCC (for review, see Sordillo *et al*., 1997). Thus, the SCC is a direct reflection of the degree of the inflammatory reaction and can therefore with advantage be used as an inflammatory indicator in the diagnosis of SCM. However, other milk parameters that change during inflammation, such as certain proteins, enzymes and the milk conductivity, can also be used for diagnosis of SCM (Sandholm, 1995b).

Immediately after calving, the SCC is high, but drops during the first week of lactation, staying low until the end of the lactation period. The milk from a healthy quarter in mid-lactation should contain less than 100 x 10^3 somatic cells per ml (for review, see Schukken *et al*., 2003). Minor changes in SCC can occur due to physiological factors (for review, see Harmon, 1994) such as stage of lactation, age, breed and milk yield and there is also a day to day variation. Season and stress factors may also affect the SCC to some extent, but the factor with the most significant impact on SCC is the infection status. Under practical conditions, a cut-off value of 200 x 10^3 cells per ml for diagnosing SCM has been found appropriate to use, to minimize diagnostic mistakes (for review, see Schukken *et al*., 2003).

Mastitis – common worldwide

Mastitis causes great economic losses to the dairy sector worldwide, mainly through reduction of milk yield (for review see Seegers *et al*., 2003). Mastitis has, in principal, been considered a problem in high producing cattle in developed countries, but through field investigations and improved recordings it is now recognized as a significant problem even in low yielding animals in developing countries, with a detrimental effect on animal production and public health (Harouna *et al*., 2009; Mdegela *et al*., 2009; Tesfaye *et al.,* 2010). The prevalence of mastitis varies between countries and geographical regions, usually the highest prevalence being found in countries with a poor developed dairy sector and lack of udder health control programs. SCM is considered as a larger problem than CM. The prevalence of cows/udder quarters with SCM (based on SCC) has been reported from surveys in different countries: Uruguay 52.4/26.7 % (Gianneechini *et al*., 2002); Hungary 49.7/22.6 % (Janosi & Baltay 2004); Ethiopia 34.4 %/17.9 % (Almaw *et al.,* 2008); Tanzania 51.6/30.0 % (Mdegela *et al.,* 2009) and Vietnam 88.6/63.2 % (Östensson *et al*., 2012). From the Netherlands, Lam *et al*. (2013) reported a cow prevalence of 22 % and in Finland Pitkälä *et al*. (2004) found that an average of 31 % of the cows (within a herd) were afflicted with subclinical mastitis. In Sweden no survey on SCM has been performed, but 25.8 % of the individual cow measures of milk SCC; in the monthly milk recording were found to indicate SCM ($\geq$ 200 x 10^3/ml; Swedish Dairy Association, 2012b). A threshold SCC value of > 200 x 10^3/ml or > 300 x 10^3/ml or a positive CMT reaction has been used as indicative of SCM in the studies referred to.

Effect of mastitis on yield and milk composition

Mastitis causes a reduction in milk yield (for review, see Seegers *et al.,* 2003). The most significant effect on milk production comes from the subclinical form because it has a chronic character and can remain undetected for long periods of time since it is not associated with visible symptoms. This, combined with the high prevalence of SCM, produces a strong

negative impact on the dairy economy (for review, see Halasa *et al*., 2007; Tesfaye *et al.*, 2010). Production losses based on different studies have been suggested to amount to approximately 375 kg per clinical mastitis case and in subclinical mastitis approximately 0.5 kg per 2-fold increase of SCC at cow level (for review see Seegers *et al*., 2003). The reduction in yield is, in general, considered to be directly correlated to the increase in SCC, but it has been found that production also remains impaired after the recovery from SCM. Furthermore, mastitis leads to changes in the composition of the milk, which in turn may reduce the milk quality and durability (Korhonen & Kaartinen, 1995). The content of lactose and casein decreases, while the concentrations of fat, serum proteins and minerals increase. The inflammatory reaction is associated with increased lipolysis and proteolytic enzymatic activity which result in deteriorated quality and processing properties of the milk.

Intramammary infection

The main pathway for bacteria to infect the udder is through the teat canal (Sandholm & Korhonen, 1995). More pronounced mastitis reactions most probably originate in bacterial invasion even if the bacteriological culturing of the milk is negative when sampling takes place - while modest and/or short lasting inflammatory reactions may be induced by other insults than microorganisms and management factors (for review, see Harmon 1994).

In many cases of mastitis, particularly the subclinical form, bacteria cannot be isolated from the milk. In ***CM*** cases, a frequency of 26-27 % bacteriologically negative samples has been found in several studies (Nevala *et al*., 2004; Koivula *et al*., 2007; Bradley *et al*., 2007). Eriksson Unnerstad *et al.* (2009) observed a lower figure of 11.3 %. All studies reported less than 8 % contaminated samples, a category that should be considered since it also might contain true negative and/or positive samples. In ***SCM***, several authors (Sobiraj et al. 1997; Koivula *et al*. 2007; Nam *et al.*, 2010, Persson *et al.* 2011, Östensson *et al*., 2012) have found a frequency of culture-negative samples ranging between 16 and 32 % while others (Janosi & Baltay 2004; Bradley *et al*., 2007) have observed higher values of 39.0-41.1 %. In the study by Persson *et al.* (2011), 17.8 % of the samples were given as contaminated which was generally higher than in the other studies referred to.

It has been suggested that negative bacteriological culturing of milk from mastitis cases may be due to the infection being eliminated although the SCC has still not declined (Sandholm, 1995a). Factors such as low bacteria concentration in the milk, incorrect sampling technique, the bacterial culturing methods or, probably more rarely, that the mastitis is actually not caused by infection are other possible explanations (Hogan *et al*., 1999). Recent results from bacteriological examination of mastitic milk samples by the PCR technique have shown that mastitis pathogens may be present in high concentration in culture-negative samples (Taponen *et al*., 2009). This indicates that the high frequency of culture-negative milk samples from mastitis cases might be attributable to shortcomings in the culturing method but to clarify this more research is required. Common mastitis pathogens can, principally, be divided into two groups depending on their primary and main reservoir; infected udders or the environment (for review, see Carrillo-Casas & Miranda-Morales, 2012). The pathogens harboured in infected udders are considered as contagious and are transmitted from cow to cow via for example milking equipment. *Staphylococcus aureus* and *Strept. agalactiae* belong

to this group. The environmental pathogens are mainly transmitted from their main reservoir in the cow's immediate environment to the udder, usually between milkings. Mastitis pathogens in this group are *Strept. uberis* and coliforms such as *Escherichia (E.) coli* and *Klebsiella* species. *Strept. dysgalactiae* primarily resides in the udder but can behave as both a contagious and an environmental pathogen (for review see Calvinho *et al.*, 1998). Coagulase negative staphylococci (CNS) constitute a third group of mastitis pathogens considered to be opportunists and minor pathogens although the pathogenicity among the species in this bacteria group may vary. They are normally present on e.g. the bovine skin (Devriese & De Keyser, 1980). Good milking hygiene practices are the most effective measures to prevent spreading of the contagious pathogens, but it is also important to prevent the import of pathogens to a healthy herd via, for example, the introduction of new animals. Environmental bacteria are best kept in check by maintaining good overall hygiene with a clean environment and animals (Honkanen-Buzalski & Pyörälä, 1995). The opportunists may, in general, be dealt with by not giving them favourable opportunities to infect the udder (for review, see Carrillo-Casas & Miranda-Morales, 2012). Transmission from one infected udder to another may, per se, occur with any mastitis pathogen no matter the original main source.

The most common bacteria isolated from mastitis cases are staphylococci and streptococci, according to surveys from different parts of the world (Gianneechini *et al.*, 2002; Botrel *et al.*, 2010; Nam *et al.*, 2010; Persson *et al.*, 2011).

Staphylococci

Staphylococcus aureus

S. aureus is a major pathogen often causing severe mastitis reactions of different forms (Pyörälä, 1995), ranging from peracute to chronic. It has the ability to survive phagocytosis by "hiding" and staying alive inside epithelial cells and white blood cells (leukocytes). Furthermore, it is capable of forming so-called L-forms without a cell wall, thereby becoming inaccessible to antibiotics and is very difficult to eradicate from the udder tissue (Pyörälä, 1995). Hence, *S. aureus* mastitis often becomes chronic/subclinical and constitutes a severe problem with great losses in milk production.

S. aureus is also a zoonotic bacterium, and chronically infected cows act as a reservoir from which transmission to humans can occur both from physical contact (i.e. hand milking) and consumption of unpasteurized milk (Pyörälä, 1995). Some strains of *S. aureus* produce toxins that can cause severe illness in both cows and humans, and these toxins are not destroyed by pasteurization. Udder infections by *S. aureus* are therefore not only potentially harmful to the animal but also to humans. *Staphylococcus aureus* has been shown to be the most commonly isolated pathogen from mastitis cases in many countries worldwide, for example Zambia (Pandey *et al*., 1996), Uruguay (Gianneechini *et al.*, 2002) and Sweden (Persson *et al*., 2011).

Coagulase negative staphylococci

Coagulase negative staphylococci constitute a group of several species, which were previously considered as a minor mastitis problem. During the last decades CNS have appeared with increasing frequency among isolates from mastitis cases (for review, see Pyörälä & Taponen, 2009) and are currently considered as important mastitis pathogens all

over the world (Mdegela *et al.*, 2009; Nam *et al.*, 2010; Persson, *et al.*, 2011; Abrahamsén, 2012). They are predominantly found on the teat skin and apex, but also in milk (Devriese & De Keyser, 1980). The species found on skin differ from those which have been isolated from milk. The most frequently isolated CNS from SCM cases are *S. epidermidis, S. simulans, S. chomogenes, S. xylosus* and *S. haemolyticus* (for review, see Pyörälä & Taponen, 2009). *Staphylococcus epidermidis* has also been isolated from CM cases in Zambia, being the fourth most commonly isolated pathogen (Pandey *et al.*, 1996).

