Sveriges lantbruksuniversitet
Fakulteten för veterinärmedicin och husdjursvetenskap
Institutionen för kliniska vetenskaper

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Uppsala

2013

Examensarbete inom veterinärprogrammet

ISSN 1652-8697
Examensarbete 2013:74

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Mjölkningsrutiner och mjölkningshygien bland småskaliga mjölkbönder i distrikten Mapepe, Choma och Batoka i Zambia

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

Key words: milking hygiene, milking routines, small scale dairy farms, Zambia
Nyckelord: mjölkningsrutiner, mjölkningshygien, småskaliga mjölkbönder, Zambia

Online publication of this work: http://epsilon.slu.se
ISSN 1652-8697
Examensarbete 2013:74

SAMMANFATTNING

Småskalig mjölkproduktion är ett system som främjar regelbundna inkomster och spelar en viktig roll för att minska fattigdomen, ger anställningsmöjligheter och skapar välfärd samt säkrar en näringsrik livsmedelsförsörjning. Det finns cirka 2500 småskaliga mjölkbönder i Zambia men deras kunskap om bra mjölkningsrutiner och hygien är ofta dåliga. Bra skötselrutiner och mjölkningshygien är viktigt för att uppnå en god juverhälsa och mjölkproduktion, och för att inte förorena mjölken. Subklinisk mastit är en vanlig sjukdom bland mjölkkor, som medför förändrad sammansättning av mjölken och minskad mjölkproduktion vilket orsakar stora ekonomiska förluster. Syftet med denna studie var att undersöka skötselrutinerna, med fokus på mjölkningen, hos småskaliga mjölkbönder i tre olika områden i södra Zambia samt att undersöka om det fanns något samband mellan skötselfaktorerna och juverhälsan (subklinisk mastit). Syftet var också att ge bönderna råd om hur de kan förbättra sina skötselrutiner och därmed juverhälsan. En fältstudie gjordes där 26 gårdar ingick. Hälften av dem hade ett mjölkcelltal på $\leq 400 \times 10^3$/ml och hälften på $> 400 \times 10^3$/ml i besättningsmjölken. Mjölkprover togs från samlingsmjölk från hela juvret samt från varje juverdel separat (n=432) från 111 kor utan symtom på klinisk mastit. Korna var antingen korsningar av lokala raser (*Bos indicus*) eller korsningar av lokala och exotiska raser (*Bos taurus*). Celltalet mättes i alla mjölkprover med DeLavals cell counter och bakteriologisk odling gjordes på alla juverdelsprover. Bönderna intervjuades om mjölkningsrutiner och hygien och observationer avseende detta gjordes på varje gård i samband med mjölkningen. Prevalensen av subklinisk mastit på ko- respektive juverdelsnivå var 48,7 % och 26,9 % och prevalensen av intramammär infektion var 57,7 % och 31,5 %, vilket tyder på måttliga juverhälsoproblem i dessa regioner i Zambia i jämförelse med andra studier av småskalig mjölkproduktion under liknande förhållanden i andra länder. Det finns dock en avsevärd förbättringspotential. Juverhälsostatus var tydligt förknippad med ras. De lokala korsningsraserna hade påtagligt lägre genomsnittligt celltal samt prevalens av subklinisk mastit och intramammär infektion både på ko- och juverdelsnivå. Detta har observerats även i andra studier och det finns undersökningar som tyder på att det kan röra sig om en rasbetingad genetisk relativ mastitresistens baserat på egenskaper i immunförsvaret. De lokala och exotiska korsningsraserna var i många avseenden exponerade för olika skötselrutiner vilket också delvis skulle kunna ligga bakom skillnaden i mastitförekomst mellan raserna i studien. Det fanns oavsett ras allvarliga brister i vissa skötselrutiner som man vet har stor negativ inverkan på juverhälsan och mjölkens hygieniska kvalitet. Genom att förbättra juverhälsan kan kornas produktionspotential utnyttjas bättre. Med en större mängd mjölk från besättningen kan mer mjölk säljas, vilket leder till högre inkomster, utan att volymen mjölk för familjens konsumtion behöver minska. Råd om lämpliga förbättringar, som finns som bilaga, inkluderar handtvätt, rätt förberedelse av juvret, kontroll av mjölken, mjölkning med hela handen som mjölkningsteknik och utan användning av glidmedel på spenarna samt spendoppning efter mjölkningen.

SUMMARY

Smallholder dairy production is a system that promotes regular monetary earnings and plays an important role in poverty reduction, employment opportunities, wealth creation and nutritional household food security. There are about 2500 smallholder dairy farmers in Zambia but their knowledge of good milking routines and hygiene is often poor. Good dairy management routines and milking hygiene are important to obtain good udder health and avoid contamination of the milk. Subclinical mastitis (SCM) is a common disease among dairy cows, which changes the composition of the milk and gives a reduced milk production leading to severe economic losses. The aim of this study was to investigate the management routines, with focus on the milking, of small-scale dairy farmers in three different areas in southern Zambia and to investigate if any association between the management practices and udder health status (subclinical mastitis) could be observed. Overall the aim was to help the farmers improve their milk production through proper advice on improved management routines and udder health in the different herds. In a field study, 26 farms, half of them with a herd bulk milk somatic cell count (SCC) $\leq 400 \times 10^3$/ml and half of them with a herd bulk milk SCC $> 400 \times 10^3$/ml, were included. Udder bulk milk samples and quarter milk samples (n=432) were taken from 111 cows without signs of clinical mastitis. The cows were either crosses of local breeds (*Bos indicus*) or crosses of local breeds and exotic breeds (*Bos taurus*). The SCC was measured in all milk samples using a DeLaval cell counter and bacteriological culturing was done on all quarter samples. The farmers were interviewed about milking routines and hygiene in addition to observations done at each farm during milking. The results from this study with a prevalence of SCM at cow and quarter level of 48.7 % and 26.9 %, respectively, and a prevalence of intramammary infection (IMI) of 57.7 % and 31.5 % indicates moderate udder health problems in this region in Zambia compared to other studies of small-scale dairy farming under similar conditions in other countries. The results show that udder health status was clearly associated with breed. The local crossbreeds had, in general, a lower average SCC and prevalence of SCM and IMI at both cow and quarter level. This is in agreement with observations in other studies and is suggested to be attributable to a breed-associated genetic resistance to mastitis due to favourable characteristics of the immunological defence. However, the background to breed differences in mastitis prevalence may also include factors, such as management. In the present study the local and exotic crossbreeds were, to a large extent, exposed to different management routines. The management routines generally applied, regardless of the breed, had severe shortcomings in factors known to have a negative impact on the udder health status and hygienic quality of the milk. The production potential of the cows could be better utilized by improving udder health. With a larger herd milk yield more milk can be sold leading to higher cash income, still leaving milk for the families' consumption. Advice on proper milking routines, that is shown in the appendix, includes hand washing, correct udder preparation, checking the foremilk, practice a whole-hand milking technique without use of lubricants, and post-milk teat dipping.

CONTENTS

BACKGROUND

Zambia

Zambia, is a developing country in the south of Africa bordering Namibia, Angola, Zimbabwe, the Democratic Republic of Congo, Malawi, Tanzania and Mozambique. Sixtyone percent of the population reside in rural areas with agriculture as their main source of livelihood (Ndandula, 2011; Mulemba, 2009). Agriculture is the main driver of Zambia's economy, accounting for more than 70 % of the total employment (Ndandula 2011; Haambulo, 2009).

Zambia's climate is pleasant tropical and there are wide seasonal variations in temperature and rainfall (Nationsencyclopedia, 2012 & FAO, 2009). From April to August there is a cool dry season, from August to November a hot dry season and from November to April a warm wet season. October is the hottest month with temperatures ranging from 27^{o} C to 35^{o} C. The mean annual temperature ranges between 18^{o} C and 20^{o} C. Apart from very rare showers in August, the main rainy season starts in mid-November, at the height of this season it rains on seven or eight days out of ten.

Smallholder dairy production in Zambia

There are about 2500 smallholder dairy farmers in Zambia that are connected to dairy cooperatives in milk marketing (Mumba et al, 2011). These farmers are being strengthened by resource persons, materials and financial support mainly from Golden Valley Agricultural Research Trust (GART) and other non-governmental organizations (NGOs) in collaboration with the Government of Zambia. The smallholder dairy farmers, including traditional (beef) cattle keepers, contribute to about 53.5 % of Zambia's total milk production. Smallholder dairy production is a system that promotes regular monetary earnings and plays an important role in poverty reduction, employment opportunities, wealth creation and nutritional household food security for the majority of the rural population.

Poverty alleviation

According to FAO (2010), 12 to 14 % of the world population (750 to 900 million people) live in dairy farming households and approximately 200 on-farm jobs are created by the production of one million litres of milk per year on small-scale dairy farms. The majority (80

%) of the rural population in Zambia is living below the poverty line (Haambulo, 2009). The regular monetary earnings coming from milk production are an obvious advantage compared to crop production, which gives access to cash income once a season after the sole annual harvest (Ngongoni et al, 2006). These regular earnings contribute significantly to an improvement in the living standard of the rural population. For example, this income makes it possible for children to go to school instead of being forced to raise money for their families. Production of milk creates employment opportunities not only on the farm but also throughout the entire dairy chain.

The HIV/AIDS-situation in Zambia

About 64 % of the estimated forty million people infected with HIV are living in sub-Saharan Africa and are at risk of becoming ill and dying prematurely of infection by HIV/AIDS due to poor nutrition and inadequate access to food and income (UNICEF, 2012a). Since 1984 Zambia has one of the world's most devastating HIV/AIDS epidemics with a prevalence of 13.5 % among people in the age range 15-49 (UNICEF, 2012b). Because of HIV/AIDS the life expectancy at birth has fallen to 49 years (AVERT, 2012). Being HIV-positive enhances sensitivity to infections, and poor nutrition hastens the progression of AIDS and additionally makes the person more vulnerable to other infections. Diseases like HIV/AIDS have aggravated the poverty in Zambia by contributing to a decline in agricultural production and off-farm sources of livelihood and to increasing household insecurity. Zambia was hit by a food shortage in 2002 to which AIDS is believed to be the major contributor due to reduced manpower and workforce in agriculture.

Drugs for treating HIV/AIDS may produce only limited effects without the right amount of nutrients as well as easily digestible food. Drug compliance is compromised if there is no access to food because some antiretroviral drugs have to be taken with food for proper absorption and effectiveness and also to minimize the side effects. People infected with HIV/AIDS, especially if under treatment with antiretroviral drugs, have a higher need of calories and protein than uninfected persons and therefore food insecurity is especially damaging for these people (UNICEF, 2012a).

Milk is a good source of nutrition

The recommended milk consumption per person per year by FAO (2010) is 200 litres, but in Zambia the estimated consumption is only 24 litres per person per year (Mumba et al 2011). Milk contains protein, carbohydrate, fat, vitamins and minerals and is, therefore, a nutritionally complete foodstuff and a valuable supplement to a poor diet. Milk and milk products can provide a good nutritional support for people on antiretroviral therapy who live in poverty and are food insecure (Pandey & Muliokela, 2006; UNICEF, 2012a). Vertical transmission (from mother to child) of the virus causing AIDS accounts for 40 to 90 % of all HIV infections in infants and children (UNAIDS, 2004). Thirty to fifty per cent of the vertical transmission occurs by breastfeeding and the only way to prevent this transmission is to discourage or stop feeding infants with breast milk. This requires an alternative food source that is easily accessible in large quantities, affordable and socially acceptable. Infant milk formula could have been an option, but it is not available in the rural areas of Zambia or, if it is available, it is very expensive in the rural areas of Zambia. Additionally, it means an increased risk of gastrointestinal infections in the infants since there is poor access to drinking water of acceptable hygienic quality in the rural areas of Zambia. Milk from cows or goats is therefore the only realistic alternative to breastfeeding.

Golden Valley Agricultural Research Trust (GART)

Smallholder dairy farming is a system that has demonstrated the mitigating effects of HIV/AIDS (Pandey & Muliokela 2006). The farmers' organization of Zambia and the government started a joint venture in 2000, the GART, with the aim of promoting farming development. GART is a self-sustainable institution that is engaged in agricultural development and research, knowledge transfer, food security and HIV/AIDS mitigation through agricultural production (Pandey & Muliokela, 2006). In 2004, GART started a program in the Southern province in smallholder dairy development with the aims of improving household food security, income generation and HIV/AIDS mitigation. The number of dairy farmers increased five times and the milk consumption per person per year increased from 10 Kg in 2000 to 17 Kg in 2005 (Pandey & Muliokela, 2006). Improved milk consumption results in improved nutrition and consequently increased resistance against diseases, i.e. opportunistic infections (UNICEF 2012 a).

Knowledge of milking hygiene is often poor among farmers. Poor hygiene during milking and handling of milk increase the risk for contamination with bacteria that might cause disease in the consumer and also reduces the shelf life of the milk. The desired increased availability and consumption of milk requires a sustainable improved production of milk of good hygienic quality, which in turn requires advice and measures to improve udder health and milking and management routines. GART operates in order to support small farmers' milk production with this goal, and this thesis work was done with the support of, and in connection with, GART. The study was done in parallel with another study of the incidence of mastitis and mastitis-causing bacteria in the same animal population.

INTRODUCTION TO RESEARCH PROJECT

Importance of good milking practices

Good dairy management practices will ensure that milking routines do not harm the animals or introduce contaminants into milk, that milking is carried out under hygienic conditions and that the milk is handled properly after milking (FAO & IDF, 2011). A good milking routine means removing milk efficiently from the cow with minimal risk to udder health (Blowey & Edmondson, 2010). Cows are easily stressed and therefore it is important to have a calm milking environment and practice a consistent milking routine. If the cows become nervous the milk let-down reflex will be affected.

