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Pure white gold

Subclinical mastitis in dairy camels in Kenya with a special focus on *Streptococcus agalactiae*

Dinah Seligsohn

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Cover: Camel with suckling calf being milked in Isiolo County, Kenya
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Abstract

In the drylands of the Horn of Africa, camels are fundamental for food and nutritional security due to their ability to produce milk despite limited access to feed and water. Subclinical mastitis (SCM) is common in the region and has a negative impact on food security and household income. In this thesis project, we investigated the prevalence, aetiology and potential biomarkers of SCM. Antibiotic susceptibility was assessed and the molecular epidemiology of *Streptococcus agalactiae* (SRA) investigated through genomic and phylogenetic analysis. In total, 804 udder quarters in 206 lactating camels were screened using the California Mastitis Test and milk was sampled for bacteriological culture. Whole-genome sequencing was carried out on 122 SRA genomes collected from camel milk and extramammary sources. The prevalence of SCM at quarter, camel and herd level was 26%, 46% and 100% respectively and SCM was associated with a higher age, later stage in lactation and lesions on the udder or teats. The most common udder pathogen was SRA, followed by non-aureus staphylococci and *Staphylococcus aureus.* Tetracycline resistance was widespread among SRA isolates. Most SRA isolates from milk belonged to sequence type (ST) 616 and showed signs of adaptation to the mammary gland. However, there was a high nasal prevalence in healthy camels and the same STs were found in milk and extramammary isolates, suggesting a more complex epidemiology than previously assumed. Udder health in camels could be improved with the development of informed control strategies adapted to the local context.

Keywords: *Camelus dromedarius*, antibiotic susceptibility, whole-genome sequencing, GBS, molecular epidemiology, pastoralist, NAGase, LDH, SCC

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Rent vitt guld - Subklinisk mastit hos mjölkkameler i Kenya med fokus på *Streptococcus agalactiae*

Sammanfattning

I torrområdena på Afrikas horn är kamelen viktig för livsmedelsproduktionen tack vare sin förmåga att producera mjölk trots en begränsad tillgång på foder och vatten. Subklinisk mastit (SCM) är vanligt bland mjölkkameler och påverkar mjölkmängd och hushållsinkomst negativt. I detta doktorandprojekt undersöktes prevalens, etiologi, riskfaktorer och biomarkörer för SCM samt antibiotikaresistens. Den molekylära epidemiologin för *Streptococcus agalactiae* (SRA) i kamelhjordar studerades genom analys av sekvensdata. Totalt undersöktes 804 juverfjärdedelar från 206 kameler med California Mastitis Test och mjölken provtogs för analys. Helgenomsekvensering utfördes av 122 SRA-isolat, insamlade från mjölk och andra vävnader. Prevalensen av SCM på juverdelsnivå, individnivå och hjordnivå var 26%, 46% och 100% och risken ökade med åldern, senare i laktationen och vid skador på juver/spenar. Den vanligaste juverpatogenen var SRA, följt av koagulasnegativa stafylokocker och *Staphylococcus aureus*. Tetracyklinresistens var utbredd hos SRA. De flesta SRA-isolat från mjölk hörde till sekvenstyp (ST) 616 som visade tecken på att vara anpassad till juvret. Många kameler var asymtomatiska bärare av SRA i noshålan och samma ST:s isolerades från mjölk och extramammära källor vilket kan ha betydelse för förekomsten av mastit. För att förbättra juverhälsan hos kameler behöver vetenskapligt underbyggda kontrollstrategier utvecklas, anpassade till kamelhållarnas förutsättningar.

Nyckelord: *Camelus dromedarius*, antibiotikaresistens, helgenomsekvensering, GBS, molekylär epidemiologi, pastoralist, NAGase, LDH, SCC

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Dedication

To my family

“I may not have gone where I intended to go, but I think I have ended up where I needed to be”

- Douglas Adams

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Seligsohn, D.*, Nyman, A-K., Younan, M., Sake, W., Persson, Y., Bornstein, S., Maichomo, M., de Verdier, K., Morrell, JM., Chenais, E. (2020). Subclinical mastitis in pastoralist dairy camel herds in Isiolo, Kenya: Prevalence, risk factors, and antimicrobial susceptibility. *Journal of Dairy Science*, 103, 4717-4731.

II. Seligsohn, D.*, Younan, M., Larsen, T., Morrell, JM., Chenais, E., Nyman, A-K. Detection of subclinical mastitis in camels (*Camelus dromedarius*) using somatic cell count, N-acetyl-β-D-glucosaminidase and lactate dehydrogenase. (Submitted manuscript)

III. Seligsohn, D.*, Crestani, C., Forde, T.L.., Chenais, E., Zadoks, R.N. Genomic analysis of group B *Streptococcus* from milk demonstrates the need for improved biosecurity: a cross-sectional study of pastoralist camels in Kenya (Accepted for publication in BMC Microbiology)

IV. Seligsohn, D.*, Crestani, C., Gitahi, N., Lejon Flodin, E., Chenais, E., Zadoks, R.N. Extramammary sources of Group B *Streptococcus* reveals unusual ecology and epidemiology in camels (Submitted manuscript)

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*Corresponding author.

The contribution of Dinah Seligsohn to the papers included in this thesis was as follows:

I. Actively involved in planning and coordination of the study. Constructed the sampling protocol and questionnaire, participated in the sampling, performed all laboratory analyses and statistical analyses. Drafted the manuscript and finalised it with the co-authors. Corresponded with the journal.

II. Actively involved in formulating the research idea, planning the study and constructing the sampling protocols. Performed the statistical analysis of the data and drafted the manuscript. Finalised it with input from the co-authors. Corresponded with the journal.

III. Actively involved in formulating the research idea, and planning and coordinating the study. Performed most of the laboratory and all of the statistical analyses and drafted the manuscript. Finalised the manuscript with input from the co-authors. Corresponded with the journal.

IV. Formulated the research idea. Planned and coordinated the study, performed the data collection and all laboratory and statistical analyses. Drafted the manuscript and finalised it with input from the co-authors. Corresponded with the journal.

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Abbreviations

AMR	Antimicrobial resistance
BA	Blood agar
CAMP	Christie-Atkins-Munch-Peterson
CI	Confidence interval
CM	Clinical mastitis
CMT	California Mastitis Test
EA	Edward's agar
ECOFF	Epidemiological cut-off value
GBS	Group B S*treptococcus*
IMI	Intramammary infection
IQR	Interquartile range
LDH	Lactate dehydrogenase
LMICs	Low and middle income countries
MALDI-TOF MS	Matrix-assisted laser desorption/ionisation-time of flight mass spectrometry
MIC	Minimum inhibitory concentration
NAS	Non-aureus staphylococci
NAGase	N-acetyl-β-D-glucosaminidase
OR	Odds ratio
PCR	Polymerase chain reaction
SCM	Subclinical mastitis
SCC	Somatic cell count
SLV	Single locus variant
SNP	Single-nucleotide polymorphism
SRA	*Streptococcus agalactiae*
ST	Sequence type

SVA	National Veterinary Institute
TH	Todd Hewitt
WGS	Whole-genome sequencing

1. Introduction

The livestock sector is fundamental to global food production and to the livelihood of millions of smallholders and pastoralist households worldwide. Globally, an estimated 200 million people are pastoralists, who are directly dependent on the keeping of livestock for sustenance (FAO, 2001). Pastoralism, i.e., herding of livestock on pasture, makes use of grazing resources and converts them to nutrients to be utilised for human consumption. Pastoralism has traditionally been a form of animal husbandry practised by nomads who move with their animals in search of good grazing grounds. Pastoralists often live in marginal ecological zones and livestock are their main material assets (Jenet et al., 2016). In light of ongoing climate change, global warming and subsequent unpredictable or extreme weather, livestock-keeping is becoming more vulnerable (Dong et al., 2011). Regions prone to drought, such as the arid and semi-arid lands of East Africa, are experiencing prolonged droughts and desertification at the expense of both animal and human lives.

Many pastoralist communities in East Africa have kept camels for centuries for the production of milk, meat and hides, for transport and for cultural reasons (Catley et al., 2016). The camel is highly valued among many pastoralists due to its remarkable ability to adapt to harsh climatic conditions and produce milk despite limited availability of feed and water (Bekele et al., 2011). Camel milk is widely regarded as “the white gold of the desert” (Wernery, 2006).

Aside from frequent droughts, camel pastoralists face numerous challenges, such as diseases among the animals and limited access to animal health services (Onono et al., 2010). One of the main constraints of milk production in camels is mastitis, inflammation of the udder (Farah et al., 2007). Mastitis is most commonly caused by an intramammary infection

(IMI) and *Streptococcus agalactiae* (SRA) is a common bacterial infection in camel udders (Wahinya et al., 2014). The negative implications of mastitis are multifaceted; it reduces the yield, quality, shelf life and nutritional value of the milk, and may impact on animal welfare (Saleh et al., 2013). The economy of households that are dependent on milk sales will thus be adversely affected (Kashongwe et al., 2017). Understanding the epidemiology and risk factors of the disease, as well as establishing methods for accurate detection of mastitis, are fundamental to improving udder health in camels.

The new knowledge generated from the studies in this thesis will expand the knowledge basis of mastitis in camels and contribute to informed control strategies for improving camel udder health.

2. Background

2.1 *Camelidae*

The *Camelidae* family is thought to have evolved from small hare-like animals in North America 40-60 million years ago during the Eocene era. Camelids belong to the order Artiodactyla (even-toed ungulates), suborder Tylopoda (pad-footed). The *Camelidae* is now the only remaining branch of the family of Tylopoda and contains three different genera, *Camelus, Lama* and *Vicugna*, which include seven species (Stanley et al., 1994). Although camelids are foregut fermenters with multi-compartmentalised stomachs, regurgitating and masticating their cud, they are taxonomically separate from, and anatomically different to, the suborder Ruminantia (the "true ruminants") (Zarrin et al., 2020).

The genus *Camelus*, generally referred to as the Old World Camels, includes the one-humped *Camelus dromedarius* (dromedary camel), the two-humped species *Camelus bactrianus* (Bactrian camel), and *Camelus ferus* (the wild two-humped camel) (Ji et al., 2009). Dromedary camels are tall and slender with a relatively thin coat and can be found in hot deserts throughout Central Asia, the Middle East, and northern and eastern Africa. Australia has a large feral population as a result of their introduction to the continent from Central Asia in the 19th century. Globally, the dromedary camel population is thought to comprise over 35 million animals, with the highest concentrations in the Horn of Africa and north-central Africa (FAOSTAT, 2019; Faye, 2020).

The Bactrian camel is stockier, with a thick coat adapted to the cold deserts of Central and East Asia. The population consists of approximately 1 million animals in total, with most of them in China and Mongolia. Due to

losses of habitat, the wild two-humped camel is critically endangered. The total population is estimated to be around 800 to 1,000 animals, all in the Gobi desert (Zarrin et al., 2020).

The genera *Llama* and *Vicugna*, or the New World Camels, are represented by the two domestic species, *Llama glama* (llama) and *Vicugna pacos* (alpaca), and their two wild counterparts, *Lama guanacoe* (guanaco) and *Vicugna vicugna* (vicuña). The New World Camels were originally found in the Andean altiplano where they were kept and bred for meat, fleeces and transport (Wheeler, 1995).

All camelids are adapted to survival in extreme climates and share some common features. They have padded feet that cause little damage to the ground, and a cleft upper lip for selective foraging. Their characteristic elliptical erythrocytes are a unique feature among mammals. The oval shape improves the resistance of erythrocytes against osmotic stress, which is an advantage for animals experiencing periods of limited access to water (Vap and Bohn, 2015).

Camelids have the same number of chromosome pairs and can interbreed. Interbreeding occurs naturally in geographical regions where the habitats of the different species overlap, such as areas in the Andes where cross-breeding of llamas and alpacas results in hybrids, and parts of Central Asia where Bactrian camels and dromedary camels sometimes produce hybrids (Wheeler, 1995). Hybrids between dromedary camels and llamas have also been produced through artificial insemination (Skidmore et al., 1999).

2.2 Camel pastoralism in East Africa

The first molecular evidence of the domestication of the dromedary camel in the Arabian Peninsula dates back 5000 years (Almathen et al., 2016; Orlando, 2016). They were introduced to Africa somewhere between 3000 BC and 500 AD; dromedaries are thought to have first arrived in East Africa in around 1000 BC (Epstein, 1971). Dromedary camels will be referred to below as “camels” unless specified otherwise in the text.

In the deserts and drylands of North and East Africa, camels are fundamental for food security. In addition to household sustenance, camels and camel products, such as milk, meat, hides and wool/fibre, can be traded for other goods. They can be used for transport and fill important sociocultural roles in many camel-keeping societies (Nyariki and Ngugi,

2002). The camels' ability to adapt to high ambient temperatures, browse plants that cannot be utilised by other types of livestock and minimise water loss, make them uniquely adapted to the harsh climate. They provide a source of milk all year round (Ali et al., 2019; Engelhardt et al., 1986; Kartzinel et al., 2019; Wu et al., 2014) (Fig. 1). In addition to supporting pastoralist livelihoods, browsing camels contribute to biodiversity and grassland carbon sequestration (Dabasso et al., 2014; Seid et al., 2016). The negative impact on land degradation and soil erosion from camels has been shown to be smaller than that from cattle, sheep and goats. The camels' soft padded feet cause less soil compaction and their naturally very mobile foraging behaviour, where they browse and graze over large areas, puts less pressure on the land (Al-Dousari et al., 2019).

The Horn of Africa is home to the largest populations of dromedary camels in the world (FAOSTAT, 2019.; Faye, 2020). Camel-keeping communities can be found throughout the region with some keeping only camels, but many herds comprise mixed livestock species (camels, cattle, sheep and goats). Pastoralist herds regularly cross national borders in search of good grazing land. Trading of live camels across land borders is common, but there is also extensive export of camels from the coastal cities in Somalia and Djibouti to the Arabian Peninsula, predominantly for meat and racing (Aklilu and Catley, 2009; Alary and Faye, 2016; Younan et al., 2016).

Figure 1. A camel being milked in the dry season, while a group of camels seek shade in the background. Photograph: Dinah Seligsohn

2.3 Camels in Kenya

Camels have been present in Kenya since between the 10th and 13th centuries, with the first archaeological proof being found in the Chalbi desert in Marsabit County (Stiles, 1988). Today, the Kenyan camel population is estimated to be 4.73 million heads, making it one of the largest national camel herds in the world (FAOSTAT, 2019). However, no credible camel census has been carried out in Kenya since the 1969 national livestock census (Musinga et al., 2008). Most camels are kept extensively by traditional pastoralists or agro-pastoralists, and only a fraction is kept on ranches (Field, 2005).

Arid and semi-arid regions make up 80% of Kenya's land mass and are of limited agricultural use (Fig. 2). An estimated 30% of Kenya's human population live in these areas, the majority depending on livestock for their food security (Amwata et al., 2016). In the northern arid and semi-arid counties, many communities have a long tradition of keeping camels, some of them almost entirely depending on camels for their food security (Sato, 1980), each with their own traditional management routines. Other communities have turned to keeping camels more recently as a means of improving their resilience (Bollig, 1992; Kagunyu and Wanjohi, 2014; Sperling, 1987; Volpato and King, 2019).