The frequency of CNS isolates is generally higher in SCM than in CM cases (for review, see Pyörälä & Taponen, 2009). In Sweden, CNS are the second most common pathogens causing SCM being isolated from 16 % of quarter milk samples with high SCC (Persson *et al.*, 2011). The CNS are considered less virulent than *S. aureus* though in some cases it is the other way around. Usually, the tissue damage after an infection by CNS is mild and the prognosis is therefore good (for review, see Pyörälä & Taponen, 2009).

Antimicrobial resistance

Some staphylococci produce β-lactamase, an enzyme which makes these bacteria resistant to penicillin. This is of serious concern. Penicillin is the drug of choice for treatment of gram-positive bacteria. It is a narrow spectrum antimicrobial belonging to one of the safest therapeutic groups and is considered to have a low *de novo* resistance driving effect (Olsen *et al.*, 2006). Persson *et al.* (2011) found that of staphylococci isolated from Swedish SCM cases, 35 % of the CNS and 4 % of the *S. aureus* strains were producing β-lactamase. This relation between the bacteria is in accordance with studies performed in several other countries in northern Europe, that penicillin resistance of *S. aureus* is lower than that of CNS (for review, see Taponen & Pyörälä, 2009). In contrast, studies from other parts of the world have shown that resistance to penicillin is more widespread among *S. aureus* than CNS strains (Botrel *et al.*, 2010; Pitkälä *et al.*, 2004; Abrahamsén, 2012). Regarding resistance to other commonly used antimicrobials, *S. aureus* isolates have generally been shown susceptible while a majority of CNS isolates are resistant to one or more antimicrobials (for review, see Taponen & Pyörälä, 2009).

Streptococci

There are three major streptococci causing mastitis; *Strept. agalactiae, Strept. dysgalactiae* and *Strept. uberis* (for review, see Carrillo-Casas & Miranda-Morales, 2012). They are not equally spread over the world, but cause varying problems in different geographical areas.

Streptococcus agalactiae

Strept. agalactiae is an obligatory udder bound pathogen and is considered to be very contagious (Keefe, 1997; for review, see Carrillo-Casas & Miranda-Morales, 2012). It has several virulence factors and can cause both CM and SCM. Penicillin is generally very effective against *Strept. agalactiae*, and since the bacterium's survival outside the udder is very restricted it is possible to eradicate it from an infected herd. Many countries have had successful eradication programs and the majority of their dairy herds are free from *Strept. agalactiae* (for review, see Carrillo-Casas & Miranda-Morales, 2012). As examples, in Sweden, 0.2 % of quarter milk samples diagnosed with SCM have been reported to show

growth of *Strept. agalactiae*, and in Uganda 3.3 % (Persson *et al.*, 2011; Abrahamsén, 2012). In Vietnam, an exceptionally high frequency of isolates with *Strept. agalactiae* has been reported, 21 %, although the study included all udder quarters in a population of cows irrespective of their SCC (Östensson *et al.*, 2012).

Streptococcus dysgalactiae

Strept. dysgalactiae was originally isolated from infected mammary glands, and particularly teat injuries. Later several extramammary reservoirs have been identified but no studies of potential environmental sources have been done (for review, see Calvinho et al., 1998). It was previously described as contagious, but is today commonly classified as an environmental mastitis streptococcus although it exhibits characteristics of both a contagious and an environmental pathogen (for review see Calvinho *et al.*, 1998 and Schukken *et al.*, 2011). It is not as contagious as *Strept. agalactiae*, which is why prevalence in an infected herd is usually lower than is the case with *Strept. agalactiae*. *Strept. dysgalactiae* is commonly associated with so called summer mastitis, but can cause mastitis all year round (Pyörälä, 1995).

Streptococcus uberis

This streptococcus can be found almost anywhere in the environment surrounding the cow, but also on the cow's teat skin and in the rumen (for review, see Schukken *et al*., 2011). Since the cow's environment is a reservoir for *Strept. uberis*, teat dipping and other frequently used methods for mastitis control, are not very effective against this bacterium (Pyörälä, 1995). Although its main source is the environment, a contagious behaviour of the bacteria with transfer between animals has been observed lately (for review, see Schukken *et al*., 2011).

Antimicrobial resistance

To treat streptococcal infections, both clinical and subclinical, penicillin is the drug of choice. Almost all streptococcus strains are susceptible to penicillin (Pandey *et al*., 1996; Denamiel *et al.*, 2005; Bengtsson *et al.*, 2009; Persson *et al.*, 2011).

Coliforms

Escherichia coli (E. coli) and *Klebsiella* spp are coliforms that can cause mastitis (for review, see Schukken *et al.*, 2011). These bacteria are part of the normal bovine intestinal flora and contaminate the environment, often bedding material, via faeces. During the puerperal period, the cow is especially sensitive to coliform infections since the immune defence at this time is lower than normal. All gram-negative bacteria produce endotoxin and an IMI usually results in a severe inflammatory response. *Klebsiella* mastitis often occurs as a herd problem. *Klebsiella* spp in general cause serious but less rapidly developing CM cases than *E. coli* but with a longer duration of infection and may give rise to SCM. *E. coli* most frequently induce acute CM, often of serious character with a rapid progress and sometimes with a fatal outcome (Sandholm & Pyörälä, 1995). *E. coli* can also cause SCM, although less frequently, but these strains are different than those causing CM (Dogan *et al.*, 2005). The frequency of *E. coli* and *Klebsiella* spp isolated from mastitis cases may vary considerably depending on herd and country. In Sweden, *E. coli* and *Klebsiella* spp have been found in 2.9 % and less than 1.0 %, respectively, of quarter milk samples diagnosed with SCM (Persson *et al.*, 2011)

while in CM cases the corresponding figures were 15.9 % and 4.2 % (Eriksson Unnerstad *et al.*, 2009).

Antimicrobial resistance

According to Persson *et al.* (2011) most *E. coli* strains isolated from SCM cases in Sweden are in general susceptible to common antimicrobials used in mastitis therapy. However, resistance patterns show great variations between countries (Hendriksen *et al.*, 2008). The strains causing chronic infections tend to be less resistant to antibiotics than those causing CM (Dogan *et al.*, 2005).

AIMS OF THE STUDY

The main objectives of this study was to investigate the prevalence of SCM based on SCC among cattle kept in small scale dairy production in southern Zambia and the prevalence of udder pathogens. The study was conducted in parallel with a study on milking and other udder health related management routines (Linda Olofsson, examensarbete i veterinärprogrammet, SLU 2012). This enables, to some extent, comparison of the results from both studies to gain a wider perspective of the udder health situation in this part of Zambia and an added value to each of the studies.

MATERIAL AND METHODS

The study was concentrated to three different areas; Mapepe in Lusaka province and Batoka and Choma, both in Southern Province. The altitude in these areas is around 1000 meters above sea level, and the annual rainfall is 800-1000 mm. The field work was conducted between mid-September and mid-October 2012, i.e. at the end of the dry season in Zambia and the temperature was around 35ºC and 20ºC during daytime and night time, respectively.

Selection of farms and cows

The selection of the farms was made at the GART-provided milk collection centres (MCCs) in Mapepe, Batoka and Choma. A milk sample was taken from the herd bulk milk delivered from each farmer and immediately tested for SCC. Since the effect of milking and management routines on udder health were investigated in a parallel study it was desirable to include farms with different udder health status. Thus, farms representing herd SCC ranging from low to high were selected. The aim was that half of the farms should have above and the other half below 400 x 10^3 cells/ml in the herd milk. Studies have indicated that at a SCC of 400 x 10^3/ml in herd milk up to one-third of the cows will be infected contributing with abnormal milk, while at 700 x 10^3/ml the corresponding figure is approximately two-thirds (Brolund, 1985). Official limits set for milk intended for human consumption may vary

between regions in the world. The SCC level chosen in this study is applied for example in New Zealand and the European Union (Regulation No 853/2004).

Due to practical reasons, farms with very remote locations were not included. The intention was to conduct the study on farms with approximately equal herd size. However, this was not possible since correct information about herd size was often not obtained from the farmers, possibly because cattle are considered a symbol of wealth and status (Ndandula, 2011).

Samples were taken from a maximum of 6 cows at each farm. In farms with ≤ 6 lactating cows, all were included. In farms where the number of milking cows was higher, samples were taken from the first 6 cows to be milked, because of logistic and linguistic difficulties. If a cow showed signs of CM it was excluded and another cow was selected. If the farm had ≤ 6 lactating cows, only the udder quarter/quarters with signs of CM was excluded and samples were taken from the remaining quarters to obtain as equal a number of samples from each herd as possible.

Milk sampling and treatment of samples

Before milking was started, milk samples were taken from each quarter after discarding the foremilk into a control vial. If the milk contained flakes or if the quarter showed any signs of CM, that quarter was excluded. The quarter milk samples were taken aseptically into sterile test tubes. When milking of a cow was finished, an additional sample was taken from the udder bulk milk, for measuring SCC at cow-level (cow SCC). All samples were kept in a cooling box during transport. At the laboratory, SCC was measured in each milk sample (quarter SCC + cow SCC), and the quarter milk samples were thereafter kept frozen until bacteriological culturing. In the bacteriological laboratory, the samples were thawed in room temperature. To assure that the milk samples were properly mixed, the tubes were turned several times before bacteriological culturing.

Threshold value to diagnose subclinical mastitis

Two different SCC threshold values were used for evaluation of the udder health status: Milk with SCC < 100 x 10^3/ml was considered to emanate from healthy quarters. Taking into account that the SCC to some extent may be affected by non-pathological factors, a slightly higher SCC value has been found more relevant to use in practice for diagnosing SCM (for review, see Schukken *et al.*, 2003). Thus, SCM in the present study was diagnosed based on a milk SCC of > 200 x 10^3/ml.