Milk from a healthy udder contains only few bacteria, but milk is a perishable product which is very easily contaminated and invaded by bacteria (for review, see LeJeune & Rajala-Schultz, 2009, Pandey & Voskuil, 2011). Milk is also an ideal medium for microorganisms. The contamination of milk can occur at different stages in the milking procedure and from different sources, mainly from the external surface of the udder and teats, and from the surface of the milking utensils (Murphy & Boor, 2000). Additionally, contamination can occur from animal the shed and environment, other body parts of the animal, the milker, handling of the milk, storage and transport (for review, see LeJeune & Rajala-Schultz, 2009; Pandey & Voskuil, 2011). Bacteria in the milk may also comprise mastitis pathogens from the udder. In general, pathogens are not capable of growing in low temperatures and therefore play a minor role when the milk is kept cool. In conditions with high ambient temperature and lack of cooling of the milk during storage and transport, the pathogens can multiply and become a real health hazard for the consumer. Milking hygiene has an impact on the hygienic quality and shelf-life of the milk, but also on the occurrence of mastitis and risk of spreading mastitis infections. Mastitis, in turn, negatively affects milk yield, composition and quality. For small-scale farmers to be able to practice good milking routines it is of great importance that they are given proper advice and assistance (Pandey & Voskuil, 2011).

A total bacterial count may commonly be up to 50 000 per ml, but it is possible to milk cows under primitive conditions in such a clean way so that the raw milk contains less than 1000 bacteria per ml (Pandey & Voskuil, 2011). *Lactococcus* (previously *Streptococcus) lactis* is a family of lactic acid bacteria commonly found in milk (van Hylckama Vlieg 2006; Pandey & Voskuil, 2011). When the milk is kept at ambient temperatures after milking these bacteria multiply and grow very fast, producing lactic acid from lactose and thereby causing the natural souring of milk. These bacteria usually come from the environment (air, dirty equipment, dust etc.). The degree of contamination and the temperature of the milk are the main factors of how rapidly the milk turns sour. Proper cleaning and sanitizing procedures and hygienic milking routines are therefore essential to control the quality of milk and the milk should be cooled as soon as possible after milking.

Mastitis

Mastitis means inflammation in the mammary gland and may occur with or without infection. The inflammatory reaction is the body's defence against an insult such as trauma or invading microorganisms and causes alterations in the udder tissue and milk. It may also give rise to systemic immunological reactions. Mastitis is the most common disease of dairy cows with negative consequences for economy and animal welfare. It may occur in a clinical or subclinical form. Clinical mastitis (CM) is associated with local symptoms from the udder and might also give systemic effects such as fever. Subclinical mastitis (SCM) is silent with no visible symptoms or milk alterations, and laboratory methods are necessary for diagnosis. SCM is the most common form of mastitis. The incidence may vary between countries but e.g. in Sweden approximately two-thirds of the cows are affected by SCM at least once during a lactation (Swedish Dairy Association, 2011). A characteristic feature is a decline in milk production. Because of the difficulty of detecting SCM it has a significant impact on milk production over time and is economically the most detrimental form. (Seegers et al., 2003; Sandholm, 1995)

Mastitis causing agents

Although mastitis may be present without infection, most mastitis reactions probably have an infectious history. Bacteria, fungi, yeasts and even possibly virus, can cause infection of the udder, but the main infectious agents are bacteria (FAO, 1989). The most common bacterial pathogens are *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Escherichia coli* and *Klebsiella spp*. but other pathogens may also cause intramammary infection (IMI). The above-mentioned bacteria are divided into two broad categories: contagious pathogens and environmental pathogens, depending on their main source of transmission.

No matter which infectious agent is involved, a mastitis infection can be easily transmitted between cows or udder quarters within the cow during milking, for example via contaminated milk, the milking cluster or hands of the milker and udder cloths. *Staphylococcus aureus* and *Streptococcus (Str.) agalactiae* are the major contagious pathogens, with infected udders as their main reservoir (for review, see Carrillo-Casas & Miranda-Morales, 2012; Honkanen-Buzalski & Pyörälä, 1995). *S. aureus* and *Str. agalactiae* will therefore spread mainly during preparation of the udder or on hands and milking machines. While these pathogens may colonise and multiply in teat sores and in teat ducts, the degree of exposure of the teats to bacteria greatly increases. *S. aureus* and *Str. agalactiae* usually cause chronic infections that persist in a subclinical form but could occasionally become clinical.

Other udder pathogens reside mainly in the environment and can be transmitted from there to the udders between milkings, for example via contaminated beddings, licking of the udder and teats, contacts with the udder with the tail or legs and flies. Pathogens like *Str. uberis*, *Str. dysgalactiae*, *Escherichia (E.) coli* and *Klebsiella* species belong to this group of "environmental bacteria". These bacteria normally do not colonise the teat skin and they multiply mainly in organic bedding materials. Therefore infections with these bacteria are most common in housed cattle. *Str. dysgalactiae* is also able to colonise and multiply in teat lesions, but the main primary site is not the milk of the infected quarters but other bovine sites. To prevent transmission, cows with a high somatic cell count and diagnosed IMI ought to be separated from healthy cows with a low cell count.

Coagulase Negative Staphylococci (CNS) are present on the cows skin and in the environment. Although they have traditionally been considered as opportunists and minor pathogens, they are isolated from mastitis cases with increasing frequency and are today regarded as a major mastitis-causing organism. In many countries CNS have become the most common bacteria isolated from bovine mastitis. These species of bacteria are not as pathogenic as the other principal mastitis pathogens and infection mostly remains subclinical. Knowledge of the CNS species causing bovine mastitis is limited and it is therefore difficult to determine whether they behave as contagious or environmental pathogens (Pyörälä & Taponen, 2008).

While different bacteria have a common route of transmission there are different effective measures to prevent spreading. For example the most effective measure to prevent spreading of contagious udder pathogens is good milking practices. It is also of great importance to prevent importation of pathogens into a healthy herd when introducing new animals to the herd. To control the spreading of environmental pathogens, overall good hygiene with clean animals and environment should be practiced (Honkanen-Buzalski & Pyörälä, 1995). Opportunistic bacteria (e.g. CNS) are best controlled by not giving them favourable opportunities to infect the udder (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.

Somatic cell count – an indicator of mastitis

Somatic cell count (SCC) is the most commonly used indicator of udder health and SCM. Somatic cells are cells originating from the body (in contrast to bacteria cells in the milk) and in bovine milk consist mainly of leukocytes. In normal milk the SCC is low. During inflammation, which is the body´s response to an insult such as trauma or bacterial invasion, the milk SCC increases due to leukocytes migrating to the udder and milk to neutralize the insult (for review see Sordillo et al., 1997). Increased SCC in milk is seen in both clinical and subclinical forms of mastitis and the degree of increase is a direct reflection of the degree of inflammation in the udder. Therefore SCC is being used as an inflammatory indicator in the diagnosis of SCM.

The somatic cell count of milk from a healthy bovine udder quarter in mid-lactation should be less than 100 x 10^3 cells per ml of milk (for review, see Schukken et al, 2003). The SCC can be influenced to some extent by physiological factors such as milk yield, stage and number of lactation and breed. It may also vary from day to day. Even season and stress factors may affect SCC to some extent, but status of infection is the factor with the most significant impact on SCC. In practical udder health work a milk SCC of > 200 x 10^3/ml to indicate

SCM has been found relevant to use to minimize diagnostic errors (Brolund, 1985; for review see Schukken et al., 2003). During mastitis the SCC may increase up to many millions of cells per ml of milk. The highest SCC is associated with CM while the SCC elevation in SCM is generally more modest and variable.

Effect of mastitis on milk yield, composition and quality

To achieve high yield and quality products in the dairy processing industry a raw milk of high quality is required (Forsbäck et al., 2009). Mastitis causes reduced milk synthesis (for review, see Seegers et al., 2003) and impaired milk quality and shelf life (for review, see Korhonen & Kaartinen, 1995). This is due to alterations of different kinds caused by the inflammatory process, such as an impaired blood-milk barrier, increased content and activity of leukocytes in the milk, and disturbed functioning of the milk-synthesizing epithelial cells resulting in reduced production and an altered composition of milk constituents.

The effects on milk quality and composition are strongest in clinical mastitis but SCM without visible signs and with moderately increased SCC also results in deteriorated quality of the milk (Forsbäck et al., 2009). It is rare that all udder quarters of the cow are afflicted with mastitis but milk from single quarters with high SCC can affect the entire udder composite milk. On the other hand, single quarters with increased SCC (SCM) may not be detected by measuring the composite udder milk SCC due to dilution of low SCC milk from other healthy quarters. The alterations on milk quality have been shown to occur in milk from separate udder quarters even when the cow composite SCC is as low as 100-200 x 10^3/ml, a SCC that would not draw much attention in practice (Forsbäck et al., 2009).

Milk yield

Mastitis is the most frequent and costly production disease in dairy herds (Seegers et al., 2003) mainly through losses in milk production. Bovine mastitis, both the clinical and subclinical forms, is the disease with the largest impact on milk production (for review, see Seegers et al., 2003; Mdegela et al, 2009). SCM is the most prevalent form and while it is silent and can remain undetected for a long time it is considered to be the form with the strongest impact on dairy economy (Halasa et al., 2007; Tesfaye et al., 2010). According to Seegers et al (2003) production losses due to a clinical mastitis case have been suggested to be approximately 375 kg and approximately 0.5 kg per two-fold increase of SCC at cow level in a case of subclinical mastitis. In general, the reduction in milk yield is considered to be directly correlated to the increase in SCC but it has also been found that milk production remains impaired after recovery from mastitis (Rajala-Schultz et al., 1999). Milk yield begins to decrease at a SCC that exceeds 100 x 10^3/ml (Korhonen & Kaartinen, 1995). The decrease is linearly in relation to the logarithmic value of the SCC.

Milk composition and quality

Inflammatory reactions do not only change the quantity of the milk but also the quality (Korhonen & Kaartinen, 1995). As the SCC reflects the change in milk yield it also reflects the changes that occur in the composition of milk. There is an increased permeability of the blood-milk barrier due to mastitis, with damaged epithelial cells and wider tight junctions, which will result in an influx of serum proteins and enzymes to the milk (Forsbäck et al., 2009). The components of blood filter from the blood circulation into the mammary gland and the more increase in inflammation the more the chemical composition of milk approaches that of blood. The changes in the composition of the milk reduce milk quality and durability (for review, see Seegers et al., 2003; Ogola et al., 2007; Sandholm, 1995). The inflammatory process and activity of leukocytes results in an increased concentration of proteolytic and lipolytic enzymes with a detrimental effect on e.g. the protein and fat content of milk.

Fat content

Mastitis decreases the fat content by less than 10 % but the changes in the fat content of milk caused by mastitis are diverse (Korhonen & Kaartinen, 1995). However, the changes in fat composition is significant and decreases the quality of milk products, mainly due to the increase in free fatty acids while the total amounts of fatty acids remain unchanged. There is a decrease in the amount of phospholipids due to a decrease in the amount and size of fat globules. The amount of short-chain fatty acids (C4-C12) increases and there is a corresponding drop in the amount of long-chain fatty acids (C16-C18). However, the amount of unsaturated long-chain fatty acids is higher in mastitic milk than in normal milk and all the changes in the lipid phase increase the lipolytic sensitivity in mastitic milk, which is also intensified by an increased lipase activity.

Proteins

Only when the SCC exceeds 1 000 x 10^3/ml will there be a decrease in the total amount of proteins in the milk (Korhonen & Kaartinen, 1995), but Le Roux et al (1995) showed that the altered protein composition starts at as low level as a SCC of 250 x 10^3/ml in udder quarter milk. Generally, the proportion of casein diminishes as a consequence of increased proteolytic activity, while the proportion of whey proteins increases. The amount of whey proteins is related to the degree of the inflammatory process. Many of the whey proteins originate from blood and as the degree of inflammation increases, the filtration into the mammary gland increases. The influx of serum proteins and enzymes will lead to increased proteolysis and an altered protein composition with lower casein number and higher whey proteins in affected udder quarter (Forsbäck et al., 2009; Urech et al., 1999). Proteolytic enzymes such as plasmin, elastase, cathepsin and collagenase lower the casein content by degradation (Forsbäck et al, 2009; Le Roux et al., 2003).

Lactose

There is a highly significant negative correlation between the lactose level and SCC in mastitic milk and mastitis causes a decrease in lactose content of approximately 10 % (Korhonen & Kaartinen, 1995). While lactose is the most important osmotic gradient in milk the osmotic balance between milk and blood will be disturbed by mastitis, resulting in an influx of sodium and chloride ions with the aim of maintaining the balance. Therefore, the content of sodium and chloride in mastitic milk may increase tens of times beyond the normal level.

Minerals and trace elements

Mastitis causes an increase in the passive permeability over the udder epithelium and a weakened ability of the udder epithelium to concentrate ions, leading to a change in the content of minerals and trace elements in the milk (Korhonen & Kaartinen, 1995). The result is a higher concentration of sodium and chloride, while magnesium, calcium, potassium and phosphorus decrease considerably. These changes have significant importance both for the processing properties of milk and also its nutritive value. For example, the curdling ability of milk is reduced due to the decrease in the amount of calcium and phosphorus and the change in their ratio. The heat tolerance of milk and its organoleptic properties is reduced because of changes in lactose and salt balance. Copper and iron are bound to whey proteins and their content will therefore increase in mastitic milk, while zinc that is bound to casein will decrease in content.

There is also a loss of 10-15 % of riboflavin and ascorbic acid (water soluble vitamins) content in mastitic milk affecting the bacteriological fermentation processes and lowering the quality of cheese and sour milk products.

Enzymes

Enzymatic and biochemical activity increase due to mastitis and some of these characteristics have for many years been used to detect mastitis (Korhonen & Kaartinen, 1995). The enzymes originate from the blood, the mammary gland tissue and/or the infecting microbe and the enzymatic activity in the mastitic milk may to some degree be reflected by the site of origin of the enzymes. The activity of these oxidizing, lipolytic and proteolytic enzymes causes a faulty fermentation of sour milk products and induces various quality problems such as altered odour, taste and structural faults.