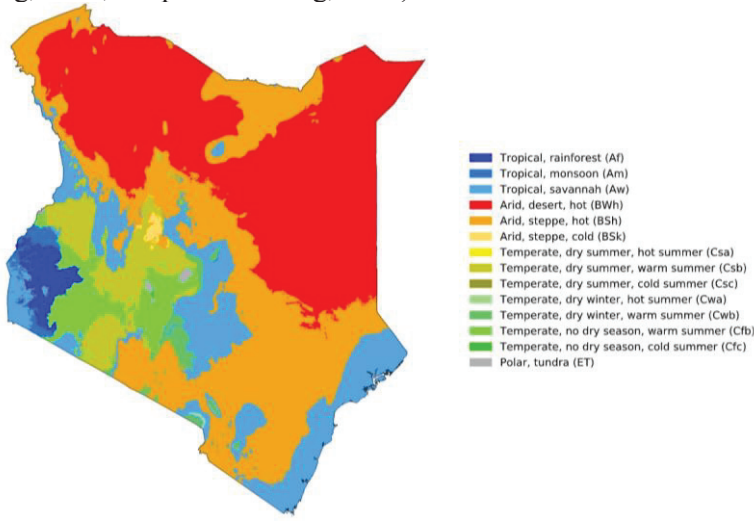

Figure 2. Köppen-Geiger climate classification map for Kenya 1980-2016 visualising its climatic zones. Arid and semi-arid lands are indicated as a colour gradient, ranging from yellow to red. Source: Beck, H.E., Zimmermann, N. E., McVicar, T. R., Vergopolan, N., Berg, A., & Wood, E. F. - "Present and future Köppen-Geiger climate classification

maps at 1-km resolution". Nature Scientific Data. DOI:10.1038/sdata.2018.214. Published under the Creative Commons Attribution 4.0 International license.

Although there are no indigenous standardised camel breeds in Kenya, four distinct types are recognised (Mburu et al., 2003). The Gabbra and Rendille types (sometimes referred to as the common Gabbra-Rendille type), and Turkana types are all small and very hardy, whereas the Somali type is tall and slender. The Somali type has the highest potential for milk production. There have been a few imports of camels from Pakistan and the Arabian Peninsula, with the purpose of enhancing production traits and improving the genetic material. They are mainly kept on ranches (Hülsebusch et al., 2002; Musinga et al., 2008).

Globally, Kenya is the second largest producer of camel milk, with an approximate annual production of 854,669 tonnes (FAOSTAT, 2019) and an estimated total value of 16.5 billion KSh (US$ 0.165 billion) (Nyariki and Amwata, 2019). In addition to food security and nutritional value, camel milk is a valuable asset for marketing, as its price exceeds that of cow milk, particularly in the dry season (Musinga et al., 2008.; Nathan et al., 1996). Camel milk contributes significantly to the daily calorie intake of pastoralists in Isiolo County (Elhadi et al., 2015). Traditionally, camel milk was only used for household consumption and selling it was regarded as something that would bring bad luck. This custom changed a few decades ago. What initially started with unorganised female vendors hawking surplus milk to individual households has now developed into a more intensified production, with a value chain involving a wide range of stakeholders. The commodification of camel milk has been viewed partly as a response to the increasing sedentarisation of nomadic pastoralists, and in part to their subsequent adaptation strategies, such as livestock diversification (Anderson et al., 2012).

The camel milk value chain in Kenya is largely informal and not governed by a regulatory framework (Muloi et al., 2018). Camel milk is sourced in pastoral rangeland and often sold locally in rural towns and villages. The development of milk hubs and predominantly female-led milk cooperatives has further transformed the market structure. Peri-urban semi-commercial herds can now be found clustered around urban centres and milk hubs (Anderson et al., 2012). With the relocation of traditional camel pastoralists to the country's larger cities, the urban demand for camel milk has created supply chains from remote regions such as Isiolo, Laikipia and Garissa

Counties, delivering thousands of litres of milk to Nairobi daily. There is small-scale export to neighbouring countries and attempts have been made to enter the international market (Anderson et al., 2012; Muloi et al., 2018; Musinga et al., 2008.). Nationally, camel milk is marketed as a healthy alternative to cow milk, thus reaching new consumer segments. The demand for camel milk is projected to increase over the next ten years (Robinson and Pozzi, 2011).

2.3.1 Camel husbandry

Although new market opportunities have emerged and the camel milk industry in Kenya is undergoing significant changes, camel husbandry still largely adheres to traditional practices. These practices vary across different communities, although some general strategies overlap. Herds are usually split into migratory herds, kept in more remote areas, and smaller herds kept closer to settlements and used for milk production and transport. During the day, camels are herded onto pasture, to forage and sometimes visit watering points. To supply their high need for salt, camels consume halophytic plants or are herded to salt licks. In more peri-urban systems with access to agricultural equipment retailers, commercial salt/mineral mixtures can be purchased. At night, the camels are kept in bomas, traditional enclosures constructed from thorn bushes and branches, to protect the herds from predators. Calves are penned separately from their mothers during the night but move with the herd during the day (Fig. 3).

Figure 3. Calves being separated from their mothers overnight in a traditional boma made from branches. Photograph: Dinah Seligsohn

Milking is usually carried out in the morning and evening, but this may vary depending on the available pasture and the subsequent production status of the camels. To stimulate milk let-down, the calves are released from their boma to suckle the dams (Musinga et al., 2008). The duration of the milk ejection reflex is short in camels (Boujenane, 2020) and to maximise milk offtake camels are traditionally milked by two milkers simultaneously, one milking from each side (Fig. 4).

Figure 4. Camel being milked by two herders simultaneously. The calf is physically restricted from suckling during milking. Photograph: Dinah Seligsohn

All milking is done by hand (Musinga et al., 2008). The calf is essential for continued milk production and thus the high calf mortality reported in northern Kenya is a threat to food security since it negatively impacts milk production and affects animal welfare (Kaufmann, 2003). To balance the competition for milk between humans and calves, different strategies are employed. In some cultures, the camel is milked only on two teats and the other two are left for the calf. The use of "anti-suckling devices" or "teat tying" is another solution to ensure milk availability for humans. Two or sometimes all four teats are tied together with plant fibres or strips of cloth (Fig. 5a-b), or even rolled on sticks and fastened with string (Abdurahman et al., 1995; Bakheit et al., 2008; Obied et al., 1996). In some communities, a cloth or a basket is tied around the udder to prevent the calf from suckling. In dairy cattle, frequent milk removal stimulates production and the suckling of the calf efficiently empties the udder (Wall and McFadden, 2008). For camels, efficient milk removal has been shown to stimulate milk production positively, but the importance of calves has not been thoroughly evaluated (Atigui et al., 2014). Apart from adverse effects on teat skin and teat function (Fig. 5c-d), anti-suckling devices are likely to have a negative impact on milk production.

In all traditional camel-keeping communities, household duties are to some degree divided according to gender. Commonly, the responsibilities of tending to, and milking, camels rest with men or boys. Camel milk however, is the property of women and they are responsible for its handling and distribution (Anderson et al., 2012; Toroitich, 2013). Camel milk is traditionally consumed raw, without prior pasteurisation, or in a fermented form, which is known as *suusac* in Kenya (Wayua et al., 2012).

Camel pastoralism is labour-intensive, but from an economical perspective is regarded as a "low-input" system, requiring minimal investment in already existing herds. The central costs listed in a report summarising the developing milk market in Isiolo town, were contracted herders (wages and provisions), watering fees, supplementary feed and occasionally health-care costs, such as veterinary drugs (de-wormers, acaricides and antibiotics) (Musinga et al., 2008).

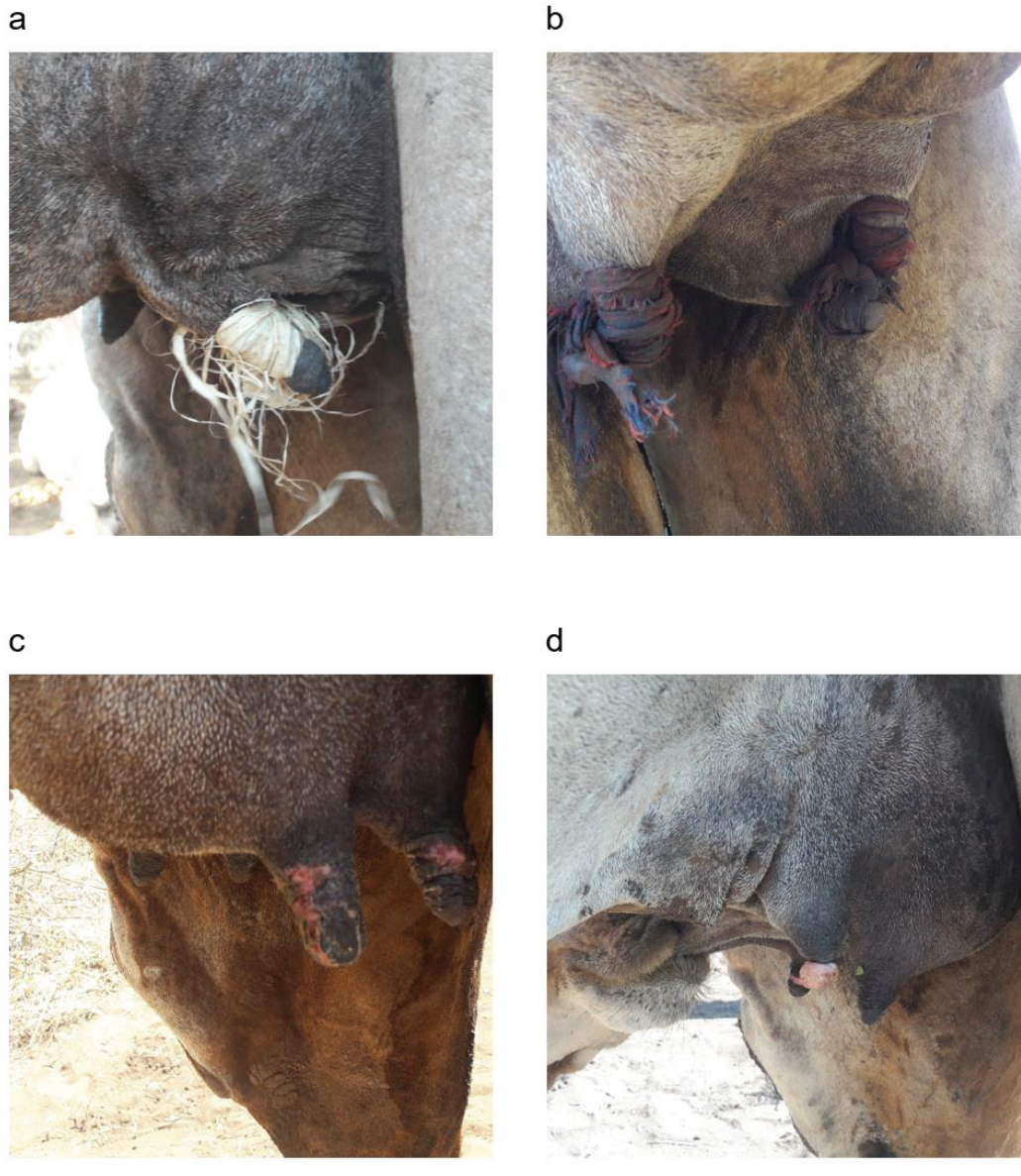

Figure 5a-d. a) Plant fibres used to tie off two teats to restrict suckling by the calf; b) strips of cloth being used to tie off all four teats to prevent the calf from suckling; c) lesions on teats due to frequent teat tying; and d) inverted teat apex due to loss of functional teat sphincter. Photograph: Dinah Seligsohn

2.4 The camel as a dairy animal

In the Horn of Africa, the most important commodity of the domestic camel is its milk. Most of the global camel milk production comes from pastoralist systems with suboptimal production settings, but intensified management systems have emerged, operating under conditions similar to cattle dairy production in industrialised countries. Modern camel milk enterprises can now be found, most notably in the Middle East but also in North Africa, Australia, USA and Europe (Nagy and Juhasz, 2016).

The camel udder is located between its hind legs with the attachment close to the abdominal wall. Udders vary greatly in shape with some being more pendulous than others. The anatomy of the camel udder differs in some respects from that of other dairy species. The udder is divided into four quarters, each one containing two to three glandular complexes with separate teat canals. The teat canals are longer and narrower than in cattle (Saleh et al., 1971). The udder cistern is tiny, holding only a small fraction (4-20%) of the total milk volume, with most of the milk (80-96%) stored in the alveolar compartment of the udder parenchyma (Atigui et al., 2014; Ayadi et al., 2013, 2009). Consequently, milk let-down and availability are strongly dependent on continuous oxytocin release during the milking process, as reviewed by Nagy and Juhasz (2016). During milk ejection, the milk is released into the teat cisterns, resulting in a visible change in the appearance of the teats: they expand longitudinally and increase in circumference. Any disturbances to milking will interrupt the milk flow (Atigui et al., 2014). A steady release of oxytocin is best evoked by suckling by the calf, but it is possible to train camels to respond to tactile stimuli and to be milked by conventional milking machines (Nagy and Juhasz, 2016).

Camels have a slow reproductive rate. Sexual maturation in the female occurs at around three years of age but most camels are not bred until they are four to five years old (Skidmore, 2011). The gestational period is long, lasting on average 384.5 days. (Nagy and Juhász, 2019). A new pregnancy cannot overlap with lactation as milk production will terminate within four months of conception (Nagy et al., 2015). Natural lactation lasts between 12 and 18 months on average and requires the presence of a calf. In the event of the death of the calf, milk production will decline rapidly (Abdalla et al., 2015; Bekele et al., 2002). The lactation curve in camels, as has been described in several studies, is similar to the lactation curve in cattle, with very little difference between management types (Abdalla et al., 2015;

Almutairi et al., 2010; Musaad et al., 2013; Nagy et al., 2012). Peak lactation is reached around four to five months into lactation and the persistency coefficient, the ability to retain milk production after peak lactation, is higher than in cattle.

The production potential in camels is being intensively researched and involves many different aspects, such as management system, genetic potential and environmental influences, as well as camel parameters, such as number of lactations/calvings, stage of lactation (days or months from calving) and udder health status. Existing data on production are difficult to compare due to methodological differences in how milk yield is assessed, the units used for measuring, and the production system studied, all of which obscure direct assessment. A review by Faye (2005) summarises production data from both intensive and pastoralist systems across various regions. Individual production ranges from 1,000 to 12,000 L per lactation. In an intensive system, an average daily milk yield of 5.8-6.9 kg has been reported (Nagy and Juhasz, 2016). This is surprisingly close to the volumes/amounts reported from pastoralist systems. Bekele (2002) reported a daily average milk yield of 4.1 kg in pastoralist camels in Ethiopia and, similarly, Bakheit (2008) found an average daily milk yield of 3.14 L in Sudanese camels. Slightly higher yields were found in Pakistani camels, at 7.4-8.2 L, although the authors did not specify methods for assessing the volume (Ahmad et al., 2012a; Faraz et al., 2020). External factors may influence the milk yield. For camels on pasture, season is a strong determinant of daily production. Simpkin (1996) and Bakheit (2008) showed that traditionally kept camels in Kenya and Sudan display a bimodal lactation curve with peaks corresponding to the wet seasons when richer pasture is available. For camels in intensive production systems, with a more balanced food supply, feed supplementation does not seem to increase milk yields further. Nevertheless, both photoperiod and calving season have been shown to affect production under these conditions (Nagy and Juhasz, 2016).

Traditionally, no systematic breeding programmes have been in place to improve the genetic potential for milk production; selection has been based on other factors, such as survival abilities. Significant differences in milk production potential have been observed among different breeds/ecotypes of camels. With the development of more large-scale enterprises, it is likely that this area will receive more attention and focused efforts. Nonetheless, in

view of the slow reproductive rate in camels, measurable progress will take some time.

2.4.1 Camel milk composition and properties

Camel milk has a sweet, sharp and sometimes salty flavour, mainly depending on the fodder (Farah, 1996). Mean pH is 6.8 (Khaskheli et al., 2004). The appearance is glistening white in comparison with cow milk, which has been attributed to the low concentration of carotene (Abu-Lehia, 1989). The shelf life of raw camel milk of an acceptable hygienic level is reported to range between two and five days at room temperature, and up to 42 days if kept refrigerated at 4 °C (Omer and Eltinay, 2009). Overall milk composition is similar to cow milk. The mean values and standard deviations for the main components in camel milk (in g/100 mL) are total protein 3.35±0.62, fat matter 3.82±1.08, lactose 4.46±1.03, ash 0.79±0.009 and dry matter 12.47±1.53, as reviewed by Konuspayeva et al. (2009).