Infection was diagnosed as growth of one or more mastitis pathogens after bacterial culturing of the milk sample.

Analysis of samples

SCC

The SCC was measured using a DeLaval cell counter, DCC (DeLaval, Tumba, Sweden), which is a portable instrument. One disposable cassette is used for each milk sample. When the milk passes through the cassette it is mixed with propidium iodide, a fluorescent, DNA-specific dye. After staining, the cell nuclei are counted by the instrument based on their

fluorescence. The equipment was handled according to the manufacturer's instructions. When a result was questionable (i.e. zero) a new cassette was used and the milk sample was re-tested.

Culturing

The bacteriological laboratory work, biochemical testing and preliminary bacteriological diagnosis were done in Zambia, according to the routines at the National Veterinary Institute (SVA), Uppsala, Sweden. All milk samples were cultured on blood (5 % bovine blood) agar plates with esculine and incubated in 37ºC for 48 hours. To be classified as bacteriologically positive, at least three CFUs were needed for all genera except *S. aureus* where even growth of a single CFU counted as positive (Ericsson Unnerstad *et al.*, 2009). Each agar plate was first examined regarding culture purity and quantity of growth, which was categorized as mild (<10 CFUs), moderate (10-50 CFUs) or abundant (>50 CFUs). Subsequently, the bacteria was classified according to colony morphology and hemolysis (negative, α-, β-, or double). For further classification catalase test reaction, PHO test reaction or P-test reaction were used, depending on the genera. Criteria for classification according to colony morphology and chemical reactions (Hogan, 1999) are shown in table 1 and 2, respectively. Plates with growth of three or more different pathogens were categorized as mixed cultures.

Table 1. Criteria for bacteriological diagnosis according to colony morphology

Genera	Morphology
Staphylococci	Yellow, white or grey, round colonies, 2-4 mm after 48 h
Streptococci	Small, dewdrop like colonies, 0.5-1 mm after 48 h
Coliforms	Large, grey colonies, 2-4 mm after 24 h.

Table 2. Criteria for bacteriological diagnosis according to chemical reactions

	Hemolysis	PHO	Catalase	P-test
S. aureus	α or double	-	+	na
CNS	α or neg	-	+	na
Strept. spp.	na	-	-	na
E. coli	na	+	na	+
Klebsiella spp.	na	+	na	-

na = not applicable

To confirm the preliminary bacteriological diagnosis, all species of staphylococci and streptococci, and bacteria that could not be categorized, were sent to SVA in Uppsala, Sweden. Colony material from these isolates was transferred to transport media consisting of agar with 5 % horse serum in 4 ml plastic tubes with a screw cork. Since far more milk samples than expected were bacteriologically positive, the number of available transport media tubes was not enough for all samples with pathogen growth (according to above). Therefore, in the end of the study when there were two or more milk samples originating from the same cow, analyzed at the same time with bacteria exhibiting identical colony

morphology and chemical test results, only colony material from one of them was transferred to transport media for further examination and the others were categorized as equivalent.

The transport media tubes were stored in +6 º C for 5 weeks before being packaged and sent to SVA in Uppsala, Sweden for further typing. The transport time from Lusaka, Zambia was 4 days. At SVA, the tubes were stored cold (+8 ºC) for 19 days before being recultured onto blood agar plates and incubated in 37 ºC for 48 hours.

Typing of bacteria

Typing of the bacteria was done by the personnel at the Mastitis Laboratory, SVA using SVA's standard accredited methods (Ericsson Unnerstad *et al.,* 2009). Each *S. aureus* and CNS isolate was tested for β-lactamase production.

RESULTS

A total of 26 farms, 111 cows and 432 quarter samples (1 quarter was excluded because of CM and 11 quarters were dried off) were included in the study. The milk samples at cow/udder level could only be obtained from 76 of the 111 included cows. The missing samples are, in most cases, due to the milkers not emptying their bucket between every cow they milked. The distribution of farms, cows and quarters between the three areas is shown in table 3.

Table 3. Distribution of farms, cows and quarter samples between the three areas

Area	Farms	Cows	Quarters
Mapepe	11	37	144
Batoka	9	45	177
Choma	6	29	111
Total	26	111	432

Of the 26 selected farms, 12 had a herd SCC ≤ 400 x 10^3/ml and 14 had > 400 x 10^3/ml. The mean/median overall herd size was 29.7/20 cows and the mean/median number of lactating cows was 7/5.5. Table 4 shows data on the herd size and cows included in the study. Since it was not possible to obtain reliable information on the total number of cows in the herd, the number of lactating cows at the time of visit is given.

Table 4. Data on the herds included in the study

Herd SCC x 10^3/ml	≤ 400 (n=12)	> 400 (n=14)	Total (n=26)
Local/exotic cross breed (%)	68/32	26/74	46/54
	Mean (range)	Mean (range)	Mean (range)
Herd SCC x 10^3/ml	144.3 (15-329)	907 (540-1531)	555 (15-1531)
Lactating cows (nr)	8.6 (2-20)	5.5 (2-15)	7.0 (2-20)
Total milk yield/day	15(4-35)	21.5 (6-50)	18.4 (4-50)
Quarter SCCx10^3/ml	160.9 (0-3396)	297.2 (0-3225)	232.5 (0-3396)

The cows were of two crossbreed types; a cross between different local breeds or a cross between one local and one exotic (Holstein, Fresian, or Jersey) breed. The number and geographical distribution of the different breeds is shown in figure 1.

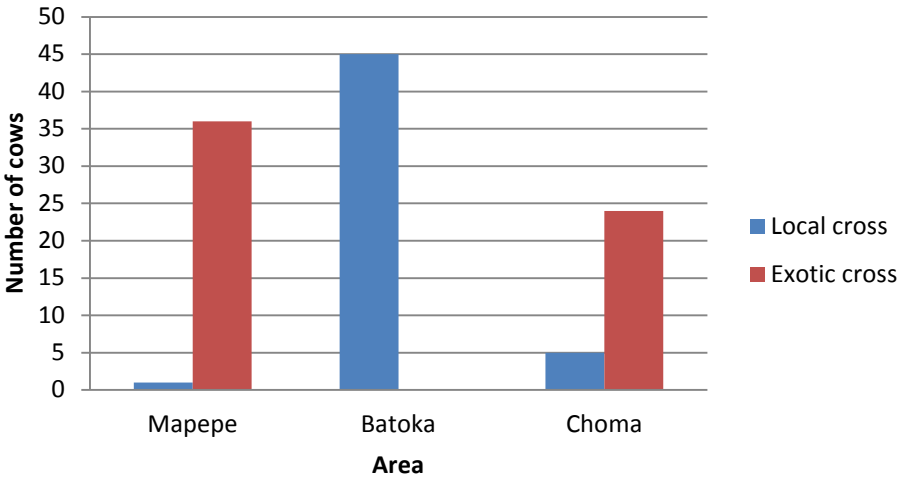

Figure 1. Distribution of the two crossbreed types between the three areas.

Prevalence of subclinical mastitis

SCC

The average herd SCC (in thousands) for all farms was 555 ranging from 15 - 1531. In the two groups with high and low herd SCC, the mean herd SCC (in thousands) was 907 (range 540 - 1530) and 144.3 (range 15 – 329) respectively.

In total, 40.8 % of the cows had SCC < 100 x 10^3/ml and 51.3 % had SCC < 200 x 10^3/ml. Hence, the overall prevalence of SCM at ***cow-level*** according to the threshold SCC value used in this study was 48.7 %. Of the 39 cows with a SCC < 200 x 10^3/ml ("not SCM") , 10.3 % had at least one ***quarter*** with SCC ≥ 200 x 10^3/ml. Of all 111 cows, 48.6 % had at least one quarter with SCC ≥ 200 x 10^3/ml.

Figure 2 shows the distribution of cow and quarter SCC in the study, and figure 3 shows the distribution of cow SCC in the two different breeds.

Overall, 65.5 % and 73.1 % of the quarters had SCC < 100 x 10^3/ml and < 200 x 10^3/ml, respectively. This gives a prevalence of SCM at quarter-level of 26.9 %.

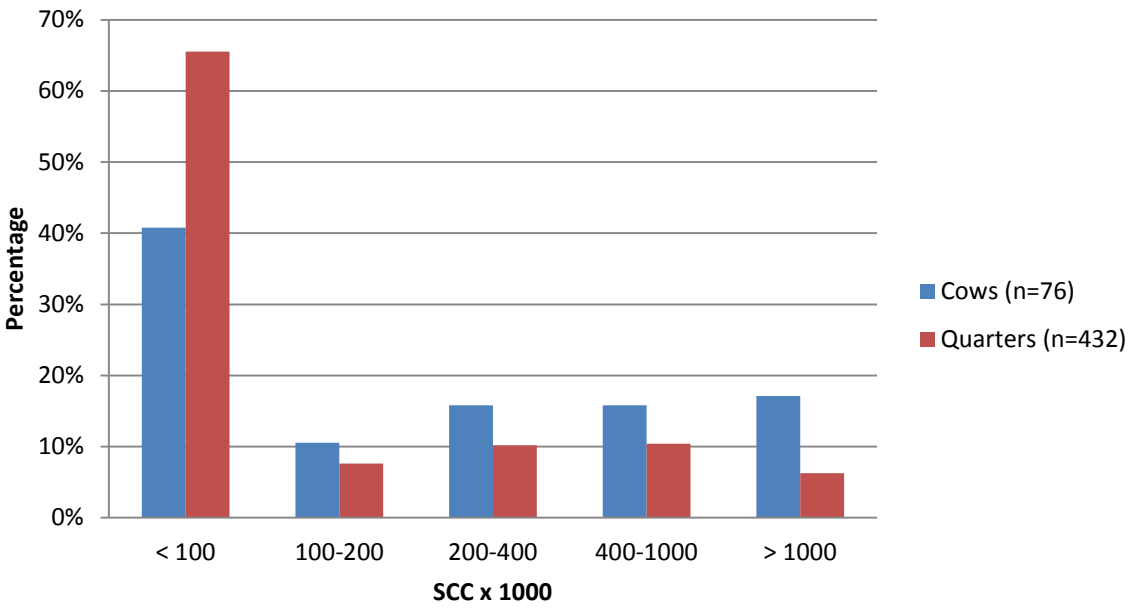

Figure 2. Distribution of SCC at cow and quarter level. Cow milk SCC was measured on 76 of the total 111 cows included in the study