Physical properties

The physical properties of milk will change due to mastitis (Korhonen & Kaartinen, 1995). The pH increases from 6.6 to over 7, mainly because of the transfer of bicarbonate ions from blood to milk. Along with the quantity of ionising salts, the specific conductivity increases and as the cell matter and serum protein content increase, the viscosity increases. As a result of the decrease in lactose content, the freezing point may increase a little and the density of milk falls. The oxidation-reduction ability decreases due to decreased oxygen content and the activity of the infecting bacteria.

Risk factors for mastitis

Predisposing factors for mastitis are associated with the individual cow, virulence of the pathogen, environment and management routines, especially milking hygiene and routines (for review, see Ali-Vehmas & Sandholm, 1995 and Barkema et al., 1999a, b; Abera et al., 2012).

The cow

Cow factors that are known to influence the risk for mastitis are breed, milk production level, udder and teat conformation and immunological status (for review see Saloniemi 1995 a). Locomotion may also play a role. If the cow moves in a clumsy fashion and is ungainly due to e.g. foot and leg disorders or pain there is a higher risk of teats being trampled on and injury to the udder. This risk is increased if the cow has, for example, a pendulous udder with long teats. There are breed differences in the disposition of mastitis. For example the Swedish Holstein is slightly more prone to mastitis than the Swedish Red (Persson Waller et al., 2008). Breed differences might be attributable to a single factor or a combination of factors of the breed that is predisposing for mastitis, such as udder and teat conformation or milk production. For example, Karimuribo et al. (2008) found that the Boran breed in Tanzania had more subclinical mastitis than the Tanzania shorthorn Zebu, a difference that was considered to be associated with the higher volumes of milk produced by the Boran (Mwatawala and others 2004). Other studies have also shown that higher producing dairy cows are at a greater risk of developing subclinical mastitis (Wilton et al., 1972; Kennedy et al., 1982). A correlation between high yielding herds and the incidence of CM has also been observed (Fleischer et al., 2001; Sato et al., 2008). Peak milk yield, lactation stage and age of the cow are other factors that have been found to be associated with higher risk of mastitis (for review, see Saloniemi 1995a; Karimuribo 2008).

The shape and position of the teat end, length and closure of the teat canal, properties of the canal keratin layer and milking ability are characteristics associated with the local defence of the udder that also influence the cow’s susceptibility to mastitis. Both very high and poor milking ability is predisposing for mastitis. The general immunological status affects the body’s ability to combat infections and insults, but the outcome of this battle is dependent on the interaction between the host and the microbe where consequently other factors such as the virulence of the microorganism also play an important role. Impaired immunological defence may occur as a result of some virus infections, malnutrition and metabolic disorders such as ketosis (Holtenius et al., 2004) and is considered to be a reason behind the increased susceptibility to mastitis around calving.

The environment

There are several studies on the impact of the production environment on udder health (Saloniemi, 1995b, Philpot and Nickerson, 2000a and 2000b) and the distribution of udder pathogens varies between countries partly because of differences in management (Ericsson Unnerstad et al, 2009). Increased pressure of different environmental factors will lead to an increased risk for udder injuries and mastitis. Examples of some environmental factors are dirty lying places that may lead to transmission of the infection to the udder and skin from the environment or from another cow. Poor cleaning of the stall as well as poor insulation and ventilation that promote the growth of udder pathogens in the immediate environment of the cow is another factor. Slippery stall floor may cause teat injuries, which gives a favourable microbial microenvironment on the udder and teat skin. When working with udder health it is important to point out environmental factors to the owner of the animal in order to prevent the cows from increased mastitis risk by the environment. It has been shown that housing systems, ventilation, lighting, the stall floor and bedding materials have a great impact on the occurrence of mastitis but most of it is not relevant for small scale dairy production in tropical areas such as Zambia since they rarely keep their animals indoor.

There are some variations in the environments in which milking is done (Yambayamba & Zulu, 2011). Some farmers have a basic infrastructure of the milking parlour, which includes a concrete floor and roofing material, while some farmers milk the cows in the open. When hand-milking is practiced, conditions that may increase the dust content in the surrounding air will increase the microbial population and thereby lead to increased bacterial contamination of the milk. In a study by Yambayamba and Zulu (2011) on the influence of the milking environment on the microbial quality of raw milk they found milking parlours with a concrete floor to be the best and an open area with a non-concrete floor to be the worst, when looking at bacterial contamination level. A milking parlour with a concrete floor is a protected area and is easy to clean and is therefore a good environment for milking.

While most of the dairy cows in small-scale production in Zambia are kept outside the climate is of great importance in the transmission of mastitis. Differences in temperature and humidity can have strong effects on bacterial counts in milk (Elmoslemany et al., 2010). According to Elmoslemany et al., (2010) in a study on dairy herds on Prince Edward Island high milk bacterial counts were found during spring and summer. Similar results were found by Sraîri et al. (2009) and this is explained by faster bacteria growth in warmer temperatures. In many African countries the climate is divided into a rainy season and a dry season. Biffa et al., (2005) found a higher prevalence of mastitis during the rainy season compared to the dry season in Ethiopia, probably due to its association with poor sanitation. It is easier to keep the cows clean during the dry period and in the rainy season the ground will be muddy which favour the proliferation and transmission of mastitis pathogens. In a study by Harouna et al., (2009) in Niger significantly greater numbers of microorganisms, particularly environmental microorganisms, were isolated during the rainy season than in the dry season.

The thermal factor of the environment may negatively affect the production of dairy cows, especially high yielding cows (Kadzere et al, 2002). When dairy cows fail to maintain thermo neutrality due to increasing ambient temperature and humidity, they may become hyperthermic and risk heat stress (André et al, 2011). Higher producing cows are more at risk than lower producing cows due to teir high metabolic activity. The dairy cows of small-scale dairying are usually not high yielding, with the result that they are not as sensitive to heat as high yielding cows. Heat stress may lead to decreased milk production and changes in milk composition, e.g. decreased fat and protein content.

In general, when talking about housing systems, loose housing systems might increase the risk for mastitis caused by environmental bacteria. At the same time, if contagious udder pathogens become established they may be harder to eradicate in loose housing systems because of difficulties in keeping strict grouping of cows and milking order (Ericsson Unnerstad et al., 2009). The incidence of mastitis caused by *S. aureus* seems to be higher in tie stalls. In any housing system it is impossible to stop the spread of contagious bacteria, if a strict milking order is not accomplished (Ericsson Unnerstad et al., 2009).

Animal management

For animals to be in a general good and healthy condition they need to be fed and watered with products of suitable quality and safety (FAO, 2011). In developing countries the water supply to dairy farms is generally of poor quality and the access to nutritious feed is usually inadequate, especially during the dry season. The cows should be fed in a clean and dry place and the drinking place should be washed and protected against contamination.

The animals and breeds should be selected in such a way that they are suited to the environment and thereby have the ability to adapt to climatic extremes, feed quality, local parasites (especially ticks) and also have an acquired resistance to endemic disease. The animals should be vaccinated according to national recommendations and regulations and there should be a vermin control program at every farm. When introducing new animals to the herd the animals should be of known health status and quarantine should be used, if indicated. People that visit a number of farms may spread disease and therefore, if possible, the access to the farm by people and wildlife should be limited. All animals should be regularly checked for disease and sick animals should be isolated and treated.

The risk factors that predispose for udder infection can be vastly different depending on the kind of pathogen, e.g. environmental pathogens versus contagious pathogens (Barkema et al, 1999a). Risk factors that hamper the first line of defence, such as the teat canal orifice or nonspecific immune mechanisms contribute to the opportunity of infection by all pathogens. In some cases one risk factor for mastitis caused by one pathogen may be a preventive factor for mastitis caused by a different pathogen (Barkema et al, 1999a). This has been indicated in a study by Lam et al. (1997) that discontinuation of post-milking teat disinfection may decrease the incidence of clinical mastitis caused by *E. coli* but at the same time there may be an increased risk of mastitis by contagious pathogens. Ericsson Unnerstad et al (2009) found that using sawdust as bedding material was a risk factor for isolating *Klebsiella spp*., but the risk of isolation of *S. uberis* was reduced. All management routines related to milking have been shown to have a strong impact on udder health (Barkema et al, 1999ab) besides the effect of hygienic measures on milk soundness and quality. To be optimal, the milking routines must be related to the milking system and other main conditions. In the following, milking routines will be described with special emphasis on small-scale dairy herds with simple conditions.

Water and feed

Among management practices that have been found to represent risk factors for mastitis is the cows' access to water. Kivaria et al (2004) found that scarcity of water was significantly associated with subclinical mastitis and Lam et al (2011) found that herds that had restricted access to drinking water had higher somatic cell counts than herds providing with drinking water ad libitum. Kivaria et al (2004) found that water contamination often occurred in the storage containers used and therefore stated that it is the microbiological quality rather than the amount of water that is associated with subclinical mastitis. Lam et al (2011) intended that dairy cows in the tropics need more water to alleviate heat stress and to increase water metabolism. Cows increase water intake not only to replace water loss via sweat, faeces, respiratory evaporation and milk, but also to cool their bodies. By providing adequate drinking water for dairy cows the impact of heat stress on milk constituents and milk SCC are reduced (Lam et al, 2011).

It is also important to keep the animals in an overall good healthy condition to prevent the risk of mastitis (FAO, 2011). The animals should be fed in clean places and the overall quality of both water and feed is an important factor for the cow's overall health and thereby its propensity to be affected by mastitis or other diseases. Barkema et al (1999a) found that nutrition was associated with the incidence of clinical mastitis. There was a decreased incidence of clinical mastitis caused by *Streptococcus uberis* and *Streptococcus dysgalactiae* when the cows had minerals in their diet.

Teat injuries

The teats should be examined for injuries. Teat lesions are classified either as congenital abnormalities, such as various hypoplasties, supernumerary or accessory teats and fistulas, or as acquired abnormalities, such as injuries, malfunction of the milking machine, incorrect milking technique, virus infections and irritating teat disinfectants (Pyörälä & Honkanen-Buzalski, 1995). Teat lesions and cracks serve as good sites for bacteria to multiply, especially contagious bacteria such as *S. aureus, Str. agalactiae* and *Str. dysgalactiae*. Acute mastitis caused by *E. coli* or *A. pyogenes* is often the result of injury of the teat canal. The cow may have poor milk let-down due to pain and may kick and defecate more frequently during milking, increasing the risk of contamination. It is also much easier to keep the teats clean when they are healthy and undamaged. Even small teat sores have been shown to be associated with an increased risk of subclinical mastitis with higher risk as the lesions approach the teat canal (Agger & Villeberg, 1986). The risk of mastitis in a quarter seems to be increased by 50 % during a 10 months follow-up period after the time of injury.

Pre-milking routines

The contamination levels and specific distribution of microorganisms, e.g. the hygienic profile of the milk, are highly correlated to pre-milking hygiene conditions and udder health (Pankey, 1989). Milking means manipulations of the teats and the main site of entry of microorganisms, the opening of teat canal (Sandholm & Korhonen, 1995). Furthermore, during milking the teat canal is wide open which facilitates the invasion of the udder by microorganisms. The prevalence of mastitis is higher in cows with poor hygiene of the milking process than in cows with good hygiene of milking process (Lakew et al, 2009).

Pre-milking routines (FAO, 1989) are focused on preparation of the udder and teats. The aim of this procedure is both to stimulate the milk ejection reflex and to clean the teats, which is important from a hygienic and udder health perspective. Bacteria on the skin of the cow may be “dirt bacteria”, which can start deterioration processes when contaminating the milk, but may also be udder pathogens emanating from the environment or infected milk which are given favourable conditions for invading the udder through the open teat canal during milking. This includes transmission of new infections to the udder as well as spread of infections between udder quarters. Dirt bacteria and pathogens may also emanate from the milker’s hand and hand hygiene is particularly important in hand milking. Thus, the cleanliness of the cow in general as well as the immediate environment during milking may also have an impact on milk hygiene and udder health, which is the reason why the properties of the cows’ environment and milking place ought to be considered as well. Before milking starts, the cow should be as clean as possible. It is not advisable to rinse the cow since this will only dissolve the dirt and make it pour down along the skin and accumulate on the udder. If the cows are very dirty in a herd this problem has to be tackled by taking measures regarding their daily environment. The milker’s hands should be washed before milking and between each cow. Proper preparation of the udder before milking should include wiping off dirt from the udder skin, cleaning the teats and inspecting the milk for alterations associated with clinical mastitis such as flakes and admixtures of blood. Such milk should not be used for human consumption.

If milking takes place inside a shed there is a high risk of contamination of the milk from the air and by insects (Pandey & Voskuil, 2011). The optimal shed should protect against rain, wind and excessive heat but at the same time have sufficient light and ventilation. The floor should be made of concrete so that it can be cleaned easily of urine, faeces, mud and feed residues. The floor should be hosed with clean water before and after each milking and the shed should therefore be located on a higher place to provide proper drainage. If these conditions cannot be met it is preferable to milk in the pasture or in a clean open field instead of milking in a dirty and/or muddy shed. The milk should be protected from contamination by foreign material such as dust, soil, dirt, manure, urine and flies (FAO, 1989). The milking area should be easy to clean and have waste handling facilities.

The cow itself is a significant source of both dirt/contamination and mastitis bacteria (Pandey & Voskuil, 2011). The skin provides a large surface for possible contamination and when urine, manure, uterine discharge, dirt, hairs and dust drop from the skin and udder, millions of bacteria can be passed into the milk. The number of mastitis pathogens on the teat end at milking has a clear impact on the incidence of IMI (Pankey, 1989). Therefore pre-milking sanitation aims to reduce the microbial population in order to decrease the risk of both contamination and spread of mastitis (Pankey, 1989). Elmoslemany et al (2009) found a strong association between udder hygiene and bacterial counts in bulk tank milk and Schreiner & Ruegg (2003) found a positive correlation between frequency of dirty udders and new IMIs.