Compared with cow milk, camel milk contains a higher concentration of long-chain fatty acids, unsaturated fatty acids and cholesterol. Protein content is also distributed differently. The main camel milk casein is β-casein, followed by αs1-casein, while the κ-casein fraction is very low, making camel milk unsuitable for cheese making. Like human milk, there is no β-lactoglobulin in camel milk (Al haj and Al Kanhal, 2010). As β-lactoglobulin is often involved in milk allergy in humans, camel milk has been suggested as an alternative milk source for people with a cow milk allergy (El-Agamy et al., 2009).

Furthermore, camel milk contains antibacterial enzymes. The concentration of lysozyme has been found to be more than double that of cow milk (Elagamy et al., 1996) while lactoferrin, lactoperoxidase and IgG content are at a similar level (Elagamy et al., 1996; Konuspayeva et al., 2007).

One of the most important properties in camel milk is the extraordinarily high vitamin C content, with levels reported as being three to five times higher than in cow milk (Farah et al., 1991; Stahl et al., 2006). This characteristic is of paramount importance for pastoralists living in areas where access to fresh fruit and vegetables is limited. In many traditional camel societies, camel milk is thought to have medicinal properties and is used as a remedy for a wide range of diseases. Similar claims have also been

made in modern research, although the scientific evidence for this is still disputed (Al haj and Al Kanhal, 2010; Yadav et al., 2015).

2.5 Mastitis in camels

Mastitis, inflammation of the mammary gland, is a complex, multifactorial disease, and a common condition in milk-producing animals worldwide. Mastitis is mostly seen as the inflammatory response to a bacterial intramammary infection (IMI) or, more rarely, is the result of external trauma (Sandholm, 2008). Mastitis can be classified as clinical or subclinical, according to presentation, and acute or chronic depending on its duration. Clinical mastitis (CM) can be graded further depending on the degree of severity of the clinical symptoms. An udder quarter with mild CM will display visible changes in the milk (colour, clotting, blood, smell); cardinal symptoms of inflammation, such as swelling, redness, pain and increased temperature of the udder can be seen in moderate CM. In severe cases, all these symptoms are present and the affected animal exhibits systemic effects, such as fever, dehydration and loss of appetite. However, subclinical mastitis (SCM) cannot be detected by clinical examination, and further diagnostic tests are required (Gruet et al., 2001).

Inflammation in the udder occurs as a response to tissue trauma. Leucocytes (white blood cells) migrate to the site of injury and, consequently, the somatic cell count (SCC) in milk will be elevated. In cows, the drop in milk production seen during mastitis is attributed to both a decline in milk synthesis in the mammary epithelial cells, as a consequence of cell damage and cytokines excreted by inflammatory cells, and a loss of lactose due to increased permeability of the blood-milk barrier (Sandholm, 2008). In a study of differential leucocyte count in camel milk, the circulating leucocytes in low SCC ($<10^5$ cells/mL) camel milk were composed of predominantly macrophages (66%) followed by lymphocytes (25%) and polymorphonuclear neutrophils (PMN) (9%), whereas in high SCC quarters (SCC$>10^5$ cells/mL), a shift was seen, with a dramatic increase in the relative proportions of PMN and macrophages (Hamed et al., 2016). Detailed knowledge of the pathophysiological processes behind mastitis in camels is scanty at present, but there have been efforts to investigate the immunological mechanisms involved in the camel mammary gland during

infectious mastitis (Abeer et al., 2016; Al-Ashqar et al., 2015; Alluwaimi, 2017; Al-Mohammed Salem et al., 2012).

Several negative implications for public health and household income are associated with mastitis. In camels, as for cows, reduced milk yield, as well as a decrease in fat and protein content, have been reported in milk from quarters with mastitis (Hadef et al., 2019; Hamed et al., 2016). These changes in composition negatively affect the milk's nutritional value, with implications for calf morbidity and mortality as well as human health. High bacterial counts, as can be found in mastitis milk, have a negative impact on storage time; post-harvest losses due to mastitis have been calculated to be 60% of the total milk yield/day/herd in pastoralist herds (Kashongwe et al., 2017). Storage time is of particular importance in view of the conditions in which most camel milk producers operate, with high ambient temperatures and limited access to hygienic storage facilities. The tradition of pooling mastitic milk with uninfected milk further contributes to milk spoilage (Kashongwe et al., 2017). Pathogens in milk can also be a public health hazard, especially when milk is consumed raw (Abera et al., 2016; Ngaywa et al., 2019; Omwenga et al., 2020; Wainaina et al., 2020). Antibiotic residues are another threat to public health as cases of CM are commonly treated with antibiotics and compliance with withdrawal times following antibiotic treatment is poor among camel pastoralists (Lamuka et al., 2017).

Camels were long thought to be less affected by mastitis than other dairy species, due to the concentrations of anti-bacterial enzymes in the milk, the high udder attachment shielding it from environmental contamination, and the long and narrow streak canals obstructing invasive microorganisms (Wernery, 2006). This field of research has received greater attention in the past two decades (Abdurahman et al., 1991), and in many geographical areas, mastitis prevalence seems to be comparable to the situation in dairy cattle, with SCM more common than CM (Abdurahman et al., 1995; Busanello et al., 2017; Mdegela et al., 2009; Persson Waller et al., 2009; Regassa et al., 2013). In the Horn of Africa, udder quarter prevalences of CM and SCM in camels are reported to be 5.4–19.5% and 20.7-39.4% respectively (Abdurahman et al., 1995; Abera et al., 2010; Almaw and Molla, 2000; Regassa et al., 2013). To boost mastitis prevention, further information is required about the risk factors for mastitis. Some risk factors associated with mastitis have been identified for camels. Age (≥10 years) and parity (≥4) are associated with an increased risk of mastitis (Ahmad et al., 2012b; Aljumaah

et al., 2011). Regassa et al. (2013) found mastitis to be more common in earlier stages of lactation, whereas Ahmad et al. (2012b) reported a higher risk both in the first month of lactation and in late lactation (month 10-12). Furthermore, skin lesions on the teats and udder are associated with mastitis (Almaw and Molla, 2000; Regassa et al., 2013). Both Regassa et al. (2013) and Almaw and Molla (2000) attribute these skin defects to the traditional practice of teat-tying. Management routines can also affect udder health and poor milking hygiene has been shown to increase the risk of mastitis in camels (Ahmad et al., 2012b).

2.5.1 Mastitis in pastoralist herds - perceptions and treatment

Clinical mastitis is usually recognised by pastoralists and is known by different names and classifications depending on the cultural context (Abbas et al., 2002; Abera et al., 2010; Raziq et al., 2010; Tuteja et al., 2011). Since SCM cannot be detected without assessing the levels of somatic cells or other changes in milk composition, which requires testing of the milk, there is generally a lack of awareness of SCM among pastoralist groups. Perceptions of mastitis and the underlying causative agents vary across geographical regions. Among Senegalese pastoralists, mastitis is thought to be caused by the boiling of milk (Prakashbabu et al., 2020); according to the beliefs of the Sahrawi pastoralists in Western Sahara, mastitis is caused by high stocking densities or retention of milk, while mastitis is generally attributed to “the evil eye” among many pastoralist groups in the Horn of Africa and Middle East (Abbas et al., 2002; Amenu et al., 2017; Volpato et al., 2015). In a study of camel pastoralists in Somalia, mastitis was listed as the main constraint for milk production (Farah et al., 2007) and has also been reported as a common problem among several other camel-keeping communities (Odongo et al., 2016; Toroitich, 2013).

Traditional remedies for mastitis include herbal medicine (Heffernan and Misturelli, 2000; Seifu and Tafesse, 2010; Volpato et al., 2015), abstaining from milking the affected quarter, removal of ticks and cauterisation of the udder (Abera et al., 2010; Ali, 2006; Mengistu et al., 2010; Seifu and Tafesse, 2010; Wanjohi et al., 2013). With the increasing availability of modern veterinary drugs to camel pastoralists, treatment with antibiotics is now common (Lamuka et al., 2017).

2.6 Detection of mastitis and intramammary infection

In addition to clinical examination of the udder, including palpation of udder tissue and a visual assessment of udder shape and of the milk, a number of direct and indirect diagnostic tests are available to detect mastitis. This is particularly important for the diagnosis of SCM.

2.6.1 Indicators of inflammation

The California Mastitis Test (CMT) is an indirect subjective method of approximating the somatic cell count in milk (Schalm and Noorlander, 1957), which is done by estimating the DNA content of the cells using an anionic detergent that dissolves cell and nuclear membranes. As a result, DNA is released and forms a gel when in contact with the detergent (Sandholm, 2008). Scoring is done on a five-point scale, where 1 represents healthy milk and 5 represents severe inflammation (Klastrup and Madsen, 1974). CMT testing does not require advanced technical equipment and is easy to learn, making it a robust animal-side mastitis test. The CMT has shown good specificity and sensitivity for identifying infected quarters when evaluated for camel milk (Abdurahman et al., 1995; Younan et al., 2001). In high-income countries, a somatic cell count (SCC) directly enumerating the cellular content in milk is routinely performed in a large proportion of dairy cattle herds to monitor the udder health status of individual cows. SCC below 200,000 cells/mL in whole udder milk samples of dairy cows is considered healthy, whereas levels above this value are classified as pathological (Schepers et al., 1997). Furthermore, SCC is widely used to assess herd udder health and as a quality indicator of bulk milk of dairy cows delivered to dairies (Wickström et al., 2009). Research suggests that it could also be a potential quality indicator for camel milk (Jayarao et al., 2004; Nagy et al., 2013). Efforts have been made to determine SCC in healthy and inflamed camel udders, with largely inconclusive results. In a study by Abdurahman et al. (1995), the mean SCC for non-infected quarters (bacteriologically negative) was 912,011 cells/mL, for quarters bacteriologically positive for minor pathogens 707,946 cells/mL, and in quarters bacteriologically positive for major pathogens 1,513,561 cells/mL. In another study under similar conditions, only a small difference in SCC was found between infected and non-infected quarters, with a mean of SCC 414,954 cells/mL and 215,774 cells/mL respectively (Guliye et al., 2002). In contrast, Saleh and Faye (2011) determined the mean SCC for non-infected quarters to be 125,000

cells/mL. One important aspect that should be considered when determining SCC in camel milk is the presence of large anucleated cytoplasmic particles. Cell fragments in milk from Bactrian camels were reported by Abdurahman et al. (1992) to account for 63-99% of the total particles in milk. Similar fragments have frequently been reported in both goat and sheep milk (Paape et al., 2001) and are thought to originate from the apocrine milk secretion seen in these species. Although the body of evidence is small and has not been verified for dromedary camel milk, it is generally recommended that DNA-specific enumeration methods of somatic cells be used to avoid overestimating the number of cells, such as the direct cell counter (DCC; DeLaval International AB, Tumba, Sweden) or direct microscopic somatic cell count stained with DNA-specific pyronin Y-methyl green.

Nagy et al. (2013) found that SCC showed a strong seasonal variation in camels kept under intensive management conditions, and that this is associated with the seasonal calving pattern of camels on the farm. Furthermore, as with other species, physiological factors such as stage of lactation, influence SCC in camel milk. Furthermore, SCC levels have been shown to be high directly after parturition, declining up to peak lactation followed by an increase as lactation progresses (Abdurahman, 1996; Nagy et al., 2013). A negative correlation between SCC and milk yield parameters has been reported in the United Arab Emirates (UAE) (Nagy et al., 2013).

Other indicators of inflammation have been evaluated for the detection of mastitis in camels, such as testing of the activity of enzymes leaking from injured mammary epithelial cells. N-acetyl-β-D-glucosaminidase (NAGase) was first found to be a reliable indicator of udder inflammation in camels by Abdurahman (1995), although the usefulness of NAGase has been challenged in later studies (Chaffer et al., 2000; Guliye et al., 2002). Lactate dehydrogenase (LDH) is a non-lysosomal enzyme that has been shown to increase in sheep, goat and cow milk from udder halves or quarters with IMI, but it has not yet been investigated in camel milk (Katsoulos et al., 2010; Larsen, 2005; Nyman et al., 2016).

During mastitis in cattle, there is an increased permeability in endothelial cells in the mammary gland, resulting in leakage of serum proteins into the milk and alterations to the milk ion content. Due to the compositional changes in milk during mastitis, electrical conductivity can be used as a diagnostic tool in cow milk, although the evidence regarding the accuracy for detecting mastitis in camel milk is contradictory (Aljumaah et al., 2020;

Younan et al., 2001). Furthermore, pH, serum albumin and ATP have been evaluated as inflammatory markers in camel milk. Ndirangu et al. (2019) report that pH testing is a reliable method for detecting SCM. Serum albumin has been shown to be of less diagnostic value (Abdurahman, 1995; Abdurahman et al., 1995; Kathiriya and Shah, 2009). Lactoferrin, an antibacterial enzyme and part of the normal milk composition in camels, has been shown to increase in quarters with SCM (Al-Majali et al., 2007).

2.6.2 Detection of intramammary infection

Bacterial culturing is usually used to detect the microorganisms causing intramammary infection. Aseptically collected milk samples are inoculated on culture media and assessed macroscopically within 24-48 hours. Further typing of relevant growth can be carried out by biochemical testing or with the more recent analytical method of matrix-assisted laser desorption/ionisation-time of flight mass spectrometry analysis (MALDI-TOF MS) (Bizzini et al., 2010) and real-time polymerase chain reaction (PCR). These methods allow for a more rapid, efficient and accurate typing procedure, compared with biochemical testing. For MALDI-TOF MS, the typing is based on the creation of a spectrum of the microorganism, which is compared to spectra in a database. This sometimes enables the identification of mastitis pathogens that have not previously been recognised. Since PCR detects DNA from microorganisms, a selection of known mastitis pathogens can be detected simultaneously in milk samples using commercial kits. The analytical accuracy of PCR for detecting IMI has been evaluated, with excellent results (Koskinen et al., 2009; Nyman et al., 2016). PCR is a rapid method with high sensitivity. Low levels of pathogens can be detected, which makes PCR particularly useful for screening of certain udder pathogens such as *Streptococcus agalactiae* (Mahmmod et al., 2013). However, since PCR analyses DNA content, no differentiation can be made between viable bacteria and dead bacteria, making it hard to interpret whether the finding is from infecting bacteria or from contamination.

2.7 Mastitis microbiology

Intramammary infections are generally caused by bacteria and, to a much lesser extent, by other microorganisms such as fungi, algae and viruses. The

overall bacterial panorama for IMI is similar worldwide, although local contexts affect the distribution of various pathogens (Gruet et al., 2001).

Mastitis pathogens are traditionally divided into environmental pathogens and contagious pathogens, although it is now suggested that this classification is too rigid since there are a number of bacterial species that fit between these two categories. For instance, the classification of non-aureus staphylococci (NAS) and *Str. dysgalactiae* is disputed and they are regarded as being somewhere between the two categories. Furthermore, niche adaptation of mastitis pathogens means that different strains within the same species could be classified as either environmental or contagious (Zadoks and Schukken, 2006). For IMI caused by environmental bacteria, the reservoir is the environment of the dairy animal and exposure to the environment is throughout the day, and during lactation and drying-off. Environmental bacteria primarily cause clinical disease. Significant bacteria causing environmental mastitis in cows include coliforms and Gram-negative bacteria such as *Escherichia (E.) coli*, *Klebsiella* spp., *Enterobacter* spp., *Proteus* spp., *Pasteurella* spp. and *Serratia* spp., but also *Streptococcus (Str.) uberis*, *Trueperella (T.) pyogenes*, *Lactococcus* spp., Gram-positive bacilli and yeast (Gruet et al., 2001).