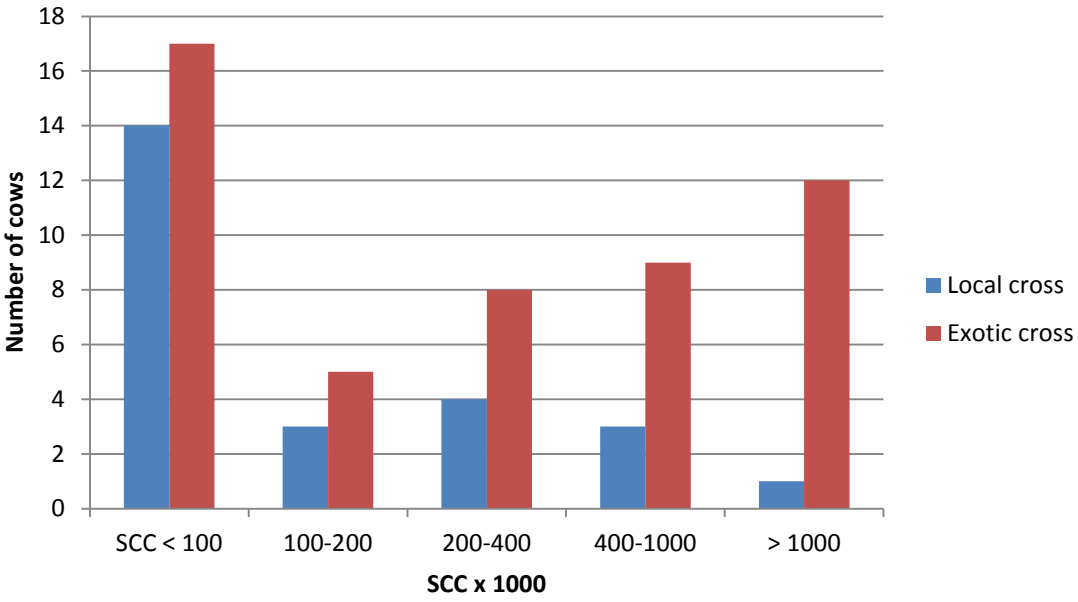

Figure 3. Distribution of cow SCC in the two types of cross breeds

Udder pathogens

Four quarter samples were bacteriologically positive after the first culturing but after reculturing at SVA they showed no growth or growth in mixed culture and were therefore excluded from the bacteriological study. Of the remaining 428 quarter samples that were cultured, 135 were positive for bacterial growth (see table 5). Out of these, nine quarter samples showed growth of two pathogens. These samples originated from 4 different farms with 1, 1, 2 and 5 udder quarters with double bacteriological diagnoses, respectively.

Table 5 shows the distribution of pathogens in relation to quarter SCC. Of the quarter samples with SCC < 100 x 10^3/ml and SCC < 200 x 10^3/ml, 12.4 % and 15.6 %, respectively, were bacteriologically positive. Of quarters diagnosed with SCM 76.1 % were positive for bacterial growth.

Of the 111 cows included in the study, 42.3 % were negative for bacterial growth in all four quarters. When looking at the different breeds, 23.3 % and 64.7 % of the exotic and local crosses, respectively, were bacteriologically negative.

In table 6, information is given for each herd concerning bacteriological findings, herd SCC and herd size. Of the 26 farms 88.5 % had at least one cow with infection in at least one quarter, and 57.7 % of the cows had one or more infected quarters.

The bacteriological results at quarter level are shown in figure 4a, and figure 4b shows the distribution of the bacteriological isolates. In figure 4c the bacteriological diagnoses in quarters *diagnosed with SCM* are shown. At quarter level, the prevalence of infection was 31.5 %. The most common infectious agent was CNS being isolated from 17.1 % (figure 4a) of the quarter samples, representing 56.3 % of the isolated bacteria (figure 4b). At cow level, 38.7 % (data not shown) were infected with CNS and these cows were distributed in 73.1 % of the farms.

The second most common pathogen was *S. aureus* (figure 4b), representing 28.5 % of the isolated bacteria. It was found in 8.6 % of the quarter samples, with 27 % of the cows being infected, distributed in 65.4 % of the farms (table 6).

Strept. dysgalactiae and *Strept. uberis* were isolated from one quarter sample each, and *Strept. agalactiae* was isolated from two quarters. These four quarters harbouring streptococci emanated from three cows, all from the same farm (table 6).

Table 5. SCC x 1000 per ml and isolated pathogens in quarter milk samples

	< 100	**100-200**	**200-400**	**400-1000**	**>1000**	**Total**
n	283	32	42	44	27	428
CNS	23	14	19	17	8	81
S. aureus	4	2	9	12	14	41
Strept. agalactiae	0	0	1	1	0	2
Strept. dysgalactiae	0	0	0	1	0	1
Strept. uberis	0	0	0	1	0	1
Other Strept. spp[1]	0	0	0	1	2	3
Corynebacterium bovis	3	0	2	1	0	6
Other[2]	4	1	0	3	1	9
Total infected quarters (%)	34 (12)	17 (53)	31 (74)	37 (84)	25 (93)	135[3] (32)

[1]*Streptococcus species that is not a typical mastitis pathogen*

[2]*4 Burkholderia cepacia, 2 Enterococcus sp, 1 Arcanobacterium pyogenes, 1 Arcanobacterium pluranimalum and 1 coagulase positive staphylococcus.*

[3]*144 bacteriological diagnoses*

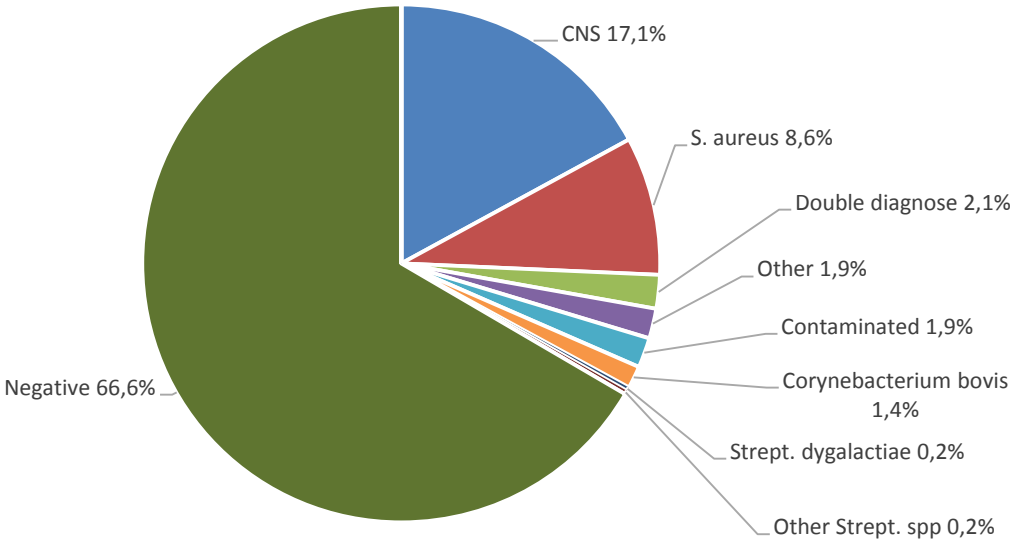

Figure 4a. Bacteriological diagnoses at quarter level (n=428).

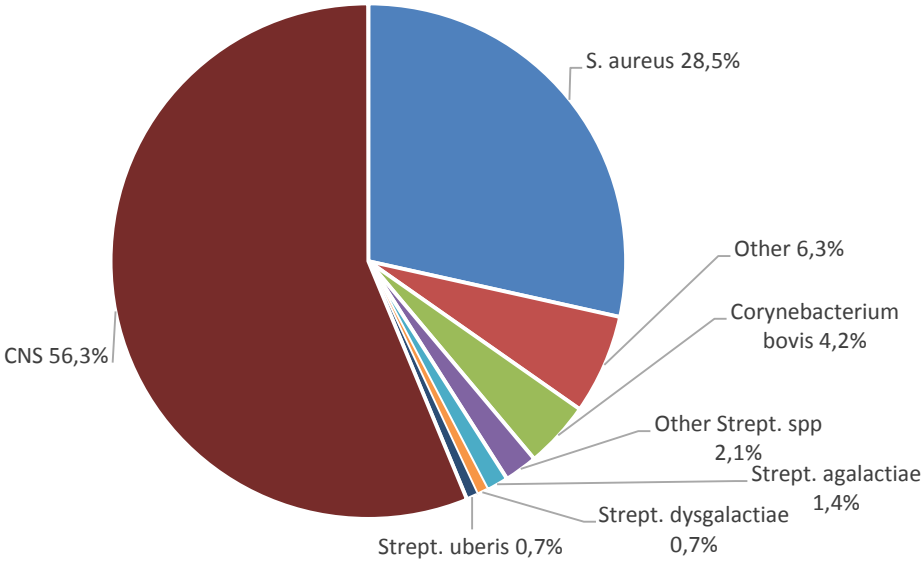

Figure 4b. Distribution of the bacteriological isolates (n=144).