The risk for contamination from the milker is higher in the case of hand milking compared to machine milking (Pandey & Voskuil, 2011). Dirty hands contaminate the teat skin and milk with dirt bacteria. When moving from one cow to another, the milker can transfer pathogenic microorganism to all cows in the herd. The milker should therefore wash the hands with soap and water and dry them with a clean towel before starting milking and handling the milking utensils, and during the milking procedure between each cow (Pandey & Voskuil, 2011).

The teats should be washed and dried before milking and only animals with clean and dried teats should be milked. Drying the udder before starting milking should be done with a single-service cloth or paper towel (FAO, 1989). Using the same cloth for more than one cow only spreads infection from cow to cow. According to Pankey (1989), manual drying of teats is a significant factor in the reduction of total bacteria count, regardless of the udder cleaning procedure.

Washing and wiping the udder and teats contribute to milk ejection. In response to tactile stimulation of the teat either by the calf, the hand or the milking machine, the neuropeptide oxytocin is released from the posterior pituitary, inducing alveolar milk ejection (Bruckmaier, 2005). Between milkings, 80 to 100 % of the milk is stored in the alveolar compartment of the udder and it is therefore important to achieve good milk ejection during milking. The release of oxytocin is necessary for fast and complete milk removal, not only in response to pre-stimulation but also throughout the whole milking. Oxytocin binds to receptors of the neuroepithelial cells that surround the alveoli resulting in contraction, thus moving of the milk to the cisternal space (Bruckmaier, 2005). For all milk to be ejected the milk has to be simultaneously removed from the udder while the cisternal space is limited, therefore ejecting further milk during the course of milking or suckling. Oxytocin needs to be elevated during the entire milking; teat stimulation by calf suckling or hands results in much higher oxytocin levels during udder emptying compared to machine milking (Millogo et al., 2012).

It takes approximately forty seconds to more than two minutes from the start of the tactile teat stimulation until onset of milk ejection, mainly depending on the degree of udder filling since much more myoepithelial contraction is needed to expel milk from a incompletely filled udder than from a more filled one. Complete emptying of the alveolar cisterna is required to maintain synthesis and secretion on a high level during the whole lactation. Milk remaining in the udder is also a favourable medium for microorganism, meaning there is a potentially increased risk of mastitis due to incomplete milking (Tancin & Bruckmaier, 2001). When milking takes place in unfamiliar surroundings there is an inhibition of the release of oxytocin because of increased plasma levels of beta-endorphin and cortisol. To achieve continuous oxytocin release throughout milking resulting in a complete udder emptying, it is important to have an emotionally stress-free environment. Feeding during milking may potentiate the release of oxytocin, and cows that have higher milking related oxytocin levels due to simultaneously milking and feeding also may have lowered cortisol levels (Svennersten-Sjaunja et al., 2004).

If the cow suffers from infectious mastitis the milk already contain harmful pathogenic microorganisms. Therefore, the milker should examine the foremilk for abnormalities (e.g. signs of clinical mastitis) at each milking with a strip cup and milk from diseased cows should be kept separate and disposed safely (FAO, 1989). This fore-stripping should be a standard food safety practice on all farms as it ensures that abnormal milk is diverted from the human food chain (Wagner & Ruegg, 2002). It is also a strong stimulus for milk let down. To detect subclinical mastitis before milking, a good practice is to use the California Mastitis Test (CMT) (Kivaria et al., 2004). CMT is a test whereby estimates of the SCC at herd, cow or quarter level can be made and therefore can give the farmers awareness of the existence of subclinical mastitis among the milking cows.

Milking

The time from the start of teat stimulation until optimal milk ejection is obtained ranges from forty seconds to more than two minutes depending on the degree of udder filling (Bruckmaier, 2005). The time for milk ejection is shortened with higher filling. Thus, there should be a certain time-lapse after starting the udder preparation until the milking should actually start. A longer time-lapse is detrimental since the milk ejection will cease.

A particular pre-milking stimulation commonly applied in herds with *Bos indicus* breeds is restricted calf suckling (Millogo et al., 2012). The natural calf suckling includes pre-stimulation, milk intake and post-stimulation and is considered to be the most optimal tactile teat stimulation for milk let down. Restricted calf suckling means that the calf is used to initiate milk ejection and is allowed to suckle after milking, emptying the last fraction of milk. In systems where calf suckling is practiced it is common to tether the calf beside the cow to stimulate milk-let down throughout milking (Ugarte, 1989; Fröberg et al., 2007), since also visual and auditory stimuli from the calf may activate the milk ejection reflex (Svennersten-Sjaunja et al., 2004). Restricted calf suckling gives a higher milk production and a better udder health (Fröberg et al., 2007 & Thomas et al., 2004). Calf suckling has been shown to reduce the incidence of mastitis, which has been explained by mechanical factors due to suckling, inhibitors in the saliva of the calf and improved udder emptying. However, there are also some predisposing factors for mastitis due to restricted calf suckling, such as increased exposure of the teat canal to bacteria and increased time the teat canal is open. Suckling can also cause dry teat skin.

According to FAO (1989) a correct technique for hand milking is to ensure that the milker's hands are clean and dry and that he/she uses a method that does not cause pain or injury to the animal. The milk should be drawn directly into the bucket when milking (Pandey & Voskuil, 2011). Hand milking is the oldest technique to remove milk from lactating animals and in many parts of the world, where farmers do not have access to electricity or technology for machine milking, milking by hand is common (Millogo et al., 2012). Machine milking is generally considered to be more gentle to the teats and udder than hand milking is but single studies have indicated the opposite, that hand milking causes less damage to teat tissue (Manninen, 1995). Hand milking can be done with different techniques. In the "stripping" technique the milker encircle the teat with his/her forefinger and thumb and then pulls the teat towards the bucket when milk is expressed and often also sliding the fingers along the teat. With the “full hand grip” technique the milker uses all fingers and put pressure on the teat from the base of the teat to the teat end by closing the hand around the teat gradually, without pulling the teat. Milkers that use the “thumb in” milking technique folds the thumb into the palm and presses the teat in the hand between the fingers and the thumb, putting pressure gradually as in the “full hand” technique. According to Millogo et al (2012) the amount of milk removed from the udder depends mainly on the milker and how well the milking technique works for the individual milker and they did not observe any effect on teat treatment by milking technique. Kivaria et al (2004) stated that a stripping (or a pull down) milking technique causes microscopic trauma of the teat epithelium, which might have been a cause of elevated SCC for small-scale farmers in Kenya. But Karimuribo et al (2008) found that the stripping technique of hand milking was associated with a significantly lower prevalence of CMT-positive quarters. The stripping technique of hand milking seems to be the most common. Some milkers use lubricants, which may be any kind of ointment, to provide better glide during hand milking. Lubricants might have a softening effect of the teats, but there is a risk of contamination from the lubricants and therefore an increased risk of infection. Teat lubricants should be used appropriately and according to national recommendations and regulations (FAO, 2011).

The milker itself has an effect on milk yield (Millogo et al., 2012) and the choice of persons for milking in hand-milking systems is as important as the choice of machine milking equipment in machine milking systems. The dairy labourers may be one of the most substantial risk factors for subclinical mastitis (Kivaria et al, 2004).

Post-milking routines

The post-milking routines are significant for udder health. Since the teat canal is open after milking for at least half an hour the cow should be prevented from lying down during this time. This can be achieved by feeding the cows after milking which is an important measure to minimize udder infections from environmental pathogens. The area where the cow can lie down and rest should be as clean as possible. After milking, teat dipping with a gentle antiseptic solution is a good udder health measure (Pandey & Voskuil, 2011). According to Murphy and Boor (2000) teat dipping after milking can reduce the numbers of bacteria on the teat skin, help to protect the stressed teat orifice after milking and also prevent bacteria from entering the teat canal. Teat dipping immediately after milking with a proper teat-dip solution has been shown to reduce new mastitis infection rate by 50 % (Chamberlain et al 1989). Teat dipping should be practiced according to national recommendations and regulations (FAO, 2011).

The equipment that is used must be suitable for effective cleaning and sanitization (Pandey & Voskuil, 2011). Buckets should be non-corrosive and easy to clean and disinfect (FAO, 2011). Thus materials that are non-absorbent and non-corrosive with smooth surfaces, minimal joints and free from dents are recommended (FAO, 2011; Pandey & Voskuil, 2011).

Stainless steel is expensive but by far the best option. Plastic equipment will become scratched on the surface after some time, be nearly impossible to clean and is therefore not advisable (Pandey & Voskuil, 2011).

Any milk residues on the utensils will allow microorganisms to grow rapidly and therefore the equipment used during milking should be cleaned immediately after use (Pandey & Voskuil, 2011). A clean brush with good bristles, hot water and a detergent should be used during washing. The equipment should be rinsed with clean water and dried immediately, preferable upside down and in the sun.

AIMS OF THE STUDY

The management routines used by small dairy farmers and the possible association between management routines and udder health status have not been studied in Zambia. The main purpose of this study was therefore to investigate the management routines of small-scale dairy farmers in Mapepe, Batoka and Choma districts in Zambia with focus on the milking.

The aim was also to investigate if any association could be observed between the management practices and udder health status (subclinical mastitis) of the herds as indicated by milk SCC and the frequency of bacteriologically positive udder quarter milk samples.

The overall aim was to help the farmers to improve their milk production through proper advice on improved management routines and udder health in the different herds. To get a picture of the households included in the study, some information was additionally collected about the family situation.

MATERIAL AND METHODS

Study area and population

The study was conducted in three GART dairy cooperatives in Mapepe, Batoka and Choma areas. Mapepe is located in Kafue district in Lusaka Province, while Batoka and Choma are in the Southern province of Zambia. The altitude in these areas is around 1000 meters above sea level, and the annual rainfall is 800-1000 mm. The study was conducted from mid-September to mid-October, i.e. during the hottest and driest period. The temperature was around 35ºC during the daytime and around 20ºC at night. The study population consisted of milking cows belonging to smallholder farmers connected to a GART-provided milk collection centre each in Mapepe, Batoka and Choma. All cows were milked by hand. The areas represent slightly different dairy farming, for instance Mapepe generally stands for exotic crossbreeds and rather small herd size and Batoka for local crosses and large herds.

The study was run in parallel with another study on the udder health situation in general in the same cow population (Eriksson, 2013). Thereby, some data and results from sample analyses could be utilized in both studies.

Study design

Selection of farms and cows

In total, the SCC in herd milk delivered from 50 farmers was tested at the collection centres. Since the purpose of the study was to investigate the influence of milking and management routines on udder health, it was desirable to include farms with different udder health status. Based on the herd SCC, 26 herds of the 50 tested were selected of which half had a SCC below 400 x 10^3/ml and half of them above 400 x 10^3/ml. The SCC level of 400 x 10^3/ml was chosen based on a limit set for milk aimed for human consumption, applied in, for instance, New Zealand and the European Union (Regulation No 853/2004).

In the final selection of farms for the study, those with very remote locations were excluded to make it possible to keep to the time schedule. The intention was to conduct the study on farms with approximately the same herd size. However, this was not possible to fulfil 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).

The farms were visited once between mid-September and mid-October 2012, mostly during the morning milking for data collection and milk sampling. In Batoka all farms were visited in the morning while five farms in Mapepe and three in Choma were visited in the afternoon. The number of farms and cows studied was based on similar previous investigations (Abrahamsén, 2012; Östensson et al., 2012).

The aim was to perform a clinical examination of the udder and to take milk samples from 6 cows at each farm. If there were ≤ 6 lactating cows at a farm, all cows were included. In farms with > 6 lactating cows, samples were taken from the first 6 cows to be milked. This simplified selection rule was used mainly due to logistic and linguistic difficulties. Cows that showed signs of clinical mastitis were excluded and, if additional cows were available, another cow was selected. If a cow with clinical mastitis came from a herd with ≤ 6 lactating cows, only the affected udder quarter/quarters was/were excluded and samples were taken from the remaining quarters to obtain as equal a number of samples from each farm as possible.

Observations and interviews

In addition to examination of cows and milk, data were collected through observations during the farm visit and interviews with the farmers. Two different protocols were used, one for recording cow and management data and one for collecting some data of the family. These protocols are attached as appendices.

Milking practices and hygiene were observed during the entire milking. Milk yield per cow was measured at the milking from which the daily milk yield/cow was calculated (Millogo et al., 2003; Chládek et al., 2011). The farmers were asked about milking and management factors that were not observable. Also data for individual cows including breed and milk yield were collected.

Moreover, the farmers were interviewed about some family characteristics, experience of dairy farming, the family's milk consumption habits, employees if any, use of antibiotics, and daily herd milk yield in different seasons. They were also asked to score the importance of milk production (earnings from milk) for the household. At some farms, where the farmers did not speak English, the driver was used as interpreter.

Sampling of milk and treatment of samples

Before milking of each cow was started, the udder quarters were inspected and palpated and the foremilk was checked in a control vial and then discarded. If there were signs of clinical mastitis either from the udder or the milk, the quarter was excluded from the study. Quarter milk samples were taken aseptically into sterile test tubes for bacteriological culturing and for analysis of SCC. Additionally, a sample was taken from the udder bulk (composite) milk after careful stirring for analysing SCC at cow level (cow SCC). The samples were kept in a cooling box during transport to the laboratory, where SCC was measured. Until the time for bacteriological culturing all the quarter milk samples were kept frozen. In the bacteriological laboratory, the samples were thawed at room temperature and before bacteriological culturing each sample was turned several times to assure that the milk was properly mixed.