The udder serves as the main reservoir for contagious mastitis pathogens. Transmission occurs primarily from animal to animal, mainly during milking. Contagious udder pathogens are known to cause both CM and SCM. The most important bacterial species responsible for contagious mastitis in cows are *Str. agalactiae* (SRA), *Staphylococcus (S.) aureus*, *Corynebacterium (C.) bovis* and *Mycoplasma* spp. Mastitis in camels is often contagious. *S. aureus* and SRA have been reported in many camel-keeping countries at a quarter prevalence ranging between 5 and 36% and between 7 and 49% respectively (Abdurahman et al., 1995; Abera et al., 2010; Ahmad et al., 2012b; Almaw and Molla, 2000; Obied et al., 1996; Regassa et al., 2013; Toroitich et al., 2017). Furthermore, NAS have been shown to be prevalent in camels with IMI. In most studies, NAS account for 7-15% of bacteriological diagnoses (Abdurahman et al., 1995; Ahmad et al., 2012b; Guliye et al., 2002; Obied et al., 1996), although some studies have reported NAS to be the most common cause of mastitis (SCM and CM) (Almaw and Molla, 2000; Seifu and Tafesse, 2010). The prevalence of *Str. dysgalactiae* and *Corynebacterium* spp. has been reported to be 11-17% and 3-7%, respectively (Ahmad et al., 2012b; Almaw and Molla, 2000; Guliye et al.,

2002; Regassa et al., 2013). For environmental mastitis, the bacterial spectrum differs from the situation in dairy cattle. Coliforms, *Str. uberis* and *T. pyogenes* are rarely reported, whereas *Micrococcus* sp. and *Bacillus* spp. are regarded as more common causes (Abeer et al., 2016; Ahmad et al., 2012b; Guliye et al., 2002; Obied et al., 1996; Regassa et al., 2013).

2.7.1 Antimicrobial resistance in mastitis pathogens

The emergence of antimicrobial resistance (AMR) is a global threat to public and animal health and to food security (Laxminarayan et al., 2013). Increasing AMR has negative effects on therapeutic treatment and is associated with economic losses. Selection pressure and the spread of resistance genes among microbial populations are the main mechanisms for acquisition of AMR in bacteria. Resistance patterns depend on bacterial properties and the antibiotic agent concerned (Levy and Marshall, 2004). Innate resistance within the bacteria to a certain antibiotic agent is referred to as intrinsic resistance. One example is *Mycoplasma* spp., which exhibits an intrinsic resistance to penicillin due to the lack of a cellular wall. Acquired resistance refers to acquisition of resistance genes, commonly through the horizontal transfer of mobile genetic elements, such as plasmids, transduction via bacteriophages or transformation, or accumulations of mutations within the genome leading to resistance. The main functions of AMR genes are: i) modulation of the permeability of the bacterial cell wall, ii) active efflux of the antibiotic agent from the bacteria, iii) enzymatic modification of the antibiotic agent, iv) degradation of the antibiotic agent, v) replacement of metabolic pathways inhibited by the antibiotic agent, vi) modification of target sites, and vii) overproduction of the target enzyme. A large number of resistance genes with different functions have been identified: some encode production of β-lactamase, which causes enzymatic degradation of the active component in penicillin, *erm* genes encode prevention of binding of erythromycin to target molecules, expression of the *tet*(M) gene prevents ribosomal binding of tetracycline, and the *tet*(L) gene encodes for efflux pumps of tetracycline (van Hoek et al., 2011).

Mastitis is one of the most common diagnoses to warrant antibiotic treatment in the dairy industry, and AMR in mastitis pathogens has been reported worldwide (Oliver et al., 2010). For example, the prevalence of *S. aureus* and NAS strains resistant to penicillin is 25% and 29% respectively in a pan-European antimicrobial susceptibility monitoring programme of

pathogens from cases of clinical mastitis. Some of these strains harbour the *mec*A gene, classifying them as methicillin resistant (de Jong et al., 2018). Likewise, in a limited study from Uganda, β-lactamase production was found in 80% of NAS-isolates from cow milk (Björk et al., 2014), and in California penicillin resistance was found in almost 50% of *Str. uberis* isolates from cow milk (Rossitto et al., 2002). Knowledge about AMR of mastitis pathogens in camel milk is limited. An intermediate susceptibility to penicillin of 36% of NAS isolates from SCM and CM cases was reported in the UAE (Al-Juboori et al., 2013), and high levels of resistance to oxacillin, cefoxitin and ampicillin in *S. aureus* were reported in Pakistan (Ali et al., 2018). Furthermore, tetracycline resistance, carried by the *tet*(M) gene, was found in SRA isolated from camels with IMI in Kenya (Fischer et al., 2013).

Antibiotic-resistant bacteria in milk pose a threat to public health, particularly in settings where milk is consumed raw. Multi-antimicrobial resistance has been found in *S. aureus* and *E. coli* isolated from camel milk in northern Kenya (Jans et al., 2017; Nato et al., 2019; Omwenga et al., 2020).

2.8 *Streptococcus agalactiae*

Streptococcus agalactiae (SRA), often referred to as Group B *Streptococcus* (GBS) in human medicine, is a facultative anaerobic non-motile chain-forming Gram-positive catalase-negative coccus (Quinn et al., 2011), the only species belonging to Lancefield group B. Morphologically, colonies are small, round and translucent, normally displaying a clear β-haemolysis on blood agar, although some strains are non-haemolytic (Brown, 1939). A capsule composed of polysaccharides forms the outer layer of the organism and its antigenic properties are used for further sub-classification of different strains according to serotype (Ia, Ib, II-IX) (Segura, 2004).

Streptococcus agalactiae is a robust and versatile pathogen that has the ability to invade and establish opportunistic infection in a multitude of different terrestrial and aquatic species, ranging from elephants, horses, dolphins and cattle to fish and frogs (Delannoy et al., 2013; Eisenberg et al., 2017; Lyhs et al., 2016; Yildirim et al., 2002). In veterinary medicine, SRA is mainly recognised as a contagious udder pathogen associated with mastitis. It has been reported to cause mastitis in cattle, but also in other dairy species, such as goats and sheep (Danmallam and Pimenov, 2019; Keefe,

1997; Mbindyo et al., 2014; Zdragas et al., 2005). The name *agalactiae* refers to "no milk" and reflects the dramatic production loss often seen in connection with mastitis caused by SRA (Keefe, 1997).

Genomic investigations of the global SRA population have revealed the existence of clonal complexes, some of them host specialists and some classified more as host generalists. The wide range of host species has been attributed to the high genomic plasticity in SRA, resulting in a strong ability to incorporate accessory genome content into the chromosome, allowing strains to adapt to different hosts and ecological niches to gain an evolutionary advantage. Acquisition of mobile genetic elements, for instance carrying AMR genes, genes for substrate utilisation and virulence genes, such as genes promoting biofilm formation or pyrotoxin production, is common in SRA (Richards et al., 2019).

2.8.1 Zoonotic aspects and interspecies transmission

Streptococcus agalactiae is regarded as a commensal pathogen of the human microbiota, and asymptomatic nasopharyngeal, rectal or vaginal carriage is estimated at around 10-30% in both industrialised and low and middle-income countries (LMICs) (Cools et al., 2016; Le Doare and Heath, 2013). Nonetheless, SRA can cause opportunistic infection in humans, specifically in immunocompromised individuals or patients with underlying conditions, such as diabetes mellitus, heart disease or malignancy. Specifically, SRA has been associated with skin/soft tissue infections, bacteremia, osteomyelitis, pneumonia, meningitis and endocarditis. More importantly, since the 1960s SRA has emerged as a pathogen in infants and is now the leading cause of neonatal sepsis worldwide. To prevent this critical condition, routine screening and subsequent prophylactic intrapartum antibiotic treatment of SRA-positive pregnant women has been adopted in some countries (Le Doare and Heath, 2013).

Zoonotic transmission of SRA has been reported from various geographic regions. In South-East Asia, an SRA strain responsible for disease in aquaculture was transmitted to humans through the consumption of raw fish. This food-borne outbreak led to invasive cases of septic arthritis and sometimes lethal meningoencephalitis in healthy adults with no underlying risk factors (Barkham et al., 2019). Reports from northern Europe, North America and Colombia of SRA isolates from humans and cattle belonging to the same clonal complex indicate that humans may be involved in

introducing SRA on dairy farms (Cobo-Angel et al., 2019; Lyhs et al., 2016; Manning et al., 2010; Sørensen et al., 2019).

Bacterial factors contributing to pathogenicity include virulence genes, such as adhesins and invasins. Some specific virulence genes, *scpB* (C5a peptidase) and *lmb* (laminin-binding protein), are strongly associated with disease in humans (Gleich-Theurer et al., 2009; Sørensen et al., 2010).

2.8.2 *Streptococcus agalactiae* in cattle

This bacterial species was first described as a cause of bovine mastitis and is strongly associated with SCM in cattle, often developing into a chronic condition resulting in long-time carriers and negatively affecting SCC and milk yield (Holmøy et al., 2019; Keefe, 1997). Due to the low infection dose, SRA is regarded as highly contagious, requiring herd-level prevention strategies. In the pre-antibiotic era, SRA was a common mastitis pathogen on dairy farms, but with the arrival of antibiotics and control programmes, the pathogen has almost been eradicated in many countries. Nowadays, control programmes are based on screening and grouping of cows, antibiotic therapy, culling of persistently infected individuals, and improved milking hygiene and biosecurity (Landin et al., 2016). In countries with efficient and systematic implementation of these interventions, such as in Scandinavia, SRA is now a rare cause of bovine mastitis. In Sweden, a quarter prevalence of 0.2-0.6% has been reported in cases with CM or SCM, and herd prevalence was 3.3% in the last national screening (Ericsson Unnerstad et al., 2009; Persson et al., 2011; Växa Sverige, 2021), while in Denmark, herd prevalence is around 7% (Katholm et al., 2012). Where there are no eradication programmes, SRA is still a substantial problem for the dairy industry. The quarter prevalence of SCM is 34% in Colombia, 11.3% in Uruguay, 11% in Ethiopia and 10% in North America (Abera et al., 2012; Gianneechini et al., 2002; Ramírez et al., 2014; Wilson et al., 1997). In China and Germany, a herd prevalence of 72.3% and 30% has been reported (Bi et al., 2016; Tenhagen et al., 2006).

With the arrival of more advanced molecular techniques, the possibility of high-resolution genomic analyses has provided new insights into the epidemiology of SRA in dairy cattle. The local setting is a fundamental determinant of the epidemiology, and different transmission patterns have been described in high-income countries compared with developing dairy industries (Reyes et al., 2017). In industrialised countries,

SRA is usually introduced through the purchase of an infected animal, with one clone propagating within the herd (Agger et al., 1994; Baseggio et al., 1997; Bergseng et al., 2009; Lyhs et al., 2016; Mahmmod et al., 2015), whereas in developing dairy industries, a large strain heterogeneity is commonly seen at herd level (Carvalho-Castro et al., 2017; Cobo-Ángel et al., 2018). These contrasting situations can partly be attributed to variations in access to veterinary services, milking hygiene and biosecurity measures (Cobo-Ángel et al., 2018; Heffernan and Misturelli, 2000; Shome et al., 2012; Wolff et al., 2019).

The first reports of survival of SRA in the environment of dairy farms date back to the 1930s (Chodkowski, 1949). Traditionally, SRA was regarded as strictly udder-bound in cattle, but this paradigm is now being challenged. Molecular characterisation of environmental SRA on dairy farms (Cobo-Ángel et al., 2018; Jørgensen et al., 2016) has revealed the existence of extramammary reservoirs, and more complex transmission routes than the previously acknowledged udder-to-udder transmission route have been suggested.

The ability to ferment lactose is regarded as a key fitness advantage in the mammary gland, and some mastitis pathogens have often been shown to carry genes coding for this function. In SRA, lactose fermentation is genetically encoded on a so-called lactose operon, and genetic signatures that point to its mobility have been highlighted (Richards et al., 2011). The presence of a lactose operon within the genome was reported for all mastitis-causing SRA isolates of bovine origin in a study by Lyhs et al (2016).

2.8.3 *Streptococcus agalactiae* in camels

In camels, SRA is a common cause of mastitis and has predominantly been associated with SCM and production losses (Wahinya et al., 2014; Younan, 2002). Similar to the situation in cattle, there are indications of SRA causing chronic udder infections in camels (Abeer et al., 2016; Younan, 2002). More severe cases of infection have also been reported, such as an outbreak of gangrenous mastitis in camels in the UAE (El Tigani-Asil et al., 2020). The bacterial load in milk from camels with SRA mastitis is high and will quickly multiply during handling and transport (Farah and Fischer, 2004).

In addition to being an important udder pathogen, SRA can also cause opportunistic infections in other organ systems in camels. Contagious skin necrosis caused by SRA has been reported (Edelsten et al., 1974), as well as

septicaemia (Nour-Mohammadzadeh et al., 2010), pleuritis (Wernery et al., 2018), abscesses, puerperal metritis and septic arthritis (Younan and Bornstein, 2007). Furthermore, SRA has been isolated from the nasopharynx and vaginal mucosa of apparently healthy camels (Mutua et al., 2017; Younan and Bornstein, 2007). Genetic characterisation of extramammary isolates of SRA by Fischer et al. (2013) revealed a distinct camel clade, separated from human and cattle SRA. Moreover, there is an association between different sequence types (STs) and symptom-specific complexes (cough, mastitis, gingivitis, vaginal discharge wound infection/abscess). There is a current knowledge gap regarding the epidemiology of SRA in camel herds, and as yet, no efforts have been made to elucidate within and between-herd transmission patterns. As SRA appears to be less udder-bound in camels than has previously been assumed (Younan and Bornstein, 2007), the role of extramammary isolates in the development of mastitis requires exploration. Moreover, SRA in milk may pose a threat to human health as camel milk is commonly consumed without prior pasteurisation and cross-species transmission has been reported for other species (Barkham et al., 2019; Cobo-Angel et al., 2019).

2.9 Prevention and control of mastitis

Mastitis is a multifactorial disease and consequently complete elimination is not an achievable target. However, there are many strategies that have significantly contributed to disease reduction and improved udder health in cattle. Mastitis control and prevention are generally based on two main principles: the reduction of new IMI and the reduction in duration of already existing IMI (De Vliegher et al., 2018; Dodd et al., 1969). The five-point plan was developed in the 1960s for the cattle dairy industry and is based on the following five pillars for mastitis control: identification and treatment of clinical cases of mastitis, dry cow antibiotic therapy, post-milking teat disinfection, culling of persistently infected cows and careful maintenance of milking equipment (Bradley, 2002; Green et al., 2012; Neave et al., 1966). Mastitis therapy differs for CM and SCM. For cows with CM, therapy usually involves antibiotic drugs, chosen according to the causative agent and its susceptibility pattern. Supportive therapy, such as anti-inflammatory drugs, fluids and electrolytes, may be considered depending on the severity of disease. For SCM, treatment with antibiotics during drying-off is usually

recommended (Gruet et al., 2001.) Implementation of the five-point plan strategy has resulted in a lower incidence of IMI, primarily caused by contagious udder pathogens, and a reduction in SCC at herd level, as exemplified by the development in the UK (Tables 1 and 2). In contrast, systematic mastitis control programmes are still lacking in most LMICs. Although there is ongoing research on locally adapted intervention strategies, these are yet to be adopted at national level (Özkan Gülzari et al., 2020; Persson et al., 2019; Sah et al., 2020).

Table 1. Incidence and aetiology of clinical mastitis in UK dairy herds (quarter cases/100 cows/year) for the years 1967, 1982 and 1998. Modified from Bradley, 2002.

Pathogen	1967	1982	1998
Staphylococcus aureus	67	7	2.2
Streptococcus agalactiae	6	1	0
Streptococcus dysgalactiae	16	4	2.0
Streptococcus uberis	7	9	5.3
Escherichia coli	7	10	14.4
Other	50	9	17.7
Total	153	40	41.6

Table 2. Annual bulk milk somatic cell counts in the UK – proportion (%) of herds falling within different ranges for the years 1979, 1993 and 2001. Modified from Bradley, 2002.