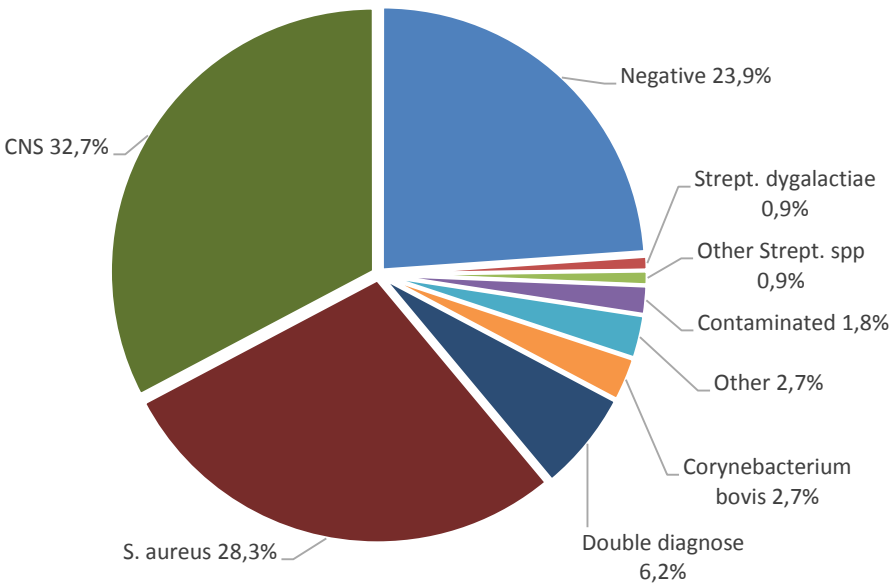

Figure 4c. Bacteriological diagnoses in quarters diagnosed with SCM (n=113)

Table 6. Data on herds and isolated pathogens in udder quarter milk samples

	Farm No																										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**Total**
Herd SCC	225	673	256	1329	329	544	846	727	602	540	1531	555	326	68	38	590	61	1361	200	15	1082	788	1530	83	80	51	
No of lactating cows	4	4	7	2	3	7	2	5	2	5	2	8	20	5	11	8	15	6	9	10	15	4	7	2	5	13	
No of quarter samples	15	15	8	4	10	20	12	19	8	20	11	22	20	8	24	8	21	24	24	24	24	16	22	8	18	23	428
CNS	2	6	3	3	2	5	5	7	0	10	3	5	0	2	1	0	2	4	5	0	1	0	12	0	0	3	81
S. aureus	1	3	1	1	2	1	3	2	1	3	3	10	1	0	0	0	0	0	0	0	5	1	1	0	0	2	41
Strept. agalactiae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	2
Strept. dysgalactiae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
Strept. uberis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
Other *Strept. spp.*	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	3
Corynebacterium bovis	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	4	0	0	0	0	0	6
Other	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	2	1	0	4	0	9
Infected quarters (%)	20	60,0[b]	50	100	40	30	66,7	57,9	12,5	65	45,5[b]	68,2[a]	5	3,8	4,1	0	9,5	16,7	20,8	0	45,8	18,8	59,1[c]	0	22,2	21,7	31,5
Nr of inf cows	3	4	2	1	3	4	3	4	1	4	3	6	1	1	1	0	1	3	3	0	4	2	5	0	1	4	64

[a] Two quarters were infected with more than one pathogen
[b] One quarter were infected with more than one pathogen
[c] Five quarters were infected with more than one pathogen

Antimicrobial resistance

β-lactamase production was tested in 71 % and 74 % of the *S. aureus* and CNS strains, respectively. Of the tested isolates, 75.9 % of the *S. aureus* and 34.5 % of the CNS were positive for β-lactamase production. The proportions of sensitive and resistant CNS and *S. aureus* are shown in figure 5.

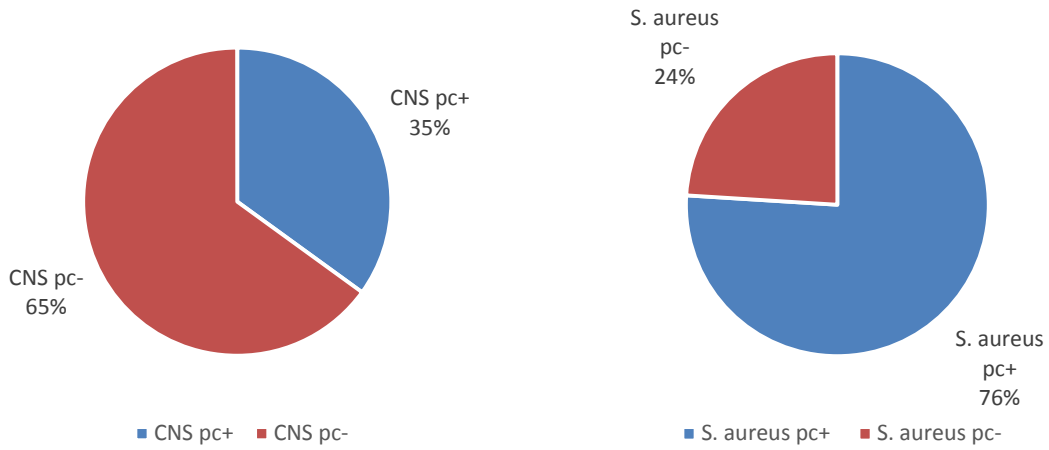

Figure 5. Proportion of β-lactamase producing (pc+) strains among 29 S. aureus and 60 CNS isolates.

DISCUSSION

SCC

Results from different prevalence studies of mastitis are somewhat difficult to compare since they have been performed in different ways, with slightly varying analysis methods and SCC levels used for definition of SCM, and different sample material. Usually, a positive CMT reaction (approximately > 400 x 10^3/ml; Schalm *et al.*, 1971) or a SCC > 200 x 10^3/ml or > 300 x 10^3/ml has been used to indicate SCM.

The reference values for SCC used to define SCM ought to be based on scientific studies (Brolund, 1985; for review, see Schukken *et al.*, 2003) such as those applied in the present study. Furthermore, they are widely used in udder health work. However, *the results* should be related to the conditions in the country or population studied. The udder health situation is not equal in different parts of the world. There are many diverse variables influencing the udder health situation in a country and, considering developing countries, the living standard and development of the dairy sector to a large extent set the preconditions for family dairy farming. The access to veterinary services and particularly udder health advisory service are critical factors. Furthermore, the udder health status, as measured by SCC, is influenced by breed, housing system, production level and climate. Thus, the situation in Zambia ought to be assessed in relation to countries with somewhat comparable conditions.

With a SCM prevalence of 48.7 % at cow-level and 26.9 % at quarter-level, the udder health situation in this part of Zambia compares rather favourably to other countries. Studies from

Finland (Pitkälä *et al.,* 2004), Tanzania (Karimuribo *et al.,* 2008; Mdegela *et al.,* 2009), Uruguay (Gianneechini *et al.,* 2002) and Vietnam (Östensson *et al.,* 2012) have shown a prevalence ranging from 51.6 % – 88.6 % at cow-level and 26.7 % – 63.2 % at quarter-level. Almaw *et al.* (2008) reported lower prevalence figures of 34.4 % at cow level and 17.9 % at quarter level, from a survey in Ethiopia.

None of the studies referred to in the previous paragraph used udder bulk milk samples and could consequently not measure a cow SCC value, but defined a positive case at cow-level as an individual with at least one SCM-positive udder quarter. Based on data from this Zambian study, the prevalence of SCM at cow-level is practically the same using either of the two definitions: 48.6 % and 48.7 % respectively. The encouraging udder health situation in Zambia is further emphasized considering the rigorous SCC limits used for diagnosing SCM in this study and that a majority of both cow- and quarter milk samples showed a SCC < 100 x 10^3/ml.

This study was conducted in parallel with a study on the effect of management routines on udder health and consumers aspects on the milk. Therefore, farms with different udder health status were selected. Half of them had a herd SCC above and the other half below 400 x 10^3/ml which is a value based on food safety considerations (see for example EU Regulation No 853/2004). It might be questioned if this selection contributed to the cows included in the study not being a representative sample of the average cow population in this area. However, the 50 farms that were screened before any selection was done were fairly evenly distributed in the two groups; 20 had a herd SCC above and 30 below 400 x 10^3/ml. Moreover, the mean herd SCC of these 50 farms and those 26 finally included in the study was in the same range, 462 x 10^3/ml and 555 x 10^3/ml, respectively. Thus, the udder health status of the herds in the study appears to have been representative of the total number of herds whose milk was investigated "by random" (with no previous selection).

At one farm (nr 5), the sampling could not start until after the cows had been milked. This could result in false high SCC from these quarters since the cell count is highest immediately after milking and 3-4 hours there after (Saloniemi, 1995). Since quarter SCC within cow was shown to range from very low to high, this effect was regarded as negligible and the quarters were still included in the study.

Bacteriology

To investigate the prevalence of mastitis pathogens, bacteriological examination has been performed in some studies only on milk samples with high SCC (diagnosed as SCM) while in other studies milk samples of a cow population have been investigated regardless the SCC.

Total prevalence – infected samples of all samples

In total, 31.3 % of all quarters in the present study, irrespective of their SCC, were positive for bacterial growth (12 % of the samples with < 100 x 10^3/ml; 16.2 % of samples with SCC 100 x 10^3 - 200 x 10^3/ml). This prevalence is similar to the results from several other surveys on the prevalence of IMI in different geographical regions which have reported values ranging 33.5 % – 45.0 % (Pitkälä *et al.,* 2004; Gianneechini 2009; Schwarz *et al.,* 2010;

Östensson *et al.,* 2012). Other studies, in South Africa (Petzer *et al.,* 2009) and Tanzania (Mdegela *et al.,* 2009), have reported much lower prevalences of IMI (15 % - 16 %) and a much higher value have been reported in a study from the Alpes in France (92 %; Botrel *et al.,* 2010). In quarters with low SCC the prevalence of IMI was slightly higher than that reported by others (Petzer *et al.,* 2009; Östensson *et al.,* 2012). This implies that the IMIs, at large, are not causing severe inflammation which is in line with the majority of the pathogens isolated being CNS, considered to be minor mastitis pathogens.