Evaluation of the udder health status

Two different threshold values were used to evaluate the udder health status. Milk with SCC $< 100 \times 10^3$/ml was considered to emanate from fully healthy udder quarters. Considering that the SCC may to some extent be affected by physiological factors, a slightly higher SCC has been found more relevant to use in practice for diagnosing subclinical mastitis (for review, see Schukken et al., 2003). Thus, a milk SCC >200 x 10^3/ml was used to diagnose subclinical mastitis in this study.

Growth of one or more mastitis pathogens after bacterial culturing of the milk sample was set as the criteria for IMI.

Analyses

SCC

A portable cell counter from DeLaval, DCC (DeLaval, Tumba, Sweden), was used to measure SCC. The equipment was handled according to the manufacturer's instructions. A disposable cassette where the milk is mixed with a DNA-specific fluorescent dye, propidium iodide, is used for each milk sample. The cassette is introduced into the instrument and each cell is counted based on the fluorescent emission from the nucleus. If a result was questionable (i.e. zero), a new cassette was used and the milk sample was tested again.

Culturing and typing of bacteria

The bacteriological culturing, biochemical testing and preliminary bacteriological diagnosis were done in a laboratory at the School of Veterinary Medicine at UNZA in Zambia (Eriksson, 2013), according to the routines used at the National Veterinary Institute (SVA), Uppsala, Sweden. Blood agar plates (5 % bovine blood) were used for culturing all milk samples, and were incubated at 37 °C for 48 hours. For all genera, a sample was classified as bacteriologically positive when at least three CFUs were growing, except for *S. aureus* where growth of a single CFU counted as positive (Ericsson Unnerstad *et al.*, 2009).

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. There, typing of the bacteria was done by the personnel at the Mastitis Laboratory, using SVA's standard accredited methods (Ericsson Unnerstad *et al.,* 2009).

Statistical analysis

The management routines among the farms included in the study were shown to be quite similar, with only slight differences concerning a few factors. There was a high covariance and dependence between different management factors and between such factors and e.g. breed. Due to this and the small size of the data set, it was not possible to statistically separate the influence of different management factors. Thus, only descriptive statistics are presented.

RESULTS

In addition to the common presentation of the results, calculations per region and breed are also shown.

Farms, cows and breeds

A total of 111 cows distributed on 26 farms were finally included in the study. Of the total udder quarters of the cows, 432 quarters were investigated. Eleven quarters were dry and 1 quarter was excluded because it had CM. The geographical distribution of farms, cows and quarters are shown in Table 1. Cow composite milk samples could only be obtained from 76 of the cows. The missing samples were due to that the milkers did not empty the bucket between each cow they milked.

Table 1. Distribution of farms, cows, quarter samples and types of cross breed among the three areas (some data in common with Eriksson, 2013)

Area	Farms	Cows (local/exotic)	Udder quarters (local/exotic)
Mapepe	11	37 (1/36)	144 (4/140)
Batoka	9	45 (45/0)	177 (177/0)
Choma	6	29 (9/20)	111 (34/77)
Total	26	111 (55/56)	432 (215/217)

The cows represented two types of cross *breeds*: crosses of indigenous breeds (*Bos indicus*) only ("local cross breeds"), and indigenous cross breeds crossed with exotic breeds (*Bos taurus*) such as Jersey, Holstein and Friesian ("exotic cross breeds"). Table 1 shows the geographical distribution of the different types of cross breeds. On a farm basis, all farms visited in Mapepe used exotic cross breeds except for a single farm with one single Boran cow. In Batoka all farms that were studied used local crosses. In Choma there were 3 farms with exotic crosses, 2 with local crosses and 1 farm with cows of both local and exotic cross breeds. There were no cows of pure breed in the study.

Data on herds included in the study and their distribution in the low and high SCC group, respectively, are shown in Table 2. It was not possible to get reliable information on the total number of milking cows in the herd and the entire herd could not be observed at the visit because they were out on the savannah. The farmers were more certain about their total number of animals. Therefore the number of lactating cows at the time of visit and the total number of heads in the herd is given to depict the herd size and structure. The mean/median number of lactating cows was 7.0/7.5 and the mean/median overall herd size including young stock and calves was 29.7/20.0 heads.

Table 2. Data on the herds included in the study (in common with report by Eriksson, 2013)

Herd SCC x 10^3/ml	≤ 400 (n=12)	> 400 (n=14)	Total (n=26)
Local/exotic cross breeds (%)	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 (No.)	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)

Data per farm

Raw data from the questionnaire and farm observations, herd SCC analyses and bacteriological examination for each farm are given in Table 3. In two herds there was no growth of udder pathogens in any of the quarter milk samples. At species level, the presentation of the bacteriological results focusses on the most frequently found pathogens, CNS and *S. aureus* (for overall bacteriological results, see Eriksson, 2013).

Some management routines were used by **no or all** farmers and are not included in the table:

- None of the milkers washed their hands before milking.
- No farmer practiced fore-stripping to check the milk for abnormalities.
- All of the milkers used a stripping type of hand-milking technique.
- No farmer used teat dipping after milking.
- The number of milkers on each farm varied between 1 and 3, and milking was always done by the same person/persons.
- All of the cows were in a calm mood during milking.
- The cow's hoof status was generally good.
- The cows were generally very clean (only single cows were slightly dirty).
- No farmer had treated any of the cows with antibiotics (or any other drug) in the last ten days when the interviews were conducted. No farmer knew what antibiotics mean.
- There were a lot of flies around the cows on each farm.
- Only a few cows belonging to 2 farms were observed to be mildly infested with ticks.

The body condition of the cows was normal in 9 herds (7 in Mapepe and 2 in Choma), thin in 12 herds (4 in Mapepe, 4 in Batoka and 4 in Choma) and very thin in 5 herds, all belonging to the Batoka area.

A majority of the farms (20) used milking equipment, i.e. buckets, of stainless steel. In Batoka, 6 farms used plastic buckets. On all farms the milking equipment was washed with cold water and 6 farmers (5 in Mapepe and 1 in Batoka) used detergent in addition. Everyone turned the utensils upside down to dry after washing.

Milk somatic cell count

Overall, the average herd, cow and quarter milk SCC in the study was 555 x 10^3/ml, 522 x 10^3/ml and 233 x 10^3/ml.

Calculated by ***geographical area*** the average herd SCC was 691 x 10^3/ml, 357 x 10^3/ml and 602 x 10^3/ml in Mapepe, Batoka and Choma, respectively. Mean cow and quarter SCC per area are shown in Figure 1.

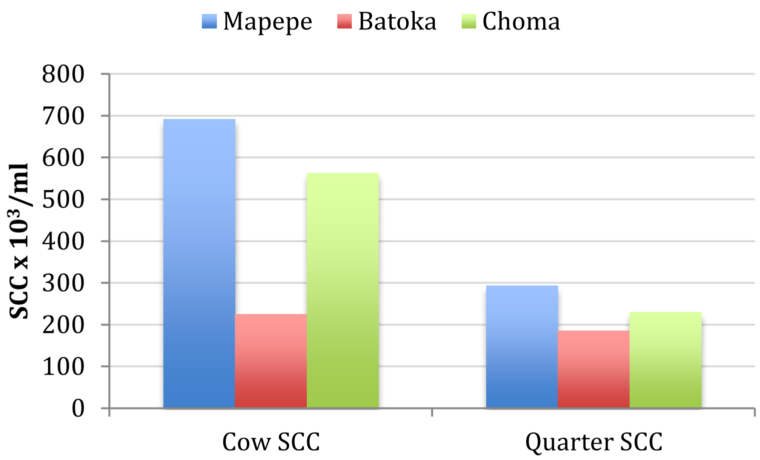

Figure 1. SCC at cow (composite milk) and udder quarter level in the different geographical areas. Data are presented as means.

The SCC was also calculated for ***each type of cross breed***. The average herd SCC was 342 x 10^3/ml and 690 x 10^3/ml in herds with local and exotic crosses, respectively. The average cow and quarter SCC, and daily yield per cow and breed are given in Figure 2.

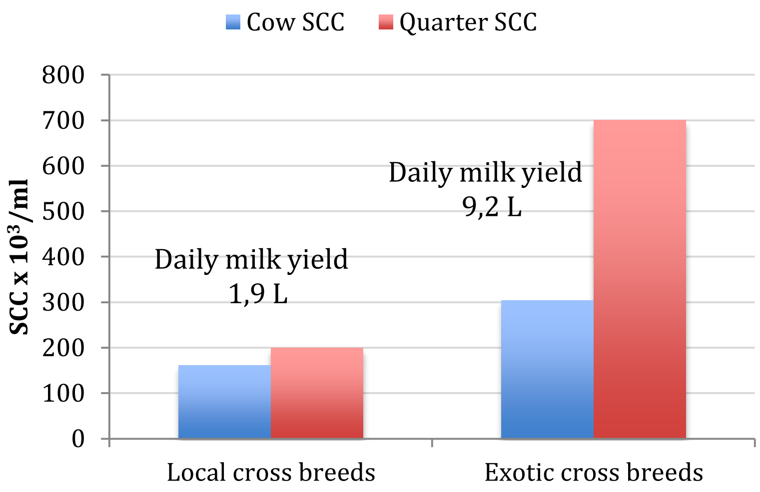

Figure 2. SCC at cow (composite milk) and udder quarter level and calculated daily milk yield for local and exotic cross breeds, respectively. Data are presented as means.

Table 3. Data on milking routines, herd SCC and bacteriological findings per farm and geographical area

** This farm used restricted calf suckling when milking the local cross bred cows, but not when milking the exotic bred cows*

Management routine	Farms Mapepe											Farms Batoka									Farms Choma					
	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	B1	B2	B3	B4	B5	B6	B7	B8	B9	C1	C2	C3	C4	C5	C6
Milkers																										
Family													X	X	X	X	X	X	X	X		X		X		
Employers	X	X	X	X	X	X	X	X	X	X	X	X									X		X		X	X
Milking environment																										
Shelter	X	X			X	X	X		X	X											X		X			
Outdoors			X	X				X			X			X								X		X	X	X
Outdoors – paddock												X	X		X	X	X	X	X	X						X
Milkings per day																										
Once				X										X	X			X	X	X				X	X	
Twice	X	X	X		X	X	X	X	X	X	X	X	X			X	X				X	X	X			X
Udder preparation																										
Wash and wipe	X	X					X							X								X				
Only wash			X	X	X	X		X	X	X	X	X									X		X	X	X	X
No preparation													X		X	X	X	X	X	X						
Restricted calf suckling													X	X	X	X	X	X	X	X				X	X	X*
Use of lubricant cream		X		X	X	X	X	X	X	X	X										X		X			
Feeding during milking	X	X	X	X	X	X	X	X	X	X	X	X	X								X	X	X		X	
Water access																										
- **Ad libitum**	X					X	X			X																
- **Restricted**		X	X	X	X			X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Tot No. of milking cows	4	4	7	2	3	7	3	5	2	5	3	8	20	5	11	8	15	6	9	10	15	4	7	2	5	13
Herd SCC x10³/ml	638	673	256	1329	329	544	846	726	602	540	1531	555	326	68	38	590	61	1361	200	15	1082	788	1530	83	80	51
Tot No. of udder quarters [local/exotic]	16 (0/16)	16 (0/16)	8 (0/8)	4 (0/4)	10 (0/10)	20 (0/20)	12 (0/12)	19 (4/15)	8 (0/8)	20 (0/20)	11 (0/11)	23 (23/0)	20 (20/0)	8 (8/0)	24 (24/0)	8 (8/0)	24 (24/0)	22 (22/0)	24 24/0)	24 (24/0)	24 (0/24)	16 (0/16)	22 (0/22)	8 (8/0)	18 (18/0)	23 (8/15)
Quarters with CNS/S. aureus [No.]	2/1	5/3	3/1	3/1	2/2	5/1	4/3	6/2	0/1	9/3	3/3	5/10	0/1	2/0	0/0	0/0	2/0	4/0	4/0	0/0	1/5	1/1	12/1	0/0	0/0	3/2
Quarters with CNS or S. aureus [%]	18.8	50.0	50.0	100	40.0	30.0	58.3	42.1	12.5	60.0	54.5	65.2	5.0	25.0	0	0	8.3	18.2	16.7	0	25.0	12.5	59.1	0	0	21,7

Prevalence of subclinical mastitis

Overall, a herd SCC > 400 x 10^3/ml, suggesting a herd mastitis problem, was observed in 53.8 % of the farms (Figure 3). Cow and quarter SCC > 200 x 10^3/ml, indicating SCM, was seen in 48.7 % of the cow composite milk samples and 26.9 % of the quarter samples (Figure 3).

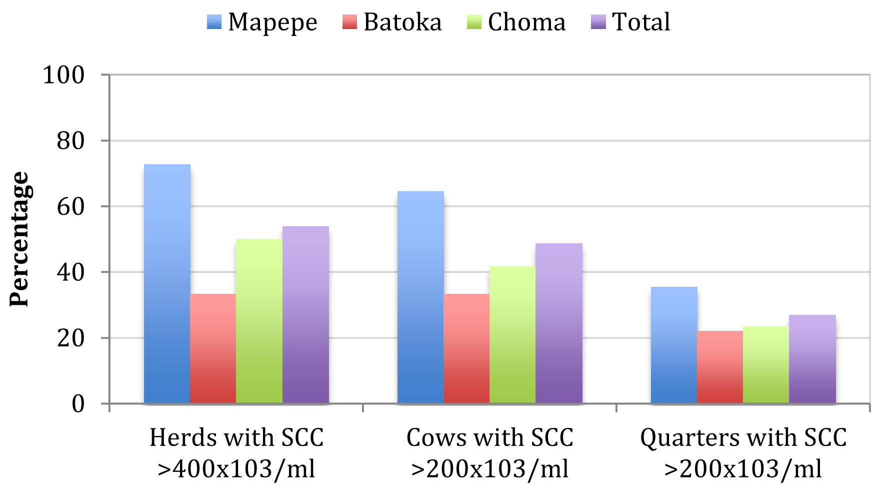

Figure 3. Proportion of herds with herd SCC > 400 x 10^3/ml, and cows (composite milk) and udder quarters with SCC > 200 x 10^3/ml. Data are presented for each geographical area and in total.