Cell count ($\times 10^3$ cells/mL)	1979	1993	2001
<200	2	26	71
200 – 399	35	47	26
>399	63	27	3

Structural transformations of the bovine dairy sector in many industrialised countries have affected management systems and routines. For example, small-scale farms with tie-stall housing are being replaced with large-scale farms with free-stall housing. Intensive management, dietary changes and new milking methods, such as automatic milking systems, have been widely introduced. Furthermore, the increasing threat of AMR calls for prevention strategies that are less reliant on antibiotic use. To adapt to these

conditions, adjustments to the original strategy have been made, expanding it into more detailed schemes for mastitis control. In addition to the above-mentioned interventions, there is now an emphasis on the following areas: milking routines that optimise udder-emptying, avoidance of milking-related injuries, a clean, comfortable and dry environment, minimisation of contact between cows and pathogen reservoirs, good external and internal biosecurity, selective treatment of infected cows, and separate risk animals from healthy cows (Østerås and Sølverød, 2009). In the National Mastitis Council's (NMC) revised five-point plan, which was later expanded into the NMC's ten-point plan, good record keeping, establishment of goals for udder health, regular monitoring of udder health status and periodic reviews of the herd's mastitis control programme are also included (Middleton et al., 2014). In addition, products to protect the udder from invasive microorganisms during drying-off, by sealing the teat canal, are now commercially available (Parker et al., 2007).

Another way of improving udder health is by selection for mastitis resistance. Such a selection is now included in many breeding programmes. Initially, the focus was on udder morphology and low SCC, but thanks to the extensive record-keeping of diseases in many countries, low incidence of clinical mastitis has been proposed as a selective breeding criterion and has been adopted in Norway since the 1990s, showing acceptable heritability (Pyörälä, 2002).

Moreover, vaccines against several mastitis pathogens have been developed as a part of attempts to reduce the risk of IMI. However, the high number of mastitis pathogens and their large heterogeneity is a major challenge in developing effective vaccines against mastitis. Vaccines against *E. coli, S. aureus,* NAS and *Str. uberis* have been released on the market, but their efficiency in disease reduction is not universally accepted. Advances in the field of immunology provide new approaches to mastitis treatment, and there is ongoing research on immunomodulating drugs supporting the local immune system in the mammary gland, as well as other biotechnological methods to combat IMI (Pyörälä, 2002; Schukken et al., 2014).

Other factors affecting resistance to mastitis are the nutritional status of the animal and stress. Zinc, copper, selenium and vitamin E are all of great importance for the normal functioning of the immune system in the udder, and deficiencies have been associated with mastitis. Hence, dietary requirements of these substances must be taken into account to improve herd

udder health (Pyörälä, 2002). Feeding zinc supplements to lactating camels has been shown to strengthen the immune system and increase productivity (Mostafa et al., 2020). Stress is known to have a detrimental effect on the immune system and make the organism more susceptible to infection. In dairy cows, stress-free handling, avoidance of over-crowding etc. are often recommended to support the immune system and prevent mastitis and other infections (Giesecke, 1985).

Despite control efforts, mastitis remains a common and costly disease in dairy industries worldwide. Bacterial causes of SCM and CM differ between countries and dairy species and consequently control efforts need to be adapted locally. Targeted control strategies must be developed at a national level but also need to be adapted to farm/herd level. Nevertheless, considering the individual variation across herds regarding management routines, facilities and the health status of the herds, and socio-economic factors as well as the farmer's motivation and behaviour, an holistic approach is necessary for successful mastitis control (Zadoks and Fitzpatrick, 2009).

3. Aims of the thesis

The main goal of this thesis was to increase knowledge about SCM in pastoralist dairy camels with an emphasis on SCM caused by SRA in selected regions in northern Kenya. More specifically, the aims of this thesis were to:

- investigate the prevalence and bacterial aetiology of SCM in pastoralist dairy camels.
- determine risk factors associated with SCM, IMI and SRA-derived IMI at camel level in pastoralist dairy camels.
- study the relationship between the inflammatory markers SCC, NAGase and LDH in camel milk and SCM.
- study the occurrence of AMR in the most prevalent mastitis pathogens from pastoralist dairy camels.
- genetically characterise SRA collected from milk and extramammary sources in dairy camel herds.
- explore the molecular epidemiology of SRA in dairy camels.

4. Materials and methods

4.1 General aspects of study design

This thesis is based on three distinct field studies, which resulted in Papers I-IV (Fig. 6). A general description of the materials and methods is included below. For a more detailed report, please see the relevant sections in Papers I-IV.

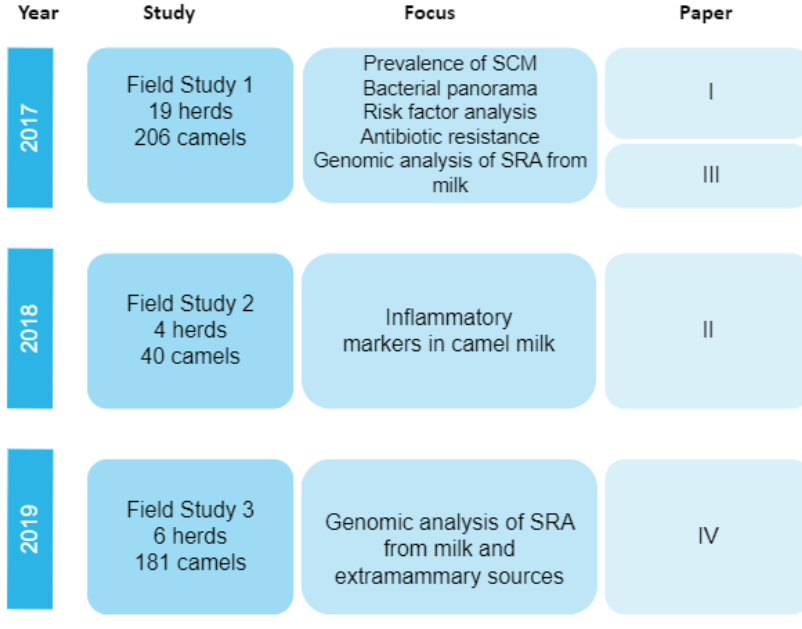

Figure 6. Schematic overview of the studies performed within this thesis, the main focus of each study, and the resulting scientific papers.

All three studies were cross-sectional. Field Studies 1 and 2 were based on convenience sampling whereas a purposive sampling strategy was used in Field Study 3.

4.2 Study areas

Sampling took place in Isiolo and Laikipia Counties, central Kenya (Fig. 7). The study areas were chosen based on the presence of extensive camel milk production in combination with a functioning infrastructure that facilitated access to camel herds.

Isiolo County covers an area of 25,382 km^2 and is mostly classified as very arid, with average annual rainfall of 150-250 mm (Mati et al., 2005). There are an estimated 45,309 camels in the region, distributed between 2,050 households (Isiolo county government and Department of Agriculture, Livestock & Fisheries Development, 2019; Musinga et al., 2008). These camel herds are mostly migratory and graze over large, remote areas where access to water is limited (Noor et al., 2013). Isiolo is the main camel milk hub in Kenya, with several milk clusters across the county. The highest density of camels can be found in the peri-urban areas around Isiolo town. Most milk is bulked in Isiolo town, with supply chains extending south to Nairobi (Muloi et al., 2018).

Laikipia County, located to the west of Isiolo County, is classified as semi-arid and has a large biodiversity. Land use is mainly divided between large-scale ranches, smallholder farmers and permanent agriculture (Georgiadis et al., 2007). The camel population is estimated to be 9,800, most of which are semi-sedentary and kept on ranches or by small holders, but nomadic pastoralists regularly cross the county border in search of grazing land during the dry season (Kenya National Bureau of Statistics & County Government of Laikipia, 2019).

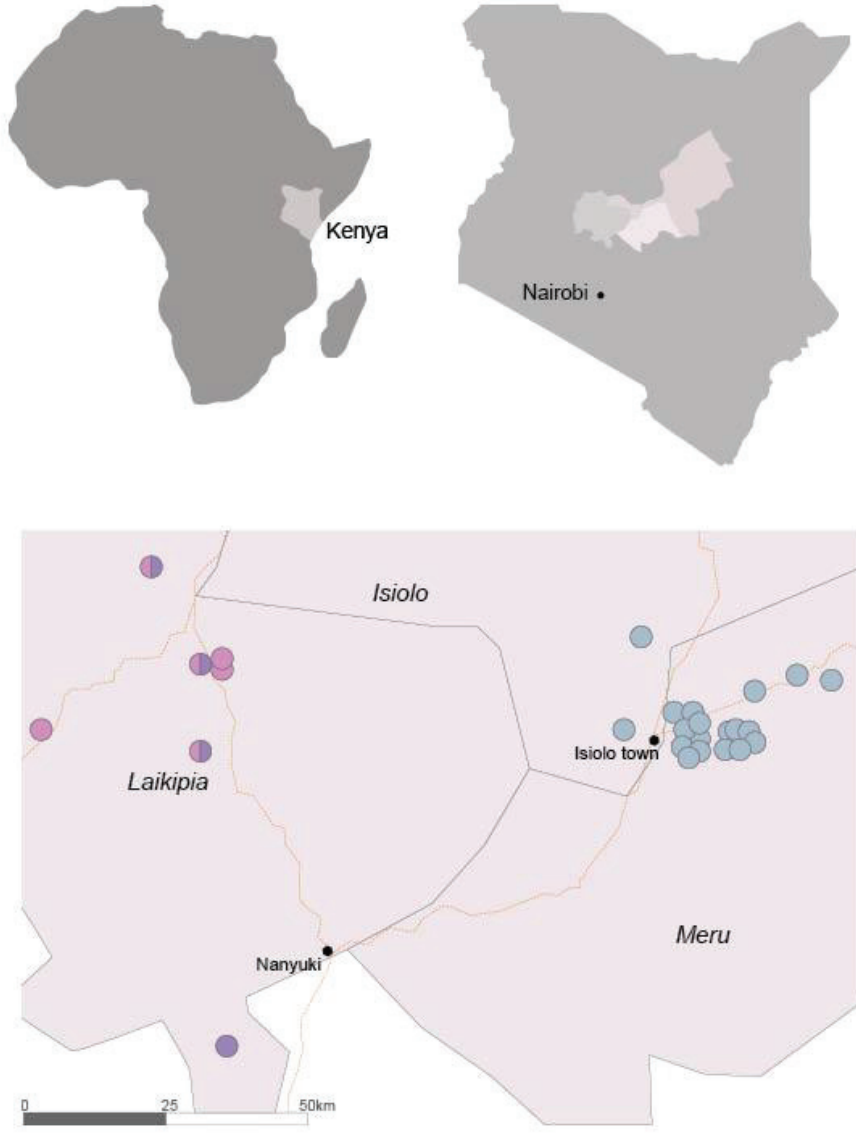

Figure 7. Map of Kenya, the selected study areas and location of herds included in Field Studies 1-3, indicated as dots. Colours correspond to the separate field studies; blue=Field Study 1; purple=Field Study 2; pink=Field Study 3. Multiple colours in a dot indicate inclusion of the herd in more than one study. Illustration: Miriam Seligsohn. © OpenStreetMap contributors. Data are available under the Open Database License (https://www.openstreetmap.org) under a CC BY-SA license.

4.3 Herd visits and sample collection

In Field Study 1, herds eligible for selection had to be managed under pastoralist conditions. Other factors were accessibility and a willingness among animal owners and herders to participate. For Field Study 2, four camel herds were selected with the inclusion criteria that the herds were kept extensively, were being milked, were geographically accessible and the herders/owners were willing to participate. In addition to inclusion criteria for Field Study 2, knowledge about mastitis prevalence in the herds from Field Study 2 was also considered in Field Study 3. In each field study, the final sample size was also based on financial constraints and time limitations. All the herds were visited once in each field study. Three of the herds in Field Study 2 were also included in Field Study 3.

Prior to sampling, animal owners and herders were informed about the purpose of the study and the sampling procedures, and their oral consent to take part was obtained. Participants were informed that participation would be anonymous and that they could withdraw from the study at any time if they wished.

4.3.1 Udder examination, CMT testing and milk sampling

Milk sampling and CMT testing took place during the first morning milking between 4 am and 8 am. Milk let-down was initiated by the suckling of the calf. Prior to sampling, a clinical examination of the udder was carried out. Signs of an acute clinical inflammation, such as swelling, redness, pain and changes in the milk (clotting, colour, smell, blood), were recorded and classified as acute clinical mastitis (ACM). In Field Study 3, the presence of induration of udder quarters in combination with CMT≥3 was classified as chronic clinical mastitis (CCM); SCM was defined as CMT≥3 with no clinical signs of inflammation. Skin lesions on udder or teat skin, teat tying and the presence of ticks on the udder were noted (Field Study 3). Lactating camels were selected for CMT testing; scoring was done according to a five-graded scale where 1 represented no changes in viscosity and 5 represented a drastic increase in viscosity with a distinct gel peak formation (Klastrup and Madsen, 1974). In Field Study 1, every second camel was tested with CMT and milk sampled at quarter level until a target of 10 camels per herd was met. For herds with fewer than 20 lactating camels, all the camels that were being milked were sampled.

In Field Study 2, the udders of all lactating camels were screened by CMT and milk samples were collected from the respective quarters from camels with a CMT score of ≥2 in one quarter and CMT<2 in the ipsilateral quarter. In Field Study 3, all lactating camels were tested with CMT and composite milk samples were collected from camels with CMT≥2 in at least one quarter. Teats were disinfected with antiseptic wipes prior to sampling, and milk samples were collected aseptically in sterile plastic vials. In Field Study 2, the vials contained colourless bronopol (SVA, Sweden) to prevent bacterial growth and improve conditions for analysis of enzyme activity. Milk samples were kept cold during transport using cool bags and were frozen at -20°C within 4 h of collection.

4.3.2 Collection of swabs

In Field Study 3, swab samples were collected from all lactating camels and their suckling calves. Calves were sampled before being released to suckle their mothers at the morning milking.

Swab samples were collected using sterile flocked nylon swabs (e-swab, COPAN Diagnostics Inc., Murrieta, CA, US). Swabs were taken from the nasal and vaginal mucosa of lactating camels, and from the nasal, pharyngeal and rectal mucosa of their calves. The nasal mucosa was sampled by flaring the nostrils and swabbing the inside of the nasal cavity of both nostrils. The vaginal mucosa was sampled by separating the labia, inserting the swab and rolling it against the vaginal wall. Oral samples were collected by inserting the swab into the oral cavity of the calf and gently rolling it against the buccal, lingual and pharyngeal mucosa. The rectal mucosa was sampled by inserting the swab in the rectum and gently rolling it against the rectal wall. Swab samples were kept refrigerated at 4-8°C and cultured within 1-10 days of collection.

4.3.3 Interviews

In Field Study 1, individual interviews were conducted with one representative owner or herder of each selected herd using a structured questionnaire. Pre-testing of the questionnaire was undertaken with six herders in two herds, and adjustments made accordingly. The questions were designed, displayed and recorded in English on a tablet using a free open-source software (KoBoToolbox, 2017). Interviews were performed in Oromo, Gabbra or Swahili by the same multi-lingual facilitator. Individual

camel data (age, parity, months in lactation, previous history of mastitis, ongoing treatments, general condition), as well as herd data (herd management and risk factors for subclinical mastitis) were recorded. The interviews were conducted during or after milk sampling.

4.4 Laboratory analyses

The laboratory analyses were performed in Kenya, Sweden and Denmark. SCC was analysed at the Mpala Research Centre (Nanyuki, Kenya) (Field Study 2). For bacterial culturing, facilities at a commercial accredited laboratory, Analabs Ltd. (Nairobi, Kenya; Field Study 1) and at the Department of Public Health, Pharmacology and Toxicology, University of Nairobi (Nairobi, Kenya; Field Study 3) were used. Enzymatic activity of NAGase and LDH was measured at the Department of Animal Science, Aarhus University (Aarhus, Denmark). Confirmation of bacterial species with MALDI-TOF MS, all phenotypic testing of antimicrobial susceptibility and lactose fermentation, and PCR were carried out at the National Veterinary Institute (SVA; Uppsala, Sweden).

4.4.1 Measurement of inflammatory markers

In Field Study 2, SCC (cells/mL) was measured using a portable direct cell counter (DCC, DeLaval International AB, Tumba, Sweden (Berry and Broughan, 2007; Gonzalo et al., 2004)) on the day of milk sampling. A subset of milk samples was frozen at -20 °C and transported on dry ice to the University of Aarhus. Assessment of enzyme activity was performed using kinetic fluorometric measurements according to Larsen (2005) and Larsen et al. (2010).