Prevalence of different pathogens

Studies on the prevalence of different mastitis pathogens in a cow population, regardless of their udder health status, are scarce. Therefore, it is difficult to compare this result from Zambia in general, with the situation in other countries. However, CNS and *S. aureus* have been reported among the most prevalent pathogens also by others (Pitkälä *et al.,* 2004; Schwarz *et al.,* 2010; Östensson *et al.,* 2012).

Bacteriologically positive samples

The distribution of different pathogens among the bacteriologically positive samples were in principal in line with other studies (Pitkälä *et al.,* 2004; Botrel *et al.,* 2010; Schwarz *et al.,* 2010) except for the frequency of streptococci. Only 5.1 % of the isolates in the present study were streptococci and only half of them constituted classical mastitis pathogens (i.e. *Strept. agalactiae, Strept. dysgalactiae* and *Strept. uberis*). All these isolates emanated from the same farm, which indicates that streptococcal infections are not widespread but occur locally. This in turn implies that it should be possible to prevent streptococcal udder infections from becoming an emerging problem in dairy herds. The frequency of streptococcal isolates among the bacteriologically positive samples was low compared to several other surveys which have found 10-20 % (Botrel *et al.,* 2010; Schwarz *et al.,* 2010) or even higher (Östensson *et al.,* 2012), but are similar to results by Pitkälä *et al.*(2004) and Guélat-Brechtbuehl *et al.* (2010).

Samples diagnosed with SCM

From quarters affected with SCM (defined as SCC > 200 x 10^3/ml) and from those with lower SCC, 76.1 % and 15.5 %, respectively, were positive for bacterial growth. This is in accordance with other studies. In a study from Vietnam (Östensson *et al.,* 2012), the corresponding figures were 51.9 % and 10.8 %. In studies from Sweden (Persson *et al.,* 2011), Uganda (Abrahamsén, 2012) and Uruguay (Gianneechini *et al.,* 2002) of milk samples diagnosed with SCM, 60.0 %, 75.1 % and 45 %, respectively, have been reported bacteriologically positive.

Among the quarters diagnosed with SCM, the distribution of pathogens was also, in principal, according to numerous previous studies (Almaw *et al.,* 2008; Harouna *et al.,* 2008; Petzer *et al.,* 2009; Botrel *et al.,* 2010; Nam *et al.,* 2010; Persson *et al.,* 2011; Abrahamsén, 2012; Östensson *et al.,* 2012). CNS and *S. aureus* were most prevalent, and frequently observed among the isolates. The frequency rank of these two bacteria isolates may differ from country to country but there are indications of increasing frequency of CNS during the last decade in many countries (Pitkälä *et al.,* 2004; Botrel *et al.,* 2010; Nam *et al.,* 2010). The exception in

the present survey compared to the other studies of SCM referred to above, was a notably lower frequency of streptococci isolates (6.3 %). In earlier studies, a frequency of 10-20 % or more has usually been reported although Pitkälä *et al.* (2004) and Botrel *et al.* (2010) found frequencies comparable with those observed in Zambia. From cases with CM in Zambia, 26.0 % of the isolated bacteria were streptococci (Pandey *et al.*, 1996).

The prevalence and frequency of *Corynebacterium bovis* observed was at the same level as those of the streptococci. In most mastitis surveys *Corynebacterium bovis* isolates are few (not given as a specified diagnose) but Pitkälä *et al.* (2004) and Schwartz *et al.* (2010) reported several folds higher figures than those found in Zambia. Infection with coliform bacteria is considered to play a minor role in SCM (Sobiraj *et al.*, 1997) and, in accordance, no coliform bacteria were isolated from the milk in the Zambian study.

Freezing of milk samples has been suggested to influence the result of the bacteriological culturing since some bacteria, such as *Streptococcus* spp are considered to be more sensitive to freezing than others, such as staphylococci (Sol *et al.*, 2002). The relatively low frequency of isolated streptococci and high frequency of staphylococci in this study may therefore have been affected by the freezing of the milk samples. However, according to Murdough *et al.* (1995) no effect was observed on viability of either streptococci or staphylococci after freezing of milk samples for 6 weeks, and freezing is generally considered to be scientifically acceptable (Hogan *et al.*, 1999). Thus, freezing of the samples is not considered to have influenced the results, substantially.

For analysis of the quarter milk SCC a small volume of the aseptically collected milk was carefully poured off the tube before the samples were frozen pending bacteriological culturing. This procedure might have contributed to increasing the number of mixed or contaminated cultures, but it is highly unlikely that it influenced the growth of mastitis pathogens. The number of mixed or contaminated cultures was low (8 of 428) in this study, and therefore it is considered that this procedure did not affect the bacteriological results.

Of the staphylococci, 26.0 % were typed in the field due to lack of tubes with transport media. The diagnoses of these samples are still considered reliable. This was practiced only in cases when the bacterial growth of several samples from the same cow, cultured in the same batch had identical colony morphology, haemolysis pattern and chemical test results, when colony material from only one of the samples was saved for further typing at SVA.

Burkholderia (B.) cepacia was isolated from 4 quarter samples, all originating from the same cow. All the 4 quarters had SCC < 100 x 10^3/ml, which implies that this bacterium is not causing mastitis. The main clinical issue of *B. cepacia* is manifestation in the lungs of patients with cystic fibrosis, sometimes with fatal outcome. Immunocompromised patients and individuals suffering from chronic granulomatous disease are also vulnerable to infections by *B. cepacia*. Furthermore, this bacterium is inherently resistant to several types of antibiotics, and some strains have been shown to be highly virulent and easily transmitted (Jones *et al.*, 2001). In a mastitis perspective the finding of *B. cepacia* is probably not significant, but it might be worth some consideration in this study since a considerable part of the rural

population in Zambia is immunocompromised, from living with HIV/AIDS (Utrikespolitiska institutet, 2011) i.e. representing a population where this bacteria species may be more prevalent. The normal habitat of *B. cepacia* is soil, water and vegetation, but it has also been isolated from "ready-to-eat" products consisting of raw unpasteurized bovine milk (Govan *et al.*, 1996; Moore *et al.*, 2001).

Nearly 76 % of the *S. aureus* strains that were sent to SVA for further analysis were positive for β-lactamase production. This result is in accordance with a study in 1996, where 80 % of the *S. aureus* strains isolated from CM cases in the same area of Zambia were resistant to penicillin (Pandey *et al.*, 1996). Although the figures from those two studies might not be directly comparable since there may be a difference in antibiotic resistance between strains causing CM and SCM (for review, see Taponen & Pyörälä, 2009), the results still indicate that the percentage of penicillin resistant *S. aureus* strains has not increased over the last 16 years, which is a positive finding. In Uganda, the resistance to penicillin among staphylococcus strains is at approximately the same level (Byarugaba, *et al.*, 2008). When compared to Sweden, the level of resistance in Zambia and Uganda is very high; only 4 % of *S. aureus* isolated from cases with SCM were found to be resistant to penicillin (Persson *et al.*, 2011). However, Swedish figures on antibiotic resistance are very ambitious to use for benchmarking since isolates from Sweden have shown to be less resistant than the average (Hendriksen *et al.*, 2008).

Breeds

The results suggest breed differences in udder health status. When grouping the cows according to cow SCC, the distributions of the two different cross breed types were similar in the middle group (i.e. SCC of 100 x 10 ^{3}- 1000 x 10^3/ml). Of the local crosses only 4 % (1 cow) had a SCC > 1000 x 10^3/ml while a majority (56 %) had a low SCC of < 100 x 10^3/ml. Of the exotic crosses, the corresponding figures were 23.5 % and 33.3 %. Hence, the local crosses tended to have lower cow SCC than the exotic crosses. Udder infections are the major cause of high milk SCC and accordingly, a lower infection rate was found among the local cross breeds. The apparently better udder health status of the local cross breeds than that of the exotic crosses may be attributable to several factors: An udder conformation less prone to fecal or environmental contamination, that they are handled and housed differently than the exotic crosses or that they have a favourable immune system in cleaning intramammary infections.

To sum up: The prevalence of SCM in Zambia is modest compared to other studies from developing countries. Considering that the study in Zambia used a rigorous SCC limit for the diagnosis of SCM and additionally based the bacteriological prevalence on culturing of all samples, irrespective of their SCC, it can be concluded that neither the SCC nor the bacteriological results can be considered to underestimate the prevalence. This indicates that the SCM situation among the small holders in southern Zambia is reasonably good.

Overall the bacteriological pattern is similar to other countries, both developed and developing, with CNS and *S. aureus* being the most frequently occurring bacteria, but with less streptococcus diagnoses than are usually observed. In general, few primarily

environmental bacteria were isolated in this study, possibly because of the dry climate during the time when the field study was conducted. During the rainy season the result might have been different. To better control spreading of the bacteria most frequently isolated, i.e. the contagious and opportunists, hygienic measures during milking and careful treatment of the udder and teats to avoid cracking of the skin are most important.

ACKNOWLEDGEMENTS

First of all, I would like to thank SIDA for the MFS scholarship and Gulli Strålfeldts fond, without these grants this project would not have been possible. My supervisor in Sweden, Karin Östensson, deserves my greatest gratitude; the time and commitment she has given to this project is invaluable. I also want to give special thanks to Elisabeth Persson, who came up with the idea for this project, and to my local supervisor Professor G. S. Pandey at University of Zambia, without whom the field work would have been close to impossible. Thanks also to Dr Stephen Muliokela, the director of GART, Mr David Mubita, center manager for GART's Livestock Development Centre in Batoka, and the rest of the GART employees for the incredible hospitality and support I received. To all the farmers who participated in the study I am very grateful, not only for being generous with their time and resources but also for making me feel like family. I would also like to thank Susanne André and everyone else at the mastitis laboratory at SVA for help both with my laboratory training before departure and the analysis of the samples afterwards. Thanks also to Olle Selander at DeLaval for supporting with equipment. Last, but definitely not least, my thanks go to Kelvin, Abie, Abdel and Clemens, for giving me the time of my life. And Linda Olofsson, what would I have done without you?