The SCM prevalence at udder quarter level *related to herd size* was 33.7 %, 34.1 % and 14.9 % in herds with 2-4, 5-8 and 9-20 milking cows, respectively.

Geographical area

The frequency of herds with increased herd SCC and the prevalence of SCM at cow and quarter level for each geographical area are shown in Figure 3.

Breed

Prevalence of SCM was calculated for each type of cross breed. Calculated on all cows, those with a ***cow*** composite milk SCC < 100 x 10^3/ml and SCC < 200 x 10^3/ml, respectively, constituted 40.8 % and 52.3 %. The corresponding values for local crosses were 59.2 % and 70.3 % and for exotic crosses 30.6 % and 40.8 %. This gives an overall prevalence of SCM at *cow-level* of 48.7 % according to the threshold SCC value of > 200 x 10^3/ml which was used in this study to diagnose SCM. For local crosses the prevalence of SCM at cow level was 29.6 % and for exotic crosses 59.2 % (Figure 4).

At *quarter* level, calculated for all cows, 65.5 % and 73.1 % of the quarters had SCC < 100 x 10^3/ml and < 200 x 10^3/ml, respectively. The corresponding figures for local crosses were 74.4 % and 80.9 %, and for exotic crosses 56.7 % and 65.4 %. Thus, the overall prevalence of SCM (> 200 000 cells/ml) at *quarter-level* was 26.9 % and was 19.1 % and 34.6 %, for local and exotic crosses, respectively (Figure 4).

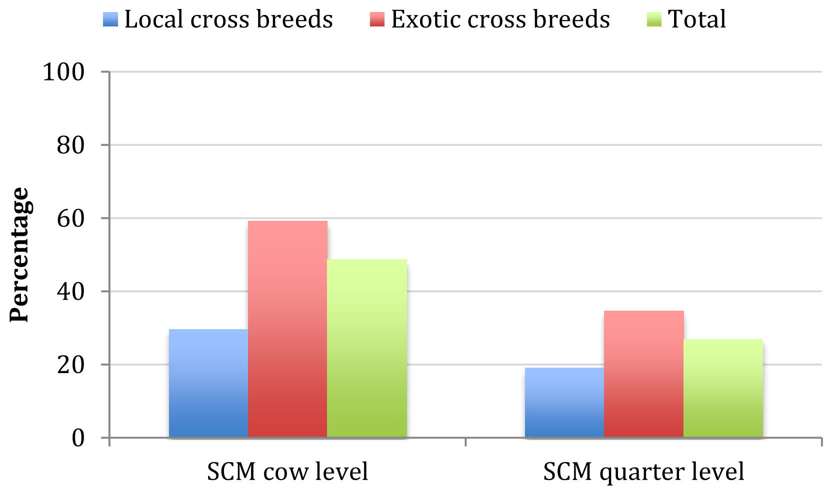

Figure 4. Prevalence of subclinical mastitis at cow (composite milk) and quarter level for the two types of cross breeds and for all cows (total). A milk SCC >200 000/ml was used to diagnose subclinical mastitis. Data are presented as means.

Of the exotic cross bred cows, 23.5 % were found in the group of cows with notably increased cow composite milk SCC of > 1000 x 10^3/ml compared to only 4 % (one cow) of the local crosses. Among cows with a low cow SCC of < 100 x 10^3/ml, 56 % were of local cross breeds and 33.3 % of exotic crosses.

Bacteriological results and prevalence of intramammary infections

Of the total 432 quarters included in the study, 428 quarters were bacteriologically examined. Out of these, 31.5 % were bacteriologically positive, 66.6 % were negative and 1.9 % were contaminated. Of the bacteriologically positive quarters, 9 (6.2 %) had double diagnoses resulting in 144 bacteriological diagnoses in total (81 CNS, 41 *S. aureus*, 7 streptococcus species, 6 Corynebacterium bovis and 9 other pathogens of 5 different species). Thus, CNS and *S. aureus* constituted an absolute majority of the bacterial isolates while other pathogens occurred in very low numbers, which is why the presentation of results will focus on these two diagnoses. Hence, the total prevalence of IMI at ***quarter level*** **(**Figure 5, total) was 31.5 % and that of CNS and *S. aureus*, respectively, was 17.1 % and 8.6 %.

The prevalence of IMI at ***cow level*** (infection in at least one udder quarter) with any pathogen was 57.7 % (64 cows), with 38.7 % and 27.0 % being specifically for CNS and *S. aureus*, respectively.

Geographical area

The overall prevalences of IMI and specifically CNS and *S. aureus*, at udder quarter and cow level, both for the whole study and split up between the different geographical areas are shown in Figure 5 and 6, respectively.

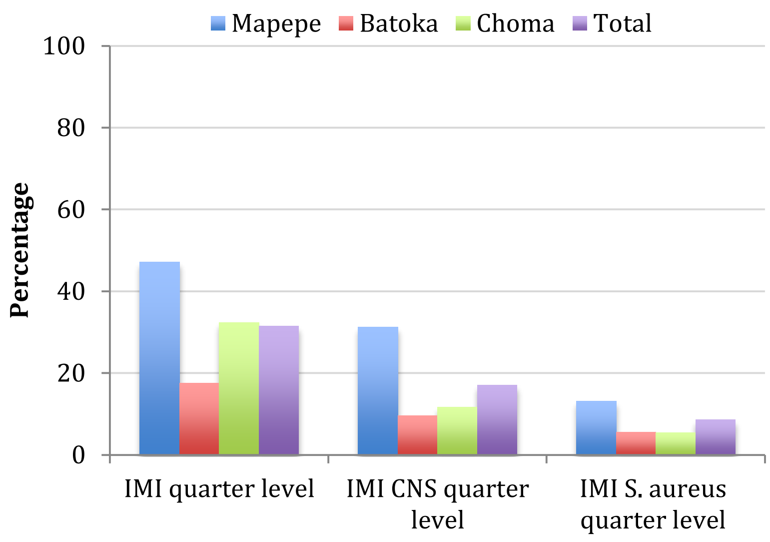

Figure 5. Prevalence of IMI at ***quarter level*** *for all pathogens and the whole study ("total"), and specified for CNS and S. aureus and each geographical area. Data are presented as means.*

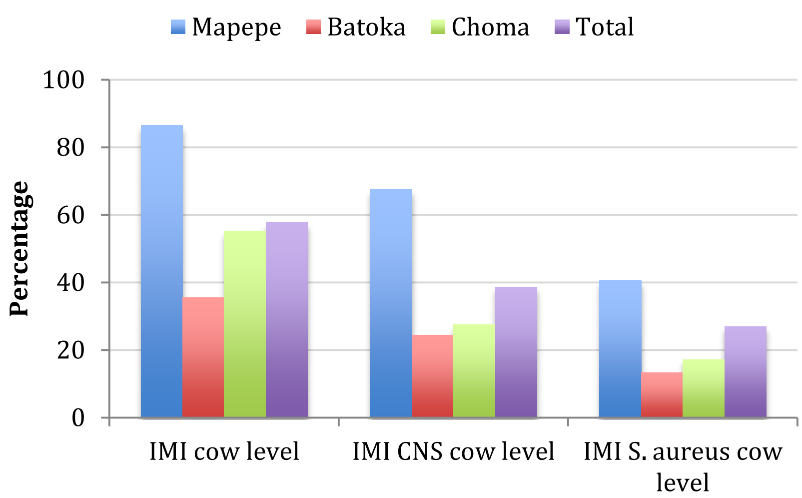

Figure 6. Prevalence of IMI at ***cow level*** *for all pathogens and the whole study ("total"), and specified for CNS and S. aureus, and each geographical area. Data are presented as means.*

Breed

Results from the bacteriological examination of quarter milk samples presented for each type of cross breeds are shown in Figure 7a and 7b. In the category "others" it should be noted that *Burkholderia cepacia* was isolated from all udder quarters of one cow of local cross breeds.

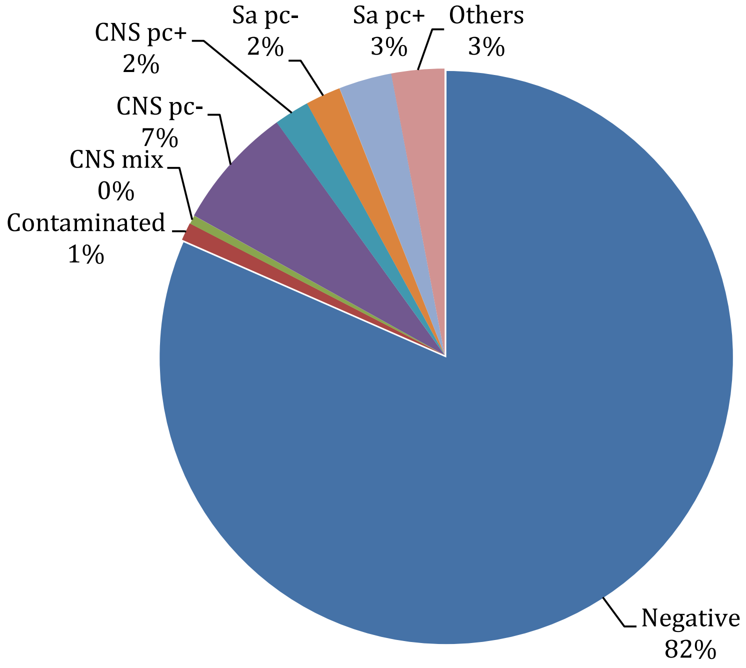

Figure 7a. Bacteriological diagnoses at quarter level for local (n=213) crossbred cows

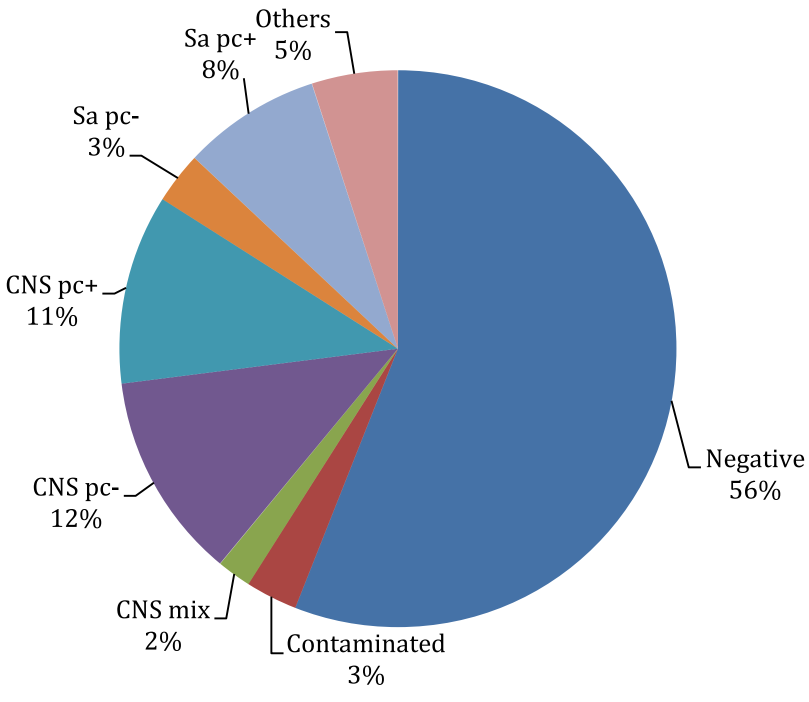

Figure 7b. Bacteriological diagnoses at quarter level for exotic (n=215) crossbred cows

There were in total 213 udder quarter samples with bacteriological results from local and 215 from exotic crossbreeds. In the local and exotic crossbreeds, respectively, 36 (16.7 %) and 90 (41.5 %) of the quarter milk samples were bacteriologically positive (i.e. the total prevalence of IMI at ***quarter basis***). Of the isolated bacteria from local and exotic crosses, respectively, 52.8 % and 62.2 % were CNS, and 30.6 % and 26.7 % were *Staphylococcus aureus.*

Of the local and exotic crossbreeds, 64.7 % and 23.3 % of the cows, respectively, were bacteriologically negative in all udder quarters. Thus, 35.3 % of local crosses and 76.7 % of the exotic had IMI in at least 1 quarter (prevalence of IMI at ***cow level***).

The ***prevalence of IMI***, in total and specified for CNS and *S. aureus*, at quarter and cow level for the two types of crossbreeds is shown in Figure 8 and 9.