4.4.2 Bacteriological analyses

All milk samples were kept frozen at -20 °C after sampling and transported to Nairobi for further processing (Field Studies 1 and 3). Milk samples were cultured within 1-10 days of collection and thawed at room temperature. Plates were incubated aerobically at 37 °C for 24 and 48 hours before final examination, unless otherwise specified in the text.

In Field Study 1, all milk samples were cultured on blood agar (BA) (CM0271, Oxoid, Thermo Fisher Scientific, Waltham, MA) containing 5%

defibrinated sheep blood, and on modified Edward's agar (EA) (Oxoid, CM0027), a medium selective for streptococci and enterococci and an indicator medium for SRA. A calibrated loop was used to plate 10 µL of each milk sample. Milk samples with CMT≥3 but displaying negative growth were recultured. For samples with negative growth on BA, 40 µL of milk was plated on BA and incubated for 24-48 h. For CMT-positive samples that were negative on EA, 1 mL of milk was added to Todd Hewitt (TH) broth (Oxoid, CM0189), an enrichment for streptococci, which was incubated for 24 h at 37 °C and then plated on EA (Fig. 8).

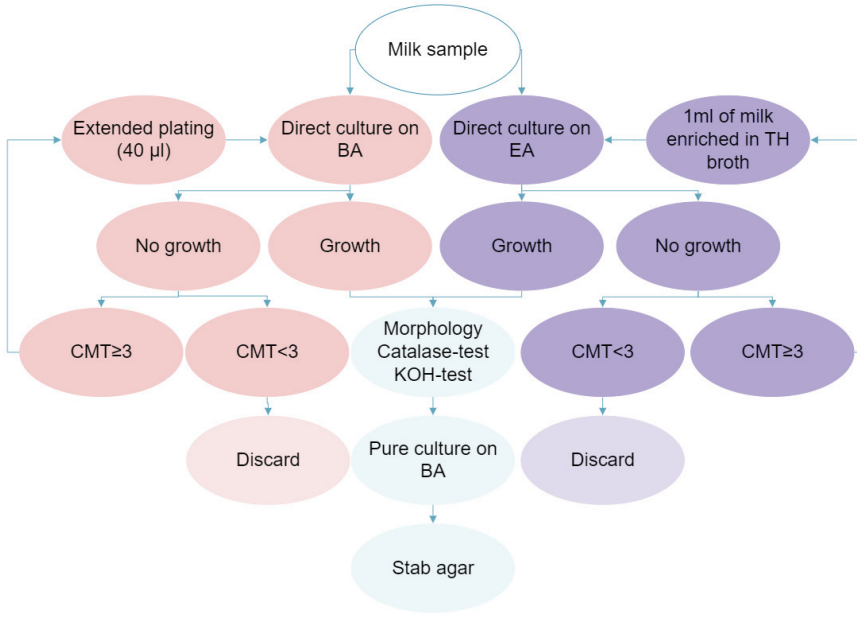

Figure 8. Schematic overview of the protocol for bacteriological analysis of milk samples in Field Study 1.

In Field Study 3, milk samples were cultured directly on EA after thawing and were then incubated. For milk samples that were negative for SRAafter direct culture, 1 mL of sample was enriched for 18 to 24 h at 37 °C in TH broth and plated on EA. Swab samples were enriched in TH broth for 18-24 h before being plated on EA.

Primary identification was based on morphology, the catalase test and the KOH (potassium hydroxide) test. In Field Study 1, growth of ≥5 CFU in pure

culture was classified as significant growth. For SRA and *S. aureus*, 1≥CFU was recorded as significant. If the plate displayed growth of three or more phenotypically different bacterial species, and no major udder pathogens were present, the sample was classified as contaminated.

In Field Study 3, blue pigmented, catalase-negative colonies with or without a clear β-haemolysis were subjected to the CAMP (Christie, Atkins, Munch, Petersen) test (Christie et al., 1944). Species confirmation of CAMP-positive isolates was performed by a slide latex agglutination test (Streptex Latex Agglutination Test, ThermoFisher Scientific Inc., Waltham, MA, USA).

In Field Studies 1 and 3, all bacterial isolates were purified on BA for 24 h at 37 °C, and inoculated on stab agars (SVA, Sweden) for 8-12 h at 37 °C before refrigeration at 4 °C. Stab agars were transported from Nairobi to SVA in Sweden at ambient temperature for 24 h. All isolates were re-cultured on BA containing 5% defibrinated bovine blood (SVA, Sweden) and checked for purity. Isolates were subjected to MALDI-TOF MS and analysed in duplicates. The following criteria were set for species identification: a score of ≥2 indicated identification at species level, a score of 1.80-1.99 at genus level, and a score below 1.80 classified as invalid (no identification). A custom-made database was used to perform the analysis, including the Bruker databases no. 5627 and no. 5989, with custom-made spectrum (MSP, Main Spectrum Profiles), for some NAS species (two *Staphylococcus devriesei*, three *Staphylococcus pseudintermedius*, two *Staphylococcys rostri*, one *Staphylococcus felurettii* and one *Staphylococcus lentus Puerto-Rico*).

In Paper III, one SRA isolate per camel out of 154 isolates originating from 65 camels in 19 herds (Paper I) was arbitrarily selected for further characterisation. In Paper IV, all SRA isolates were further characterised. All SRA isolates in Papers III and IV were subjected to lactose typing. Phenotypic lactose fermentation was assessed by inoculating each of the selected isolates on bromocresol purple lactose agar (SVA, Sweden) aerobically at 37 °C. Plates were incubated for seven days and checked for colour change after 24 h, 48 h, 72 h and 7 days. A yellow colour indicated lactose fermentation, while no colour change was classified as negative for lactose fermentation. *E. coli,* ATCC35218, was used as a positive control and *Proteus mirabilis,* CUG26767, as a negative control. The findings in the phenotypic assessment were confirmed using PCR, targeting a ≈2.5-kbp

region straddling *lacEG*. Positive and negative controls were selected from the study isolates based on genomic detection of Lac.2.

4.4.3 Antibiotic susceptibility testing

Antibiotic susceptibility was investigated by broth microdilution in 210 arbitrarily selected isolates from 81 camels and 192 udder quarters from three species groups (Paper I), and in 58 SRA isolates originating from milk and extramammary sources in 48 camels (Paper IV). To determine minimum inhibitory concentration (MIC), VetMIC panels (SVA, Sweden) (Paper I), Sensititre™ STAFSTR panels (TREK Diagnostic System, UK), Sensititre™ NLD1GNS panels (TREK Diagnostic System, UK) (Paper IV) and cation-adjusted Mueller Hinton broth (Becton Dickinson, Cockeysville, USA) were used. All testing was undertaken according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) (Clinical and Laboratory Standards Institute, 2017). A quality control strain, *S. aureus* ATCC 15019, was tested in parallel and the results were within acceptable ranges. Determination of MIC was done for the following antibiotic substances: penicillin, cephalotin, oxacillin, erythromycin, chloramphenicol, clindamycin, tetracycline, fusidic acid, gentamicin, kanamycin, ciprofloxacin and trimethoprim, enrofloxacin, nitrofurantoin and trimethoprim-sulfametoxazol. Details on the differences between the panels used are specified in Papers I and IV. All isolates were classified as wild type or non-wild type (acquired reduced susceptibility), based on bacterial species-specific epidemiological cut-off (ECOFFs) values issued by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2017). As ECOFFs are lacking for many veterinary pathogens, MIC were compared to clinical breakpoints from SVA or CLSI when necessary, the breakpoints used for each substance are specified in Papers I and IV.

4.4.4 DNA extraction and whole-genome sequencing of *Streptococcus agalactiae* isolates

In Paper III, one SRA isolate per camel out of 154 isolates originating from 65 camels in 19 herds (Paper I) was arbitrarily selected for whole-genome sequencing (WGS). For Paper IV, all collected SRA isolates were selected for WGS. The DNA extraction was performed using a magnetic bead-based method. A calibrated loop (10 µL) was used to collect colony material and suspend it in 600 µL nuclease-free water (Sigma-Aldrich, St Louis, MO,

USA) followed by mixing with 0.1 mm silica beads (BioSpec Products Inc., Bartlesville, USA). The suspension was placed in the FastPrep24 (MP Biomedicals LLC, Irvine, CA, USA) and run at 6.5 m/s for three two-minute cycles. Using the IndiMag Pathogen kit (Indical Bioscience GmbH, Leipzig, Germany), DNA was extracted and eluted in nuclease-free water. The Invitrogen Qubit 3.0 Fluorometer (ThermoFisher Scientific Inc., Waltham, MA, USA) was used to measure DNA concentration, which was then adjusted to 7.5 ng/µL. Library preparation and whole-genome sequencing were performed by Clinical Genomics, Science for Life Laboratory (Clinical Genomics, Solna, Sweden). Sequencing was done with the Illumina NovaSeq system (Illumina Inc., CA, US), generating paired-end libraries of 150bp read length.

4.5 Data analyses

4.5.1 Data management and statistical analysis

Descriptive statistics were used to summarise distribution of the CMT scores, bacteriological findings (Papers I and IV), results from the clinical examinations and distribution of AMR among bacterial isolates (Papers I and IV), as well as the distribution of sequence types (STs), capsular serotypes, AMR genes and lactose operons (Papers III and IV). Prevalence of SCM, IMI and SRA was calculated at quarter, camel and herd level. In Paper I, associations between dependent variables and independent variables were investigated using univariable mixed effect logistic regression analyses, with herd included as a random factor. To investigate associations between inflammatory markers SCC, NAGase and LDH (Paper II), a univariable linear mixed-effect regression analysis was used. Continuous variables were visually assessed if they were linearly related to the outcome. If not linearly related, dependent continuous variables were categorised using percentiles as cut-offs (Paper I), and dependent continuous variables were transformed to the natural logarithm (ln) using the lnskew0 command in Stata. In Paper I, outcomes with a *p*-value ≤ 0.20 were included in a multivariable model. Before analysis, independent variables were checked for collinearity by pairwise Spearman rank correlations. If proof of collinearity ($r \geq 0.70$) was present, the variable with the lowest *p*-value in the univariable analysis was selected. For the multivariable models, a manual stepwise-backward variable

selection was applied, with all variables included from the beginning as main effects. All plausible two-way interactions between the significant main effects were tested and all variables with a significant association ($p<0.05$) with the dependent variable were kept in the model. The variables "SCM", "IMI" and "SRA", when used as independent variables, were excluded from the respective models in the final analysis since their strong collinearity obscured other potential relations with the main effects. Model of fit of the multivariable analyses was assessed by visual assessment of diagnostic plots according to Dohoo et al. (2009). In Papers III and IV, associations between categorical variables were assessed using Pearson's chi-squared test or Fischer's exact test, when suitable. Data were edited in Microsoft Excel (Microsoft Corp., Redmond, WA). All statistical analyses were performed in Stata software (StataCorp., 2013, Stata Statistical Software: Release 13.1; College Station, TX, USA: StataCorp LP).

4.5.2 Bioinformatic analysis of sequence data

ConDeTri suite v2.3 (Smeds and Künstner, 2011) was used for trimming of reads and for filtering PCR duplicates. Trimmed reads were then assembled *de novo* using SPAdes v3.13.1 (Bankevich et al., 2012) and a quality check was performed with QUAST v5.0.2 (Bankevich et al., 2012), evaluation of the total length of the genomes, the total number of contigs and the GC content (%). Species confirmation was carried out with KmerFinder v3.2 (Cineros and Lund, 2017). Multi-locus sequence typing (MLST) was performed with SRST2 v0.2.0 (Inouye et al., 2014). When new allele variants were found, these were submitted to pubMLST (Jolley et al., 2018) for assignment of new allele numbers and subsequently the MLST profiles were submitted for ST assignment. Detection of capsular serotype was carried out *in silico*, using a standard method (Metcalf et al., 2017). The presence of AMR genes in the genome was investigated using SRS2 v0.2.0 from raw sequence reads with the ARG-ANNOT v3 database (Gupta et al., 2014). All assembled genomes were scanned for the presence of a lactose operon (Lac.2) (Richards et al., 2011) with a BLASTn v2.9.0 search (Camacho et al., 2009). Further confirmation of Lac.2-negative isolates (based on the BLAST v2.9.0 search) was performed by scanning annotation files created with Prokka v1.14.6 (Seemann, 2014) for genes carried by Lac.2 (*lacE* and *lacG* in particular) (Papers III and IV). In Paper III, assembled genomes were screened for the presence of human-associated

virulence genes *scpB* and *lmb* (Morach et al., 2018) using tBLASTn. Pairwise distances between single nucleotide polymorphisms (SNPs) in genomes belonging to ST616 were calculated using pairsnp v.0.2.0 (Tonkin-Hill, 2021) and plotted using matplotlib v3.3.2 (Hunter, 2007), with a focus on the comparison of within- and between-herd pairwise distances.

In Papers III and IV, Snippy v4.6.0 (Seemann, 2021) was used to create a core genome alignment using ILRI112 as a reference genome (accession Hf952106) (a Kenyan camel ST617 isolate). A maximum likelihood tree was inferred with RAxML-NG-v0.9.0 (Kozlov et al., 2019) under a GTR+G model to investigate phylogenetic relationships between isolates.

5. Results

5.1 Herd characteristics and management (Papers I-IV)

Field Study 1 comprised 20 pastoralist herds in the area around Isiolo town. All the herds were mobile and would be moved depending on available pasture. In Field Study 2, four herds were included: two were ranched herds (they belonged to private landowners with no tradition of keeping camels and employed workers handling their livestock) and two were pastoralist herds. Field Study 3 was conducted on three ranch herds, two smallholder herds (smaller herds managed by families and kept at the homestead, together with other types of animals), and one pastoralist herd. The total herd size ranged between 21 and 200 camels; these figures are estimates as the total number of animals was regarded as confidential in many of the pastoralist herds. The lactating herd size ranged between 7 and 52 camels.

All the herds included in the studies sold milk, except for one smallholder herd. Milking frequency varied between one and four times a day, and the herder of one herd reported that milking frequency would be adjusted depending on the season, with less frequent milkings in the dry season. In Field Studies 2 and 3, camels were watered at intervals of one to seven days (not known for Field Study 1).

A milking order was employed in eight of the 20 herds in Field Study 1, and in four of the six herds in Field Study 3. The most common milking order was based on milking the newly-calved camels first; this routine was applied in four of eight herds in Field Study 1, and in three of four herds in Field Study 3. In three herds (Field Study 1), the youngest camels would be milked first, in one herd the oldest camels would be milked first (Field Study 3), and in one herd, the herders reported that the milking order depended on the individual milking frequency for each camel (Field Study 1).

Adequate milking hygiene was largely absent in most of the study herds. In Field Study 1, 18 out of 20 herds reported that they would sometimes or always wash their hands prior to milking, but this was not observed during any of the visits. In Field Study 3, hand-washing prior to milking was not practised in any of the visited herds. In two of the ranch herds, post-milking hand washing was practised (Field Study 3). Post-milking teat disinfection was not undertaken in any of the visited herds (Field Studies 1-3).

In all the herds in Field Study 1, herders were familiar with the symptoms of CM. The treatment normally employed in the majority of herds (17 out of 20) was injectable antibiotics, most often using a combination of benzylpenicillin and streptomycin ("PenStrep", 20 out of 20 herds). Oxytetracycline was the second most used type of antibiotic, reported by 12 out of 20 herds (60%).

When the interviewees in Field Study 1 were asked what they thought the main constraints were with regard to milk production, disease was mentioned in 13 out of 20 herds, followed by drought (7 out of 20), lack of veterinary services, and security due to banditry and theft (6 out of 20 respectively) (Fig. 9**)**.