REFERENCES

Abrahamsén, M. (2012). Prevalence of subclinical mastitis in dairy farms in urban and peri-urban areas of Kampala, Uganda, Master of Science Thesis. Uppsala, Sweden: Department of Clinical Sciences, Swedish University of Agricultural Sciences.

Almaw, G., Zerihun, A. & Asfaw, Y. (2008). Bovine mastitis and its association with selected risk factors in smallholder dairy farms in and around Bahir Dar, Ethiopia, *Tropical animal health and production* 40(6), pp. 427-432.

Bengtsson, B., Ericsson Unnerstad, H., Ekman, T., Artursson, K., Nilsson-Öst, M. & Persson Waller, K. (2009). Antimicrobial susceptibility of udder pathogens from cases of acute clinical mastitis in dairy cows, *Veterinary Microbiology* 136, pp. 142-149.

Botrel, M.A., Haenni, M., Morignat, E., Sulpice, P., Madec, J.Y. & Calavas, D. (2010). Distribution and antimicrobial resistance of clinical and subclinical mastitis pathogens in dairy cows in Rhône-Alpes, France. *Journal of Dairy Research*, 65, pp. 139–142.

Bradley, A. J., Leach, K. A., Breen, J. E., Green, L. E. & Green, M. J. (2007). Survey of the incidence and aetiology of mastitis on dairy farms in England and Wales, *Veterinary Records*, 160, pp. 253-258.

Brolund, L. (1985). Causes of variation and applicability for diagnosis of subclinical mastitis, *Acta Veterinaria Scandinavica*, 80, pp. 1-123.

Byarugaba, D. K., Nakavuma, J. L., Vaarst, M. & Laker, C. (2008). Mastitis occurrence and constraints to mastitis control in smallholder dairy farming systems in Uganda, *Livestock Researh for Rural Development*, 20:1

Calvinho, L.F., Almeida, R.A. & Oliver, S.P. (1998). Potential virulence factors of *Streptococcus dysgalactiae* associated with bovine mastitis, *Veterinary Microbiology*, 61:93.

Carrillo-Casas, E. M. & Miranda-Morales, R. E. (2012). Bovine mastitis pathogens: Prevalence and effects on somatic cell count, In: Narongsak Chaiyabutr (ed.) (Chulalongkorn University, Thailand) Milk Production - An Up-to-Date Overview of Animal Nutrition, Management and Health, chapter 17. (ISBN 978-953-51-0765-1).

Denamiel, G., Llorente, P., Carabella, M., Rebuelto, M. & Gentilini, E. (2005). Anti-microbial susceptibility of *Streptococcus* spp. isolated from bovine mastitis in Argentina, *Journal of Veterinary Medicine*, B 52, pp. 125-128.

Devriese, L.A. and De Keyser, H. (1980). Prevalence of different species of coagulase-negative staphylococci on teats and in milk samples from dairy cows, *Journal of Dairy Research*, 47, pp. 155-158.

Dogan, B., Klaessig, S., Simpson, K., Oliver, S., Almeida, R. & Schukken, Y. H. (2005). Pathogenesis of chronic intramammary *Escherishia coli* infections. In : Hogeveen, H. (ed.) *Mastitis in dairy production – Current knowledge and future solutions*, pp 131, Wageningen Academic Publishers, Wageningen, The Neatherlands (ISBN : 978-90-8686-550-5).

Ericsson Unnerstad, H., Lindberg, A., Persson-Waller, K., Ekman, T., Artursson, K., Nilsson-Öst, M. And Bengtsson, B. (2009). Microbial aetiology of acute clinical mastitis and agent-specific risk factors, *Veterinary Microbiology 137*, pp. 90–97.

EC: Regulation No 853/2004 of the European Parliment and of the Council of 29, 2004. Laying down specific hygiene rules for food of animal origin. Official Journal of the European Union L226, 22-82.

FAO – Food and Agriculture Organization of the United Nations, Livestock sector brief – Zambia (2005-03) [online] http://www.fao.org/ag/againfo/resources/en/publications/sector_briefs/lsb_ZMB.pdf [2012-12-18]

Gianneechini, R., Concha, C., Rivero, R., Delucci, I. & Moreno López, J. (2002). Occurrence of clinical and sub-clinical mastitis in dairy herds in the West Littoral region in Uruguay, *Acta Veterinaria Scandinavica,* 43, pp. 221-230.

Govan, J. R. W., Hughes, J. E. & Vandamme, P. (1996). *Burkholderia cepacia:* medical, taxonomic and ecological issues, *Journal of Medical Microbiology,* vol. 45, pp. 395-407.

Guélat-Brechtbuehl, M., Thormann, A., Albini, S., Moret-Stalder, S., Reist, M., Bodmer, M., Michel, A., Niederberger, M.D. & Kaufmann, T. (2010). Cross-sectional study of *Streptococcus* species in quarter milk samples of dairy cows in the canton of Bern, Switzerland,*Veterinary Record,* 137, pp. 211-217.

Halasa, T., Huijps, K., Østerås, O. & Hogeveen, H. (2007). Economic effects of bovine mastitis and mastitis management: a review, *The Veterinary quarterly,* 29 (1), pp. 18-31.

Harmon, R. J. (1994). Physiology of mastitis and factors affecting somatic cell counts, *Journal of Dairy Science* vol. 77:7, pp. 2103-2112.

Harouna, A., Zecchini, M., Locatelli, C., Scaccabarozzi, L., Cattaneo, C., Amadou, A., Bronzo, V., Marichatou, H., Boettcher, P. J., Zanoni, M. G., Alborali, L. & Moroni, P. (2008). Milk hygiene and udder health in the periurban area of Hamdallaye, Niger, *Tropical Animal Health and Production,* 41, pp. 705-710.

Hendriksen, R. S., Mevius, D. J., Schroeter, A., Teale, C., Meunier, D., Butaye, P., Franco, A., Utinane, A., Amado, A., Moreno, M., Greko, C., Stärk, K., Berghold, C., Myllyniemi, A-L, Wasyl, D., Sunde, M. & Aarestrup, F. M. (2008). Prevalence of antimicrobial resistance among bacterial pathogens isolated from cattle in different European countries: 2002–2004, *Acta Veterinaria Scandinavica,* 50:28.

Hogan, J. S., Gonzales, R. J., Harmon, R. J., Nickerson, S. C., Oliver, S. P., Pankey, J. W. & Smith, K. L. (1999). Laboratory Handbook on Bovine Mastitis. (National Mastitis Council Inc: Madison, WI).

Honkanen-Buzalski, T. & Pyörälä, S. (1995). Monitoring and management of udder health at the farm In: Sandholm, M., Honkanen-Buzalski, T., Kaartinen, L. & Pyörälä. S. (ed.) (University of Helsinki, Faculty of veterinary medicine), *The Bovine Udder and Mastitis,* pp. 59-75. Gummerus Kirjapaino, Jyväskylä (ISBN 951-834-047-1).

Janosi, S. & Baltay, Z. (2004). Correlations among the somatic cell count of individual bulk milk, result of the California Mastitis Test and bacteriological status of the udder in dairy cows, *Acta Veterinaria Hungarica* 52 (2), pp. 173–183.

Jones, A. M., Dodd, M. E., Webb, A. K. (2001). Burkholderia cepacia: Current clinical issues, environmental controversies and ethical dilemmas, *European Respiratory Journal* 17, pp. 295-301.

Keefe, G.P. (1997). *Streptococcus agalactiae* mastitis: a review, *Canadian Veterinary Journal,* 38(7), pp. 429-437.

Karimuribo, E. D., Fitzpatrick, J. L., Swai, E. S., Bell, C., Bryant, M. J., Ogden, N. H., Kambarage, D. M. & French, N. P. (2008). Prevalence of subclinical mastitis and associated risk factors in smallholder dairy cows in Tanzania, *Veterinary Record* 163 pp. 16-21.

Koivula, M., Pitkälä, A., Pyörälä, S. & Mäntysaari, E. (2007). Distribution of bacteria and seasonal and regional effects in a new database for mastitis pathogens in Finland, *Acta Agriculturae Scandinavica,* 57, pp. 89-96.

Korhonen, H. & Kaartinen, L. (1995). Changes in the composition of milk induced by mastitis. (ed.) (University of Helsinki, Faculty of veterinary medicine), The Bovine Udder and Mastitis, pp. 76-82. Gummerus Kirjapaino, Jyväskylä (ISBN 951-834-047-1).

Lam, T. J., van den Borne, B. H., Jansen, J., Huijps, K., van Veersen, J. C., van Schaik, G. & Hogeveen, H. (2013). Improving bovine udder health: A national mastitis program in the Netherlands, *Journal of Dairy Science*, 96 (2), pp. 1301-1311.

Mdegela, R. H., Ryoba, R., Karimuribo, E. D., Phiri, E. J., Löken, T., Reksen, O., Mtengeti, E. & Urio, N. A. (2009). Prevalence of clinical and subclinical mastitis and quality of milk on smallholder dairy farms in Tanzania, *Journal of the South African Veterinary Association*, 80 (3), pp. 163-168.

Moore, J. E., Mcllhatton, B., Shaw, A., Murphy, P. G. & Elborn, J. S. (2001). Occurrence of Burkholderia cepacia in foods and waters: clinical implications for patients with cystic fibrosis, *Journal of food protection*, 64 (7), pp. 1076-1078.