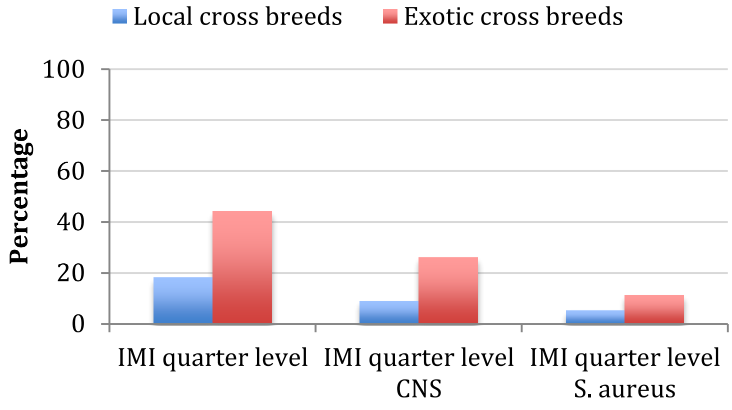

*Figure 8. Prevalence of IMI at **quarter level** for all pathogens and specified for CNS and S. aureus in the two types of cross breeds. Data are presented as means.*

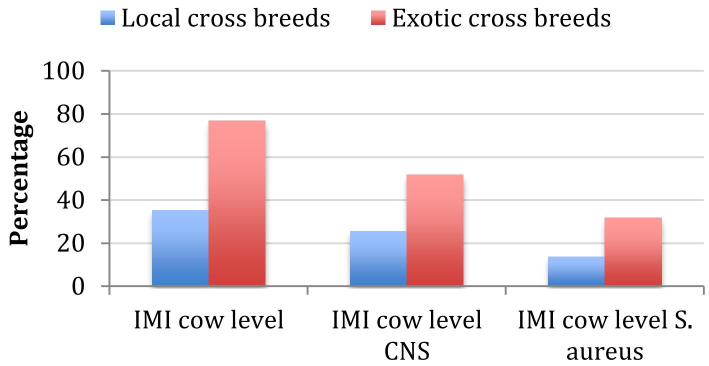

*Figure 9. Prevalence of IMI at **cow level** for all pathogens and specified for CNS and S. aureus in the two types of cross breeds. Data are presented as means.*

Management routines, breeds and udder health

Table 4 summarizes the number and frequency of farms, cows and breeds exposed to different management routines, and the udder health status (SCC data and bacteriological findings). Management routines that were used by none or all farmers are not included. Some management routines were associated with low and others with high SCC and prevalence of udder infections. Furthermore, some routines were mainly used either in local cross breeds or in exotic cross breeds. The results are further commented in the discussion.

Family data

Results from the interviews regarding the household and dairy farming are shown in figure 10 for each geographical area and in total. The average age of the farmers was 50 years (10 A), highest in Mapepe and lowest in Batoka. The average experience of dairy farming was in general short, only a few years.

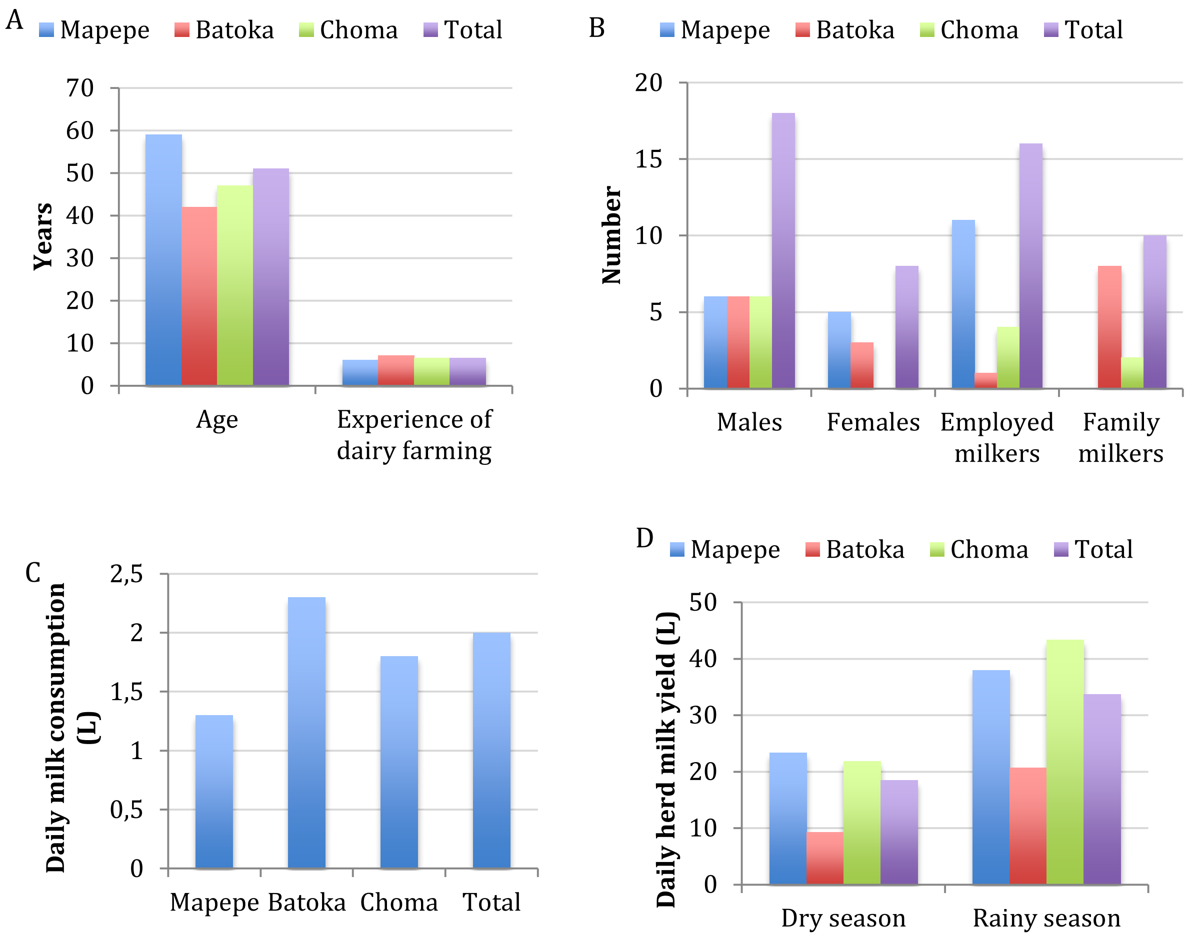

Figure 10. A) Age of the household head and experience of dairy farming (means). B) Number of farms with male and female household heads, employed milkers and family milkers. In Choma the number of farms with female heads and in Mapepe the number of farms with family milkers was zero. C) Daily milk consumption in the household (means). D) Total daily herd milk yield during the dry and rainy season (means).

A majority of the household heads were men in all regions (10 B). Female heads were most common in Mapepe. Of the total number of female household heads, 5 were widows. In Mapepe it was most common to have employed milkers which was rare in Batoka (10 B).

Table 4. Distribution of farms, cows, breeds, SCC, bacteriologically positive quarters and quarters with growth of Coagulase Negative Staphylococci (CNS) and Staphylococcus aureus, among different management routines investigated in the study. No. of bacteriologically positive quarters include all pathogens.

**"Outdoors" means milking outside in a particular milking place; "outdoors/paddock" means that the cows were milked outdoors in the paddock where they were kept regularly.*

MANAGEMENT ROUTINE	No. of farms (% of all farms) n=26	No. of cows (% of all cows) n=111	No. of cows local breeds n=55	No. of cows exotic breeds n=56	MEAN SCC x 10^3/ML			BACTERIOLOGICAL RESULTS			
					Herd	Cow	Quarter	No. of quarters n=428	No. of bact pos quarters (%)	No. of CNS pos quarters (local/exotic)	No. of *S. aureus* pos quarters (local/exotic)
MILKERS											
Family	10 (38.5)	45 (40.5)	41	4	353	216	214	175	18 (10.3)	14 (13/1)	2 (1/1)
Employee	16 (61.5)	66 (59.5)	14	52	681	691	425	253	108 (42.7)	61 (6/55)	33 (10/23)
MILKING PLACE											
*Outdoors	9 (34.6)	30 (27.0)	12	18	546	448	171	115	38 (33.0)	21 (4/17)	9 (1/8)
* Outdoors/paddock	9 (34.6)	43 (38.7)	43	0	393	234	186	167	25 (15.0)	15 (15/0)	10 (10/0)
In shelter	8 (30.8)	38 (34.2)	0	38	708	757	333	146	63 (43.2)	39 (0/39)	16 (0/16)
MILKINGS/DAY											
Once	8 (30.8)	34 (30.6)	33	1	397	161	126	134	20 (14.9)	14 (11/3)	1 (0/1)
Twice	18 (69.2)	77 (69.4)	22	55	625	626	281	294	106 (36.1)	61 (8/53)	34 (11/23)
UDDER PREPARATION											
Wash and dry	5 (19.2)	17 (15.3)	2	15	554	421	256	66	26 (39.4)	16 (3/13)	7 (0/7)
Wash	14 (53.8)	57 (51.4)	16	41	657	810	306	216	88 (40.7)	48 (6/42)	27 (10/17)
No prep	7 (26.9)	37 (33.3)	37	0	348	146	108	146	12 (8.2)	11 (11/0)	1 (1/0)
RESTRICTED CALF SUCKLING											
Yes	10 (38.5)	46 (41.4)	46	0	282	246	168	179	19 (10.6)	13 (13/0)	1 (1/0)
No	16 (61.5)	65 (58.6)	9	56	726	692	304	249	107 (43.0)	62 (6/56)	34 (10/24)
LUBRICANT											
Yes	11 (42.3)	43 (38.7)	1	42	885	814	325	165	72 (43.6)	49 (1/48)	20 (1/19)
No	15 (57.7)	68 (61.2)	54	14	313	244	175	263	54 (20.5)	26 (18/8)	15 (10/5)
FEEDING AT MILKING											
Yes	16 (61.5)	64 (57.7)	12	52	727	680	319	244	106 (43.4)	59 (5/54)	32 (10/22)
No	10 (38.5)	47 (42.3)	43	4	279	191	117	184	20 (10.9)	16 (14/2)	3 (1/2)
WATER ACCESS											
Ad libitum	4 (15.4)	17 (15.3)	0	17	539	743	254	67	30 (44.8)	22 (0/22)	8 (0/8)
Restricted	22 (84.6)	94 (84.7)	55	39	558	472	228	361	96 (26.6)	53 (19/34)	27 (11/16)

All farmers that were interviewed said that milk production was a very important source of income for the family and was the only income for most of them. The farmers were asked about how much they earned from milk each month but could not answer in most cases; therefore such information on this cannot be included in this paper.

In figure 10 C the mean milk consumption per family in the different areas is shown. All families except one boiled the milk before consumption. In a majority (19) of the families they made sour milk. Fig 10 D shows the average daily herd milk production in the dry (range 4-50 L) and rainy (range 9-95 L) season, respectively, based on information from the farmers.

The farmers estimated the average daily milk yield ***per cow*** to 3.6 L (range 0.4-10). This is less than the calculated yield based on actual measurement at the visit, 5.6 (range 0.8-20.8) L.

DISCUSSION & CONCLUSIONS

Udder health related to breeds

The results from this study with a prevalence of subclinical mastitis at cow and quarter level of 48.7 % and 26.9 %, respectively, and a prevalence of IMI of 57.7 % and 31.5 % indicates a good general udder health situation in this region in Zambia compared to other studies of small-scale dairy farming under similar conditions in other countries (Gianneechini *et al.*, 2002; Pitkälä *et al.*, 2004; Karimuribo *et al.*, 2008; Mdegela *et al.*, 2009; Schwarz *et al.*, 2010, Östensson *et al.*, 2012).

Field studies on udder health are difficult to compare in detail since they have often been performed with some differences regarding sample material, methods etc. In the studies referred to, 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. Thus, their results regarding SCM ought to be comparable with the current study although the latter applied the lower, more rigorous limit. The strict limit and the high proportion of udder quarter with low SCC (SCC < 100 x 10^3/ml) further strengthen the conclusion that the udder health in this region of Zambia is encouraging. For details about the udder health situation, at large, please see Eriksson 2013.

The most commonly isolated pathogens were CNS and *S. aureus*. This is in agreement with results from other field studies in Niger, Burkina Faso, Tanzania, Ethiopia and Mali (Harouna et al. 2009; Sidibé et al. 2004; Kivaria et al. 2004 & Mdegela et al. 2009; Almaw et al. 2008; Bonfoh et al. 2003). The CNS are considered less virulent than *S. aureus* but the group represent several species with different pathogenicity and some might cause strong mastitis reactions. The CNS are predominantly found on the teat skin and apex, hence post-milking teat dipping may help in reducing the prevalence in a herd. *S. aureus* is a contagious and major mastitis pathogen where the main source of infection is other infected udders. It is mainly transmitted from cow to cow during milking or in hand milked cows often by the milkers hand. Post-milking teat disinfectants contribute to reducing *S. aureus* mastitis and it would be of great value if the farmers in this study could introduce this practice.

Overall, the results show that udder health status was clearly associated with breed. The local crossbreeds had in general lower average SCC both at cow and quarter level and lower prevalence of SCM than the exotic crosses. Moreover, a clearly lower prevalence of IMI was found among the local breeds (at quarter and cow level 16.7 % and 35.3 %) compared to the exotic (quarter and cow level 41.5 % and 76.7 %), which is logical since udder infection is the major cause of high milk SCC. Similar differences were seen in the prevalence for the specified bacteriological diagnoses, CNS and *S. aureus.*

The comparably better udder health status of local breeds compared to exotic breeds observed in this study is in line with previous findings by Lakew et al. (2009) and Almaw et al. (2008). It has been suggested that this difference should mainly be attributable to milk yield since the zebu cows (*Bos indicus*) are low yielding compared to the exotic breeds (*Bos taurus*), a difference that was observed also in the present study. High-yielding cows are generally considered to be more susceptible to mastitis than low-yielding cows (Radostits et al (2007). This view is based mainly on comparisons among *Bos taurus* breeds where a milk yield considered as low is similar to, or even above, the yield of the exotic crosses in the present study. It is therefore doubtful if this reasoning is appropriate when comparing *Bos indicus* and *Bos Taurus* reared under similar conditions. Moreover, low yield is generally a factor that is associated with high SCC, which contradicts the argument that low yield should be a plausible explanation to the better udder health parameters recorded in *Bos indicus* breeds.

The low prevalence of mastitis observed among *Bos indicus* breeds in several studies is suggested to be attributable to a breed associated genetic mastitis resistance due to favourable characteristics of the immunological defence (for review see Cundiff 1988; Sugimoto et al., 2006; Maryam et al., 2012). However, the background to breed difference in mastitis prevalence is most probably multifactorial and may include uncontrolled factors, such as management. In the present study the local and exotic crossbreeds, respectively, were to a large extent exposed to different management routines.