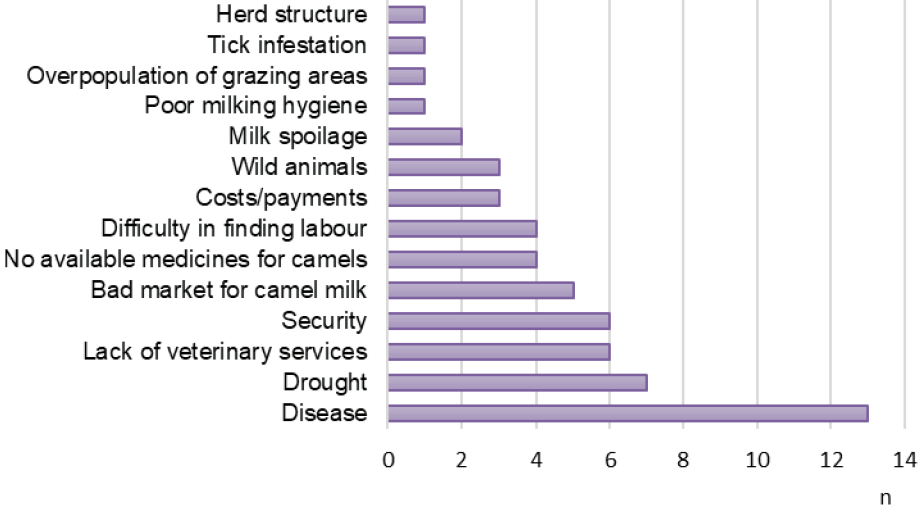

Figure 9. The main constraints to milk production, reported by interviewed camel herders and owners in 20 camel herds around Isiolo town.

In the same study, the most common disease among the camels, according to the herders and owners and mentioned in 14 out of 20 herds, was a disease syndrome called “swollen glands”. This may refer to haemorrhagic septicaemia, but the origin of this disease is not verified (Wako et al., 2016). Other conditions were ranked in the following order: skin-related conditions (8 out of 20 herds), respiratory disease and trypanosomiasis caused by *Trypanosoma evansi* (‘surra’) (6 out of 20 herds respectively). Mastitis was only mentioned as a common disease in one herd.

5.2 Clinical examination of the udder and prevalence of CM and SCM at herd, camel and quarter level (Paper I)

In Field Study 1, 804 udder quarters from 206 camels in 20 herds were included for sampling (Fig. 10). Clinical examination revealed that 17 out of 804 (2%) examined udder quarters in 11 out of 206 (5%) of the camels displayed symptoms of acute clinical mastitis (ACM; swelling or pain of the udder, changes in milk). Moreover, chronic clinical mastitis (CCM; palpatory changes in the udder tissue and induration, in combination with CMT≥3) was diagnosed in 28 out of 804 (4%) quarters in 20 out of 206 (10%) camels included in the study. Induration without an elevated cell count (CMT) was found in 10 out of 206 (5%) camels. Non-functioning quarters, so-called ‘blind teats’, were found in 13 out of 206 (6%) of the camels, and lesions on the teat or udder skin, defined as visible damage to the skin, were recorded in 18 out of 206 (9%) camels. Tick infestation of the udder or teats was noted in 28 out of 206 (14%) camels and the practice of teat tying was recorded for 7 of 206 (3%) camels. A total of 22 out of 206 (11%) camels had reportedly had CM previously.

Subclinical mastitis (CMT score of ≥3 and no clinical abnormalities) was found in 207 out of the 804 (26%) examined quarters and in at least one quarter in 95 of out 206 (46%) camels. Many of the camels had SCM in more than one quarter, 21 of the 95 SCM-positive camels (22%) had SCM in two quarters, 24 out of 95 (25%) had SCM in three quarters, and in 12 out of 95 (13%) camels, all four quarters were diagnosed with SCM. Furthermore, SCM was present in at least one camel in all 20 herds.

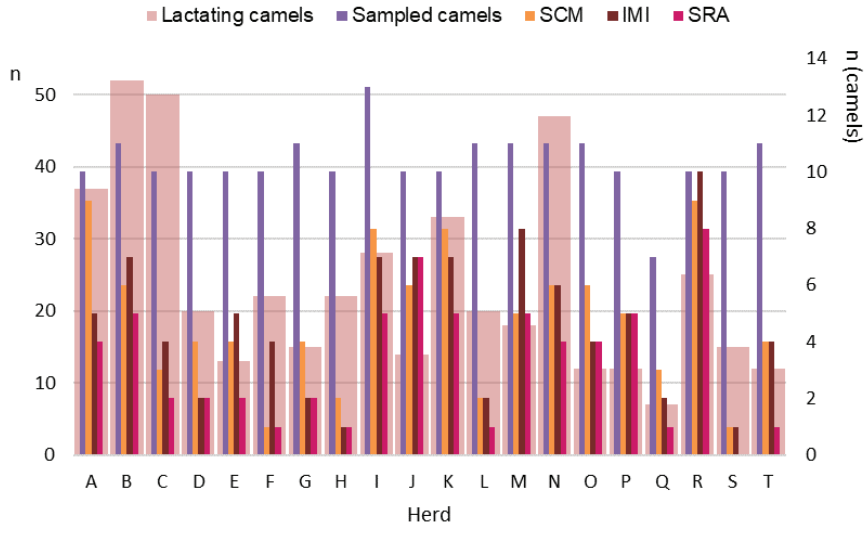

Figure 10. Distribution of the number (n) of lactating camels in each herd (A-T) (left y-axis), the number (n) of camels sampled and camels positive for subclinical mastitis (SCM), intramammary infection (IMI) and *Streptococcus agalactiae* (SRA) (right y-axis), respectively, in a prevalence study including 206 camels from 20 herds, sampled in Isiolo County in Kenya, 2017 (Paper I).

5.3 Intramammary infections (Papers I and IV)

In Field Study 1, a total of 798 milk samples from 206 camels were collected, cultured and analysed. An IMI was present in at least one quarter in 93 of the 145 (64%) camels (Fig. 10) and in a third (215 out of 798) of all quarters. A specific bacteriological diagnosis could not be confirmed due to inconclusive growth or incomplete sampling in 61 camels. A high proportion of IMI-positive camels had more than one infected quarter. Two infected quarters were found in 24 out of 93 (26%) camels, three infected quarters were found in 23 out of 93 (25%) camels, and 17 out of 93 (18%) camels had an IMI in all four quarters. The sensitivity and specificity for CMT in correctly identifying IMI-positive quarters were 82.2% (95% CI: 76.5-87.1) and 91.3% (95% CI: 88.3-93.7), respectively, when using CMT <3 and CMT >3 as cut-offs.

Culture results revealed that SRA was the most common udder pathogen, isolated from 154 out of 215 (72%) of the IMI-positive quarters. There was an overall prevalence of 19% (154 out of 798) at quarter level, 32% (62 out

of 206) at camel level, and 95% (19 out of 20) at herd level. The herd-wise distribution of SRA-positive camels is shown in Figure 10. Non-aureus staphylococci were the second most common udder pathogens. Species belonging to this group were isolated from 41 out of 215 (19%) IMI-positive quarters. The distribution of NAS was as follows: *S. simulans* was found in 16 samples, *S. delphini* was isolated from six samples, *S. rostri* and *S. chromogenes* were each isolated from five samples, *S. epidermidis* was found in three samples, *S. hyicus* in two samples, and *S. haemolyticus* and *S. warneri* in one sample each. In three samples, identification could only be done at genus level, and the isolates were classified as staphylococci spp. *Staphylococcus aureus* was found in 28 out of 215 IMI-positive quarters; in ten of these, the culture yielded a combination of *S. aureus* and SRA. Eleven milk samples yielded bacterial isolates not recognised by MALDI-TOF MS and were classified as "unidentified"; this group included the *Staphylococcus* spp. isolates. A total of 136 out of 798 (17%) milk samples were classified as contaminated.

Out of the 50 composite milk samples collected from camels with CMT≥2 in at least one quarter in Field Study 3, ten were culture-positive for SRA.

5.4 Risk factors for SCM, IMI and SRA (Paper I)

The univariable risk factor analysis for SCM included 146 camels from 19 herds. Camels with CM in at least one udder quarter were excluded from analysis, as were ten other camels due to missing data. Age (p<0.001) and lactation stage (p=0.001) were significantly associated with SCM, and camels of a higher age (>9 years) or in mid to late lactation (≥5 months) were more at risk of SCM than camels younger than 9 years and in the first to fourth month of lactation. Furthermore, skin lesions on the teats or udder were associated with an increased risk of SCM (p=0.004). In the final multivariable mixed-effect logistic regression analysis, six variables from the univariable analysis were included. The variable parity was excluded due to collinearity with the variable age. The final model showed that the odds of SCM were higher in the later months of lactation (month 12-23) in comparison with camels in the first to fourth month of lactation (odds ratio (OR)=0.008, p<0.001), and in camels in lactation month 5 to 11 in

comparison with camels in the first two months of lactation (OR=0.31, p=0.05).

The univariable risk factor analysis for IMI revealed that there was a significant linear increase in IMI with increasing age (p<0.001) and parity (p=0.006). For camels with a reported history of previous CM, there was a higher risk of IMI (p=0.003). Six of the variables investigated in the univariable analysis were included in the multivariable mixed-effect logistic regression model. Collinearity was found between the variables parity and age, and subsequently "age" was kept in the model due to the lower p-value in the univariable analysis. The only remaining variable in the final model was age, showing an increased risk in camels older than seven years compared with camels younger than seven years (p <0.001). This association was seen for the age categories 7 to 8 years (OR=3.5, p=0.04), 9 to 10 years (OR=5.0, p=0.001), and camels 11 years or older (OR=20.2, p=0.001).

As SRA was the predominant udder pathogen found in the bacteriological analysis, potential risk factors for this bacterium were investigated. The univariable risk factor analysis showed that findings of SRA were associated with parity (p<0.001), age (p<0.001), a previous history of mastitis (p=0.001) and findings of induration in the udder tissue (p=0.06). Camels with an obstructed (blind) teat were more likely to have IMI caused by SRA in at least one quarter (p=0.002) in comparison with healthy camels or camels with IMI caused by other udder pathogens. In the multivariable mixed-effect logistic regression model, seven variables were included and three remained in the final model. Collinearity was found between the variables age and parity, thus the variable age was kept in the model according to the criteria described above. In the final model, age (older than 11 years compared with camels younger than 11 years) was significantly associated with SRA findings in milk (p<0.001). Furthermore, a reported history of previous episodes of CM (OR=12.3, p=0.001) and having at least one blind quarter (OR=5.28, p=0.049) were associated with SRA-derived IMI.

The variables teat-tying and presence of ticks on the teats or udder were not significant risk factors for SCM, IMI or SRA.

5.5 Inflammatory markers (Paper II)

In Paper II, 158 quarters in 40 camels from four herds were tested by CMT, and milk samples were collected from 116 of them. The remaining 42

quarters had a CMT score of 1 and were excluded from further analyses since they could not be matched with a CMT ≥2 quarter on the same side of the udder. Median CMT for the 116 quarters included in the study was 1 (Interquartile range [IQR]: 1-3). Subclinical mastitis was defined as no clinical abnormalities of the udder and a CMT score of ≥3. Almost a third (36 of 116, 31%) of the tested quarters had SCM.

SCC was analysed in all milk samples. The median and mean values for SCC were 151,000 cells/mL (IQR: 49,500-709,000 cells/mL) and 780,974 cells/mL (SD: 1,401,466 cells/mL), respectively. Enzymatic activity of NAGase and LDH was assessed in a subset of 88 of these milk samples (originating from 34 camels), due to an insufficient milk volume in the other samples. Mean and median values for NAGase activity were 18.5 U/l (IQR:14.8-24.0 U/L) and 23.5 U/l (SD:18.6 U/l), respectively. For LDH activity, the mean and median values were 15.6 U/l (SD: 14.0 U/l) and 12.0 U/l (IQR: 8.5-16.2 U/l), respectively. Distribution of SCC, NAGase and LDH for each CMT class is shown in Table 3.

Table 3. Distribution (n=number of samples) of median, interquartile range (IQR), mean and standard deviation (SD) for the inflammatory markers somatic cell count (SCC), N-acetyl-β-D-glucosaminidase (NAGase) and lactate dehydrogenase (LDH) for CMT (California Mastitis Test)-categories in camel milk from 116 (SCC) and 88 (NAGase and LDH) quarters, in 40 and 34 camels from four herds in Laikipia County, Kenya.

CMT score	**1**	**2**	**3**	**4**	**5**
n (%)	60 (51.7)	22 (19.0)	14 (12.1)	11 (9.5)	9 (7.8)
SCC×10^3 cells/mL					
n	60	22	14	11	9
Median	54.5	170	562	1318	4202
(IQR)	(23.5-129)	(123-470)	(331-906)	(889-2018)	(3827-5359)
Mean (SD)	273 (895)	298 (232)	811 (790)	1645 (1434)	4249 (1208)
NAGase					
n	52	13	10	8	5
Median	16.5	20.8	17.9	24.8	42.1
(IQR)	(13.3-20.8)	(17.9-27.7)	(15.8-21.1)	(19.8-36.0)	(30.7-45.1)
Mean (SD)	18.7 (8.5)	22.2 (6.5)	26.9 (28.0)	32.6 (24.9)	55.6 (42.6)
LDH					
n	52	13	10	8	5
Median	8.8	13.0	13.0	23.1	57.7
(IQR)	(7.0-12.9)	(10.9-14.6)	(10.5-18.4)	(15.5-25.7)	(35.1-75.1)
Mean (SD)	10.6 (5.5)	14.6 (6.8)	14.7 (5.5)	24.7 (13.4)	58.0 (26.1)

In SCM-positive quarters, the mean value for SCC was 1,888,300 cells/mL (SD: 1,801,000 cells/mL), compared with 282,700 cells/mL (SD: 782,000) in milk from SCM-negative quarters. For NAGase, the mean value in milk from SCM-positive quarters was 35.1 U/l (SD: 31.3 U/l), compared with 19.4 U/l (SD: 8.2 U/l) in milk from SCM-negative quarters. The mean value for LDH-activity in milk from SCM-positive quarters was 27.6 U/l (SD: 22.0 U/l). In milk from SCM-negative quarters, the mean value for LDH-activity was 11.4 U/l (SD: 6.0 U/l).

Univariable mixed-effect linear regression analysis revealed a significant association between SCM and lnSCC ($p<0.001$), between SCM and lnNAGase ($p=0.001$) and between SCM and lnLDH ($p<0.001$). All inflammatory markers were positively correlated. The correlation coefficients for lnSCC and lnNAGase, lnSCC and lnLDH and lnNAGase and lnLDH were 0.54, 0.66 and 0.46, respectively.

5.6 Isolation of *Streptococcus agalactiae* from milk and extramammary sources (Paper IV)

In all, 29 out of 88 adult camels and 19 out of 95 calves were positive for SRA in at least one sample. In adult camels, SRA was detected in 24 nasal swabs and in 10 milk samples, whereas in calves it was isolated from 14 nasal swabs, seven oral swabs and three rectal swabs. All swabs were collected from clinically healthy sample sites, with the only exception of orf-like lesions on the nose and around the mouth in all calves in herd C. Most SRA isolates were collected from nasal swabs (38 out of 151). Only seven oral swabs from three herds and three rectal swabs from two herds were SRA-positive. No SRA was detected in the vaginal swabs. Furthermore, isolation of SRA in more than one sampling site in the same individual was observed in nine camels: three adults and six calves. The distribution of positive and negative samples in relation to sample site is shown in Table 4.

Table 4. Number of samples positive/negative for S*treptococcus agalactiae*, the mean prevalence, standard deviation (SD) and 95% confidence intervals (CI) for each sample category (Paper IV).