Mumba, C., Samui, K. L., Pandey, G. S., Hang'ombe, B. M., Simuunza, M., Tembo, G. & Muliokela, S.W. (2011). Economic analysis of the vialibility of smallholder dairy farming in Zambia, *Livestock Research for Rural Development*, vol. 23 (6).

Murdough, P. A., Deitz, K. E. & Pankey, J. W. (1995). Effects of freezing on the viability of nine pathogens from quarters with subclinical mastitis, *Journal of Dairy Science*, 79, pp. 334-336.

Nam, H., Kim, J., Lim, S., Jang, K. & Jung, S. (2010). Infectious aetiologies of mastitis on Korean dairy farms during 2008, *Research Veterinary Science*, 88, pp. 372–374.

Ndandula, S. N. M. (2011). Pathways to Technology Adoption: Understanding Smallholders Dairy Farmers in Southern Zambia, Institute of Social Studies, The Hague, The Netherlands.

Nevala M., Taponen, S. & Pyörälä, S. (2004). Bacterial etiology of bovine clinical mastitis – Data from Saari Ambulatory Clinic in 2002-2003, *Suomen Eläinlääkärilehti (Finnish Vet. Journ.)* 110, pp. 363-369.

Olofsson, L. (2013). Milking routines and hygiene in small scale dairy farms in Mapepe, Choma and Batoka districts in Zambia. Master of Science Thesis. Uppsala, Sweden: Department of Clinical Sciences, Swedish University of Agricultural Sciences.

Olsen, J.E., Christensen, H. & Aarestrup, F.M. (2006). Diversity and evolution of blaZ from Staphylococcus aureus and coagulase-negative staphylococci, *Journal of Antimicrobial Chemotherapy*, 57, pp. 450–460.

Östensson, K., Lam V., Sjögren, N. & Wredle, E. (2012). Prevalence of subclinical mastitis and isolated udder pathogens in dairy cows in Southern Vietnam, *Tropical Animal Health and Production*, Dec 5. [E-pub ahead of print].

Pandey, G. S. & Muliokela, S. W. (2006). Smallholder dairy farming: a tool for HIV/AIDS mitigation and food in-security. [Electronic] *Africa Forum 2006; the dual epidemics of HIV/AIDS and food insecurity*, Lusaka, Zambia, 9th May. http://www.projectconcern.net/forum/documents/Smallholder_Dairy_Farming-Pandey.pdf [2012-12-17]

Pandey, G. S., Tuchili, L. M., Sato, Y., Musonda, M. M. & Kobayashi, K. (1996). Isolation and drug sensitivity of micro-organisms from clinical bovine mastitis in Zambia, *UNZA Journal of Science and Technology*, vol. 1(1), pp. 33-39.

Persson, Y., Nyman, A. K. J. & Grönlund-Andersson, U. (2011). Etiology and antimicrobial susceptibility of udder pathogens from cases of subclinical mastitis in dairy cows in Sweden, *Acta Veterinaria Scandinavica*, vol. 53:36.

Persson-Waller, K., Bengtsson, B., Lindberg, A., Nyman, A. & Ericsson Unnerstad, H. (2009). Incidence of mastitis and bacterial findings at clinical mastitis is Swedish primiparous cows – Influence of breed and stage of lactation, *Veterinary Microbiology*, 134, pp. 89-94.

Petzer, I.M., Karzis, J., Watermeyer, J.C., van der Schans, T.J & van Reenen, R. (2009). Trends in udder health and emerging mastitogenic pathogens in South African dairy herds, *Journal of South African Veterinary Association*, 80 (1), pp. 17-22.

Pitkälä, A., Haveri, M., Pyörälä, S., Myllys, V. & Honkanen-Buzalski, T. (2004). Bovine mastitis in Finland 2001 – prevalence, distribution of bacteria and antimicrobial resistance, *Journal of Dairy Science*, 87, pp. 2433-2441.

Pyörälä, S. (1995). Staphylococcal and streptococcal mastitis. In: Sandholm, M., Honkanen-Buzalski, T., Kaartinen, L. & Pyörälä. S. (ed.) (University of Helsinki, Faculty of veterinary medicine), *The Bovine Udder and Mastitis*, pp. 143-148. Gummerus Kirjapaino, Jyväskylä (ISBN 951-834-047-1).

Pyörälä, S. & Taponen, S. (2009). Coagulase-negative staphylococci – Emerging mastitis pathogens, *Veterinary Microbiology*, 134, pp. 3-8.

Sandholm, M. (1995a). Inflammation in mastitis. In: Sandholm, M., Honkanen-Buzalski, T., Kaartinen, L. & Pyörälä. S. (ed.) (University of Helsinki, Faculty of veterinary medicine), *The Bovine Udder and Mastitis*, pp. 59-75. Gummerus Kirjapaino, Jyväskylä (ISBN 951-834-047-1).

Sandholm, M. (1995b). Detection o finflammatory changes. In: Sandholm, M., Honkanen-Buzalski, T., Kaartinen, L. & Pyörälä. S. (ed.) (University of Helsinki, Faculty of veterinary medicine), *The Bovine Udder and Mastitis*, pp. 89-104. Gummerus Kirjapaino, Jyväskylä (ISBN 951-834-047-1).

Sandholm, M. & Korhonen, H. (1995). Antibacterial defence mechanisms of the udder. In: Sandholm, M., Honkanen-Buzalski, T., Kaartinen, L. & Pyörälä. S. (ed.) (University of Helsinki, Faculty of veterinary medicine), *The Bovine Udder and Mastitis*, pp. 37-48. Gummerus Kirjapaino, Jyväskylä (ISBN 951-834-047-1).

Sandholm, M. & Pyörälä, S. (1995). Coliform mastitis. In: Sandholm, M., Honkanen-Buzalski, T., Kaartinen, L. & Pyörälä. S. (ed.) (University of Helsinki, Faculty of veterinary medicine), *The Bovine Udder and Mastitis*, pp. 37-48. Gummerus Kirjapaino, Jyväskylä (ISBN 951-834-047-1).

Schalm, O.W., Caroll, E.J. & Jain, N.C. (1971). In Bovine Mastitis, Lea and Febiger, Philadelphia, USA, p 139.

Schukken, Y. H., Günther, J., Fitzpatrick, J., Fontaine, M. C., Goetze, L., Holst, O., Leigh, J., Petzl, W., Schubert, H-J, Spika, A., Smith, D.G.E., Quesnell, R., Watts, J., Yancey, R., Zerbe, H., Gurjar, A., Zadoks, R.N. & Seyfert, H.-M. (2011). Host-response patterns of intramammary infections in dairy cows, *Veterinary Immunology and Immunopathology*, 144, pp. 270-289.

Schukken, Y. H., Wilson, D. J., Welcome, F., Garrison-Tikofsky, L. & Gonzalez, R. N. (2003). Monitoring udder health and milk quality using somatic cell counts, *Veterinary Research*, 34, pp. 579-596.

Schwarz, D., Diesterbeck, U.S., Failing, K., König, S., Brügemann, K., Zschöck, M., Wolter, W. & Czerny, C.-P. (2010). Somatic cell counts and bacteriological status in quarter foremilk samples of cows in Hesse, Germany – A longitudinal study, *Journal of Dairy Science*, 93, pp. 5716-5728.

Seegers, H., Fourichon, C. & Beaudeau, F. (2003). Production effects related to mastitis and mastitis economics in dairy cattle herds, *Veterinary Research*, 34, pp. 475-491.

Sobiraj, A., Kron, A., Schollmeyer, U. & Failing, K. (1997). Federal investigations on the distribution and in vitro resistance of udder pathogenic bacteria in the milk of cows with subclinical mastitis, *Tierarzt Praxis*, 25:2, pp. 108-115.

Sol, J., Sampimon, O. C., Hartman, E., Barkema, H. W. (2002), Effect of preculture freezing and incubation on bacteriological isolation from subclinical mastitis samples, *Veterinary Microbiology*, 85, pp. 241-249.

Sordillo, L.M., Shafer-Weaver, K. & DeRosa, D. (1997). Immunobiology of the mammary gland. *Journal of Dairy Science*, 80 (8), 1851-1865.

Swedish Dairy Association (Svensk Mjölk; 2012a). Statistik speglar produktion och marknad, [online]. http://www.svenskmjolk.se/Statistik/#.UNB3Vm-X1dI [2013-01-28]

Swedish Dairy Association (Svensk Mjölk; 2012b). Redogörelse för organisationens djurhälsovård 2010/2011 [online].

http://www.svenskmjolk.se/Global/Dokument/Dokumentarkiv/Statistik/Djurh%C3%A4lsov%C3%A5rd%202010-2011.pdf [2013-01-28]

Taponen, S., Salmikivi, L., Simojoki, H., Koskinen, M. T. & Pyörälä, S. (2009). Real-time polymerase chain reaction-based identification of bacteria in milk samples from bovine clinical mastitis with no growth in conventional culturing, *Journal of Dairy Science*, 92, pp. 2610-2617.

Taponen, S. & Pyörälä, S. (2009). Coagulase-negative staphylococci as cause of bovine mastitis – Not so different from Staphylococcus aureus?, *Veterinary Microbiology*, 134, pp. 29-36.

Tesfaye, G. Y., Regassa, F. G. & Kelay, B. (2010). Milk yield and associated economic losses in quarters with subclinical mastitis due to *Staphylococcus aureus* in Ethiopian crossbred dairy cows, *Tropical animal health and production*, 42 (5), pp. 925-931.

Utrikespolitiska institutet (2011-10-01). Landguiden, Zambia [online] http://www.landguiden.se/Lander/Afrika/Zambia [2012-12-17]

World Bank, 2011. Report no 62377-ZM, What would it take for Zambia's beef and dairy industries to achieve their potential? Finance and private sector development unit, Africa Region.