Generally applied management routines

Several factors and management routines were observed at all or no farms and a possible association between each factor and udder health in the current study can therefore not be assessed. In several aspects cow status and management routines observed were positive from an udder health perspective. At all farms milking was always done by the same person/persons, the cows were generally very clean (which was probably mostly due to the dry season), had good hoof status and were calm during milking. All these factors are known to have a positive effect on udder health (for review see Philpot and Nickerson, 2000a). It was also positive that no farmer had treated any of the cows with antibiotics (any drugs) in the last ten days when the interviews were conducted. The farmers' answers indicated that treatment with drugs was largely very uncommon.

In the milking routines generally applied among the farmers there were also some serious shortcomings, which are known since previously to have a negative impact on udder health and predispose for mastitis. None of the milkers washed their hands before milking and no farm applied proper udder cleaning before milking. Either the udder was washed and not dried at all, or dried with a dirty towel used for all cows and for several days. At 6 farms no cleaning at all, of the udder, was done before milking.

It is known that failure in hand washing and in cleaning of the udder exposes the udder and milk to microbiological contamination (Harouna et al. 2009) and increases the risk of spreading infections. According to Elmoslemany et al. (2010), not drying the teats after washing with water was associated with elevated total count of aerobic bacteria in herd bulk milk. Kivaria et al. (2004) showed a statistically significant association between the use of udder-towel and the occurrence of subclinical mastitis. Elmoslemany et al. (2010) observed higher bacterial counts when the same towel was used for drying multiple cows after washing the udder compared to using a single towel for each cow. By sharing the same cloth between cows the risk of transmission of mastitis pathogens increases and the efficiency of drying the teats is reduced.

No farmer practiced fore-stripping of milk to check for abnormalities or post milking teat dipping. Post-milking teat dipping with disinfectant to reduce the concentration of bacteria on the teat skin has been shown to be a good tool to reduce the IMI with contagious pathogens (Murphy & Boor, 2000; Chamberlain et al 1989) and is generally recommended by IDF and FAO (FAO, 2011).

All milkers practiced a stripping type of hand-milking technique, probably because of tradition. According to Kivaria et al. (2004), this technique may cause microscopic trauma of the teat epithelium. However, related to udder health status, Karimuribo et al (2008) found that the stripping technique was associated with a significantly lower prevalence of CMT-positive quarters than the whole-hand technique. Still, the stripping technique is generally considered to be more harmful than whole-hand milking and it would therefore be better if the milkers used a whole-hand milking technique.

Management, cow and herd factors associated with udder health status

Regarding management routines that were not common to all herds, some were associated with *better udder health status* compared to farms where they were not practiced, in terms of lower prevalence of SCM as well as IMI. *Milking performed by family members*, *milking outdoors*, pre-milking stimulation by *calf suckling*, and *no use of lubricant* during hand milking were each associated with clearly lower SCC at cow and quarter level and a lower prevalence of IMI, particularly with *S. aureus*.

These findings are in line with previous investigations. Thomas et al., (2004) also found that milking performed by family members was associated with better udder health compared with when employed people milked the cows. This might be due to that family members have a greater interest in good animal management than employed milkers and that milkers moving from herd to herd might contribute to spreading udder infections.

Milking outdoors is indicated to be associated with better udder health than milking indoors, under primitive conditions (Pandey & Voskuil, 2011) because it usually provides a cleaner milking environment.

Restricted calf suckling has been found associated with improved udder health compared to cows not exposed to calf suckling (for review see Ugarte 1989, Fröberg et al., 2007). This has been explained by better milk let-down and udder emptying, mechanical factors of the suckling process and microbial inhibitors of the saliva of the calf (for review see Ugarte 1989). Calf suckling as pre-milking stimulation is commonly practiced in zebu breeds to achieve proper milk let-down while a satisfactory milk ejection in exotic breeds and crosses can be obtained with good udder preparation and foremilk stripping by hand. The use of lubricants during hand milking is a great source of contamination of the teats and is generally not recommended (FAO, 1989) although lubricant can, per se, be good for the teat skin condition. When observing the milkers who used lubricants in this study, the lubricant and its containers were in most cases very dirty. Thus, such contamination of the teats can be avoided by not using lubricant, meaning a lower risk for mastitis.

Local crossbreeds were markedly overrepresented among the cows exposed to the previously mentioned management routines (milking by family members, milking outdoors, restricted calf suckling, and no use of lubricant). Local crosses constituted 91 % of the cows that were milked by family members, 94 % of those *not* exposed to lubricants and 75 % of those milked outdoors (at a particular milking place or in the paddock). Where restricted calf suckling was practiced 100 % of the cows were of local crossbreeds. Thus, even if these management routines, per se, have a positive impact on udder health, the connection observed in this study could have been due, at least partly to a genetic breed-related relative mastitis resistance of the local cross bred cows.

Some additional management, cow and herd factors were found to be associated with lower figures on the prevalence of SCM and/or IMI (compared to when these conditions were not present), such as *milking once a day, no access to feed during milking, restricted access to water, poor body condition and big herd size* (9-20 milking cows; all big herds consisted of local cross breeds only). This is strongly contradictory to results from numerous of previous studies where these factors have been associated with *impaired* udder health status (for review, see Harmon 1994; Davis et al., 1999; Kivaria et al. 2004; Thomas et al., 2004; Berry et al., 2007; Lam et al. 2011). *These results from the current study are therefore strongly questionable.* The explanation might be that also regarding these factors local cross breeds were overrepresented and in the small study material the negative effect of these factors may be masked by the breed-associated relative mastitis resistance and better udder health status of the local cross breeds.

The once daily milking of the local cross breeds was primarily due to the low milk yield. The farmers with local crosses represented the poorest families which might explain why the local crosses had a more restricted access to feed and water than had the exotic crosses. It may additionally be due to different tradition in rearing local and exotic crosses, respectively.

Furthermore a *geographical difference* in udder health status could be observed. Batoka had the lowest average quarter SCC and prevalence of mastitis and IMI and Mapepe the highest. This is probably mainly an effect of breed and that some management factors were overrepresented in Batoka while others were overrepresented in Mapepe. In Batoka all cows were of local cross breed and in Mapepe all except one cow were exotic crosses. In Choma, where both types of cross breeds were represented, the udder health status was in between that of the other two areas.

The families

The average age of the household heads was quite high but the experience of dairying was short, being only a few years. This indicates that they had started with dairy production late in life and that it was not a family tradition. It might be a reflection of the GART activities which started in 2006, to stimulate and market small-scale dairy production as an advantageous system to improve household food security and income-generation. In many households in Zambia there is only one adult left to support the family, often the woman, due to the serious HIV situation. This was reflected in the current study where approximately 20 % of the household heads were widows.

Most farms had employed milkers except among the farms in Batoka where it was uncommon. The main reason for this is probably that the farmers in Batoka were generally poorer than those in the other areas, especially Mapepe. It could also, to some extent, reflect a tradition in management of the local breeds which constituted one hundred percent of the studied cows in Batoka.

According to the farmers' information about the daily herd milk and the family's consumption, they had milk available for sale. It was not possible to get information about how much the households earned from milk production, but all farmers said that the milk production was a very important source of income. Almost all families boiled the milk before consumption. This is a common tradition in many countries, which is significant from a health perspective when the milk is not pasteurized. It is particularly important in countries such as Zambia with a high prevalence of HIV and immunosuppressed people, and of uncontrolled zoonoses.

Burkholderia (B.) cepacia was isolated from all udder quarters of one cow. The finding is most probably not important from an udder health perspective but is noteworthy considering that *B. cepacia* affects particularly immunocompromised human patients and a considerable part of the rural population in Zambia is living with HIV/AIDS (Utrikespolitiska institutet, 2011) i.e. representing a population where this bacteria species may be more prevalent. *B. cepacia* usually reside in soil, water and vegetation, but has also been isolated from products consisting of raw unpasteurized bovine milk (Govan *et al.,* 1996; Moore *et al.,* 2001).

Summary and conclusions

Although the udder health situation in this part of Zambia was encouraging compared with other geographical areas with dairy farming under similar conditions, there is still clearly room for improvement. The management routines generally applied had severe shortcomings in factors known to have a negative impact on udder health status and contribute to poor hygienic quality of the milk. By improving udder health the production potential of the cows could be better utilized. With a larger herd milk yield more milk can be sold leading to higher cash income, still leaving milk for the families' consumption. Advice on proper milking routines and other management factors for improvement are given in Appendix 3. However, achieving better udder health and improved milk production is a long-term work that requires organized regular advisory service and support to the farmers.

ACKNOWLEDGEMENTS

First of all, I wish to express my gratefulness to the MFS scholarship from SIDA and Gulli Strålfeldts fond whose financial contribution to a great extent supported this study. Special thanks to my Swedish supervisor Dr Karin Östensson, without her knowledge and dedication this project would never have been possible. I am also most thankful to Elisabeth Persson for coming up with the idea of this project and to my local supervisor in Zambia professor G. S. Pandey, who made the fieldwork possible. Thanks to the people at the veterinary department at UNZA and the people at the mastitis laboratory at SVA. 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. Furthermore, I owe the extremely friendly and kind farmers who participated in this study an enormous thank you – without you none of this would ever have taken place. Olle Selander at DeLaval thanks for sponsoring with equipment. Last, but definitely not least, my thanks go to Absolom, Kelvin, Eustace, Irene, Abdel, Clemens and every other Zambian I met, for making me have the time of my life. And Lisa Eriksson my "big sis", what would I have done without you?

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APPENDICES

Appendix 1: Observation form

ID

Cow cleanliness		1	2	3	
Udder cleanliness		1	2	3	
Tail cleanliness		1	2	3	
Handwash before milking		No wash	Wash with water		Wash with soap+water
		Disinfectant			
Washing of the udder		Yes	No		
Wipe dry after washing		Udder	Only teats		
Water Quality		1	2	3	
Wiping		With wet cloth		With dry cloth	No wiping
		Same cloth to all/many cows			One cloth to each cow
Lubricant/balm/cream		Yes	No	Type	
Foremilk elimination					
Teat dipping		Yes	No	Type	
Other postmilking routines					
Milking techniques		Whole hand	Stripping	Knuckling	Machine
Washing of equipment					
	With water	Hot water	Cold water	With dish soap	With brush
	Dry before using (turn upsidedown?)				
Type of equipment		Bucket	Can	Plastic	Stainless steel
Fly control		Yes	No		
Feeding during milking		Yes	No		
Water supply		Handpump	River	Pond	Pipe
Water quality		1	2	3	
Environment, lairage		Open	Shelter/Parlour		Muddy
Mood of the cows		calm	little stressed	very stressed	
Hoofstatus		1	2	3	
General body condition		normal	thin	very thin	

Appendix 2: Questionnaire

ID:

Age:

Sex of household head : Male Female

Married Yes No

How many (and who) are milking the cows?
- Is milking done by the family or by employees? Family Employees
- **Is milking usually done by the same person?** Yes No

How long have you been practicing dairy farming? (years)

Number of cattle

	Breed	Number	Age
Cattle, total			
Cows in milk			
Heifers			
Calves and young stock			

How often do you milk? (per day) Once Twice

What is the total milk yield on average per day? (liters)

In dry season In rainy season

Have any of the cows been treated with antibiotics (the last 10 days)?
What is the name of the antibiotic (show the bottle or the brand name)?

Is calf suckling used?
How?

How much milk do you get per cow per day? (liters)

How much milk does the family consume per day? (liters)

Is the drinking milk boiled before consumption?

Do you make sour milk? Yes From boiled milk From raw milk
No

On average how much do you earn from the milk per month? (in Kwacha)

How important is the income from milk to your household? (1=not important, 2=important 3=very important) If possible to answer: How does this compare to other income?
1 2 3

Appendix 3: Advice to farmers

The listed milking routines below were missing and/or not adequate in this study and therefore this advice is intended to improve udder health among smallholder dairy cows in Mapepe, Batoka and Choma districts in Zambia.

- **Washing of hands before milking each cow**
  The milker should wash hands with water and soap and thereafter dry the hands with a clean, dry towel. This procedure has to be done just before milking each cow. The nails of the milker should be trimmed and clean.

- **Washing of the udder and drying with a separate towel for each cow before milking**
  The udder and teats should be washed and dried before milking. It is of great importance to use an individual cloth or paper towel for drying. While washing and drying, the udder is massaged and the milk let-down reflex is stimulated. Cloths must be properly washed in a temperature of 80 C° and dried between each milking.

- **Forestripping**
  The foremilk should be checked for abnormalities, such as flakes and colour changes (e.g. signs of clinical mastitis). This should be done at each milking with a strip cup and milk from diseased cows should be kept separate and disposed safely. To detect subclinical mastitis a good practice is to use the CMT (California Mastitis Test). Cows with clinical mastitis and preferably also those with subclinical mastitis should be milked after the other cows to prevent spreading of udder infections.

- **Practice a whole-hand milking technique (not stripping)**
  By practicing a whole-hand milking technique there will be less damage to the teats. This is done by grasping the teat with the forefinger and thumb around the top of the teat. Then the rest of the teat is enclosed by the rest of the fingers, one by one. This will press the milk out. To get a good flow of milk the grip of the thumb and forefinger is released to allow the milk to flow down the teat and the process of enclosing and squeezing the teat with the rest of the fingers is repeated.

- **No use of lubricant**
  Lubricant has some positive effects on the teats but there is a big risk that the lubricant is dirty and will therefore contaminate the teats with bacteria. Therefore it is advisable not to use lubricants.

- **Post milking teat dipping**
  Dipping the teats in a disinfectant, that is approved according to national regulations, after milking will effectively reduce new infections of contagious pathogens in the udders and is a simple measure for the farmer to apply.

- **Post milking feeding**
  If possible, the cows should be given some feed after milking to prevent them from laying down the first 30-60 minutes when the teat canal is open - to reduce the risk of infections by environmental udder pathogens.