Source	Positive	Negative	Mean (SD)	95% CI
Nasal swab	38	113	0.25 (0.04)	0.18-0.32
Milk sample	10	40	0.20 (0.06)	0.09-0.32
Oral swab	6	81	0.07 (0.03)	0.02-0.12
Rectal swab	3	84	0.05 (0.02)	-0.01-0.07
Vaginal swab	0	88	-	-

5.7 Genetic characterisation of *Streptococcus agalactiae* (Papers III and IV)

5.7.1 Genetic diversity among milk isolates (Papers III and IV)

In Paper III, six STs were identified among 65 sequenced isolates from 19 herds. Half of these STs were the single locus variants (SLVs) of previously described STs (ST1652, SLV of ST617; ST1653 and ST1654, both SLVs of ST616). The ST616 variant was overrepresented among the isolates, with 54 out of 65 isolates belonging to ST616. The other isolates belonged to ST612 (n=2), ST1652 (n=6), ST1653 (n=1) and ST1654 (n=1), all of which are parts of previously described camel-associated clonal complexes. One isolate belonged to ST1, which is part of a completely unrelated clonal complex (CC1). Herd diversity varied; in most herds (n=12), isolates belonged to a single ST, whereas multiple STs were found in the remaining herds (n=7). In five herds, two STs were detected, whereas in two herds, three STs were present. In Paper IV, milk isolates belonged to three STs: ST616, ST617 and ST1652. Half of the isolates (n=5) belonged to ST1652, four of the isolates belonged to ST616, and the remaining isolate was identified as ST617. In two herds, two STs were present in milk, and only one ST was detected in milk in two herds (Table 5).

In total, four capsular serotypes (III-VI) were detected *in silico* among SRA isolates from milk (Papers III and IV). Serotype III was the most common (60 out of 75) and was found across all detected STs, with the exception of ST615. In Paper III, serotypes were associated with multiple STs and conversely, an ST could be associated with multiple serotypes. For example, four serotypes were observed within the ST616 lineage.

5.7.2 Genetic diversity among extramammary isolates (Paper IV)

One isolate (a nasal isolate) was excluded from analysis due to insufficient DNA content in the DNA extracts. In the remaining 47 extramammary SRA isolates, five STs were identified. The vast majority belonged to ST617 (n=16) or its SLV, ST1652 (n=14), followed by ST615 (n=10), ST612 (n=6) and ST616 (n=1) (Table 5). Within-host diversity, i.e. findings of isolates belonging to different STs in the same animal, was detected in six camels. In four of these cases, one of the isolates originated from milk.

Four capsular serotypes (II, III, IV and VI) were identified in extramammary SRA isolates. These were largely associated with one ST

each, with the exception of serotype IV, which was found in both ST617 and ST1652. Serotype VI was the most common (n=24), followed by serotype IV (n=12), serotype II (n=10) and serotype III (n=1).

Table 5. Distribution of sequence types (STs) of *Streptococcus agalactiae* (SRA) isolated from camel milk and extramammary sources, collected from 25 herds in Isiolo and Laikipia Counties, Kenya, in 2017 and 2019.

Sequence type	**Milk samples**		**Nasal samples**		**Rectal samples**		**Oral samples**	
	Isolates (n)	Herds (n)	Isolates (n)	Herds (n)	Isolates (n)	Herds (n)	Isolates (n)	Herds (n)
ST1	1	1	0	0	0	0	0	0
ST612	2	2	5	3	0	0	1	1
ST615	0	0	6	2	1	1	3	1
ST616	58	20	0	0	0	0	1	1
ST617	1	1	15	5	0	0	1	1
ST1652	11	8	11	3	2	2	1	1
ST1653	1	1	0	0	0	0	0	0
ST1654	1	1	0	0	0	0	0	0
Total isolates (n)	75		37		3		7	

5.7.3 Lactose typing and virulence testing (Papers III and IV)

Phenotypic lactose fermentation was detected in 49 out of 65 (75%) SRA isolates in Paper III. The results from the phenotypic testing were in complete agreement (100%) with the findings of a lactose operon in the genome and the PCR results. Lactose fermentation was associated with ST (p=0.002), with most ST616 isolates (83%, 45 out of 54) and the ST1653 isolate (n=1) being lactose fermenters. In total, three genotypic variants of the lactose operon (Lac.2) were detected, two previously known (Lac.2b, n=8; Lac.2d, n=26) and a new variant, that was named Lac.2e (n=15). For isolates originating from quarters with mastitis (CMT≥3, CM and SCM), Lac.2d was

overrepresented (p=0.001). The same association was found between isolates coming from quarters further categorised as SCM and Lac.2d (p=0.001).

In Paper IV, lactose fermentation was detected phenotypically as well as genetically in 6 out of 57 isolates (11%), originating from three herds (A, B and D). All lactose-fermenting isolates originated from milk, with the exception of one nasal isolate. There was an association between lactose fermentation and ST, with a higher proportion of isolates belonging to ST616 (4 out of 6), and one isolate belonging to ST617 and one to ST1652 (p=0.05). However, no association was found between type of lactose operon (Lac.2b, n=1; Lac.2d, n=5) and sample type.

Genomic detection of virulence genes strongly associated with disease in humans, *scp*B and *lmb,* was carried out on 65 SRA isolates originating from milk (Paper III). These genes were found in one single genome assembly belonging to ST1.

5.7.4 Phylogenetic analysis (Papers III and IV)

In Paper III, one isolate belonging to ST1 was removed from the phylogenetic tree for a better study of genetic relatedness among the remaining isolates. In the core genome phylogenetic tree, three main lineages were observed. Two lineages corresponded to a single ST (ST612 and ST1652) whereas the largest lineage was comprised of ST616 and its SLVs, ST1653 and ST1654. Within the major lineage, there was a high heterogeneity among isolates, including among isolates originating from the same herd. Within this lineage, there was very little difference in mean pairwise genetic distances (i.e. the number of single nucleotide polymorphisms) between isolates from the same herd (mean=51.33, standard deviation=24.01) compared with isolates from different herds (mean=57.86, standard deviation=20.29).

In Paper IV, phylogenetic analysis revealed five main lineages, visualised in the core genome phylogenetic tree. Four lineages corresponded to a single ST (ST 612, ST615, ST616 and ST617), whereas the fifth and largest lineage included ST617 and its SLV, ST1652. Isolates from the same herd tended to cluster within each observed lineage. For nine adult-calf pairs, both the mother and calf were SRA-positive and in six of these, isolates clustered together in the phylogenetic tree. Two of these adult-calf pairs included an isolate originating from milk. In one of these pairs, the isolate from the calf originated from a rectal swab, and in the other pair, the milk isolate clustered

with two isolates from the oral and nasal mucosa of the calf. Possible transmission routes, based on genomic studies of SRA isolated from milk and extramammary sources are shown in Figure 11.

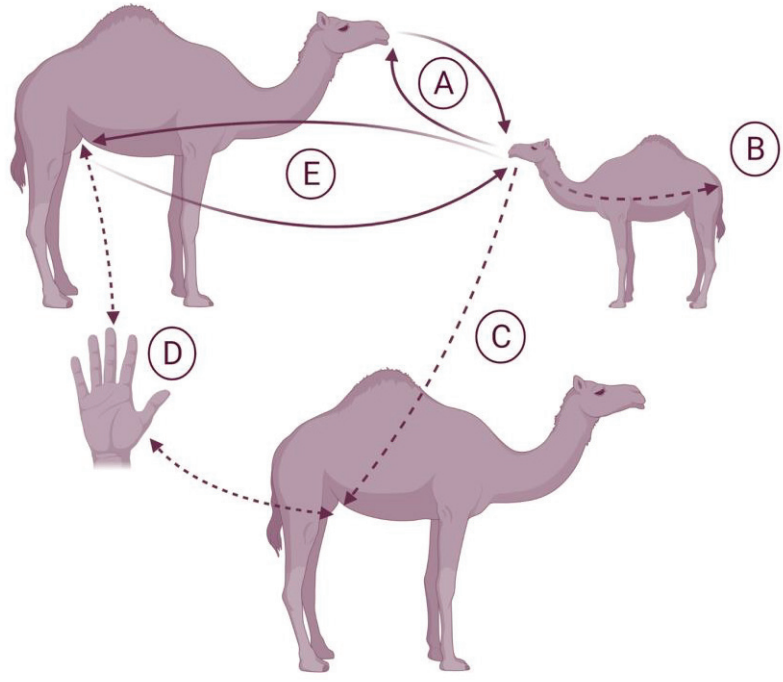

Figure 11. Possible within-herd transmission routes for *Streptococcus agalactiae* based on genomic analysis. The larger camels signify lactating adults, the smaller camel a calf. Dotted lines indicate hypothetical transmission routes. A: Transmission cycle between nasal mucosa and nasal/oral mucosa in adults and calves. B: Possible gastrointestinal passage of SRA from infected milk or nasal/oral mucosa. C: Possible contagious transmission between udders with the calf as a reservoir/vector D: Contagious transmission between camel udders through hand milking. E: Transmission cycle between the udder and nasal/oral mucosa of the calf. Illustration: Dinah Seligsohn, created with BioRender (www.biorender.com).

5.8 Antibiotic susceptibility (Papers I, III and IV)

In Paper I, a total of 210 isolates cultured from milk samples from 81 camels, encompassing three species groups, SRA (n=142), *S. aureus* (n=27) and NAS (n=41) were randomly selected for antibiotic susceptibility testing.

Among the SRA isolates, only 6 out of 142 (4.9%) showed growth at concentrations below the given ECOFF for tetracycline (wild type), whereas the vast majority of isolates (137 out of 142, 95.1%) grew at concentrations above the ECOFF, classified as non-wild type. In contrast, all SRA isolates displayed growth at MIC lower than the ECOFF for penicillin. For erythromycin, only one isolate (1 out of 142, 0.7%) displayed growth above the ECOFF. For all other substances, MIC were below available ECOFFs. The results for *S. aureus* differed slightly; 24 out of 27 isolates (88.9%) displayed growth at tetracycline concentrations below the ECOFF in contrast to the MIC for penicillin, whereas 16 out of 27 isolates (59.1%) displayed growth at concentrations below the ECOFF (wild type). All but one isolate (26 out of 27) were within wild-type ranges for gentamicin and cephalotin. For all other substances, MIC were below available ECOFFs. Most NAS species were categorised as wild type in relation to tetracycline, with 37 out of 41 (90.3%) having a MIC below the ECOFF. For penicillin, 27 out of 41 isolates (65.9%) were classified as susceptible according to the CLSI breakpoints for *Staphylococcus spp*.

In Paper III, detection of resistance genes was carried out for a subset (n=65) of the SRA isolates from Paper I. Genomic investigations revealed the presence of the tetracycline resistance gene *tet*(M) in all non-wild type isolates.

For the 58 SRA isolates in Paper IV, phenotypic susceptibility testing revealed MIC above the ECOFF (non-wild type) for tetracycline in 33 isolates. Further genomic investigations of 57 of the 58 isolates revealed the presence of the *tet*(M) gene in all non-wild type isolates. Furthermore, the tetracycline resistance gene, *tet*(L), was found in four (out of 57) of these isolates. Conversely, no resistance genes were found in any wild type isolates. Tetracycline resistance was associated with certain STs (ST616, ST617 and ST1652) ($p<0.001$) and was not detected in any of the other STs. Moreover, there was an uneven distribution of tetracycline resistant isolates across herds, and this difference was significant ($p<0.001$). For example, all isolates in herds B and C were of non-wild type, whereas none of the isolates from herd E were classified as non-wild type. There was an association between wild type/non-wild type and the origin of the isolate, with isolates from milk more likely to belong to the non-wild type (10 of 10) ($p=0.015$) than isolates originating from other sample types.

6. Discussion

6.1 General discussion

6.1.1 Subclinical mastitis – prevalence and inflammatory markers

The high prevalence of camels with mastitis seen in Field Study 1 highlights the magnitude of this udder health issue and confirms previous results from the Kenyan camel population (Toroitich et al., 2017; Wahinya et al., 2014). Furthermore, SCM was far more common than CM, as described in previous studies on camels and other dairy animals (Abdurahman et al., 1995; Busanello et al., 2017; Mdegela et al., 2009; Regassa et al., 2013; Seifu and Tafesse, 2010). The fact that SCM is impossible to detect without diagnostic tests most probably contributes to the limited awareness of the disease and its related negative impact, and also makes prevention and control more difficult. The knowledge of SCM among pastoralists is reportedly low (Abera et al., 2010), as illustrated by the finding that mastitis was never mentioned as a constraint to milk production among the interviewed pastoralists, although more than half of the examined camels were diagnosed with SCM. For diagnostic testing, CMT proved to be a useful and reliable tool for camel milk. Previous studies on camel milk showed that CMT could predict IMI caused by SRA or *S. aureus* at a satisfactory level (Younan et al., 2001), and in Paper I this was shown for IMI caused by other types of bacteria.

In addition to CMT, other inflammatory markers were found to be of potential use for detection of SCM. The average SCC in camel milk was lower than, or similar to, previously reported levels for healthy quarters, but still had a wide range for each CMT class. In cows, an SCC of <100,000

cells/mL is considered normal, whereas an SCC of >200,000 cells/mL is indicative of IMI (Bradley and Green, 2005). In the present study, the mean SCC for quarters classified as negative for mastitis was 99,000 cells/mL, which is in a similar range to the one used for classification of healthy/mastitis in dairy cows. The other inflammatory markers, NAGase and LDH, showed features comparable to findings from previous studies in camels and other dairy species. In agreement with Abdurahman (1995), but in contrast to Chaffer et al. (2000) and Guliye et al. (2002), NAGase was found to show promising potential for detection of SCM in camels. Overall NAGase levels are higher in camel milk than in cow milk and are more similar to NAGase activity levels in sheep, goat and llama milk (Leitner et al., 2004a, 2004b; Morin et al., 1995). The apocrine milk secretion in goats, resulting in high proportions of cell fragments has been suggested to contribute to the levels of NAGase activity seen in goat milk, and a similar explanation was suggested for camel milk (Abdurahman, 1995; Timms and Schultz, 1985). Moreover, LDH showed a good correlation with SCC and NAGase and was significantly higher in quarters positive for SCM compared with quarters that were negative for SCM. In cows, LDH has been shown to be a sensitive biomarker for mastitis, with an increase in activity at a very early stage of the inflammation (Symons and Wright, 1974). The potential of LDH as an early mastitis indicator in camels should be explored further. To evaluate the use of SCC, NAGase and LDH in detection of IMI in camels, bacterial culturing should be carried out in parallel.

6.1.2 Udder pathogens and risk factors

Overall, contagious mastitis pathogens were a common finding, while environmental pathogens were rarely isolated (Paper I). The contagious pathogens, SRA (72% of all bacteriological diagnoses) and *S. aureus* (13%) were the most common bacteriological findings (Paper I). This high proportion of contagious mastitis pathogens is comparable with the situation in dairy cattle in the pre-antibiotic era, when contagious IMI was the main cause of mastitis (Bradley, 2002). These results are supported by previous findings in dairy camels in Kenya and in neighbouring countries. For SRA, a quarter prevalence at around 17% was reported in camels with SCM or CM in Kenya and Ethiopia (Abdurahman et al., 1995; Almaw and Molla, 2000). However, in other studies, SRA accounts for 3.8–9.6% of the bacteriological diagnoses, which is much lower than our findings in Paper I (Abera et al.,

2010; Regassa et al., 2013; Toroitich et al., 2017). In Kenya, a prevalence of *S. aureus* of 11% was reported for pastoralist camels (Younan et al., 2001) but in a different study, *S. aureus* accounted for 36% of all bacteriological diagnoses at quarter level (Toroitich et al., 2017). Furthermore, a quarter prevalence of *S. aureus* has been reported at 5.4% in Sudan (Abdurahman et al., 1995) which is in line with the current study, and in a study in Ethiopia, *S. aureus* was detected in 49.4% of all culture-positive milk samples (Obied et al., 1996). The prevalence of NAS (19%) was comparable to findings in other studies on camels, and similar species are found in cattle (Ahmad et al., 2012b; Goncalves, 2018; Regassa et al., 2013; Wuytack et al., 2020). In cattle, multiple NAS species can be isolated from the teat apices of healthy udder quarters. Little is known about the distribution and prevalence of different NAS species in camels (Wuytack et al., 2020) and the importance of NAS as mastitis pathogens in camels remains unclear. There are several potential explanations as to why environmental mastitis seems to be less of a hazard for udder health in camels. First, the udder attachment in camels is high up and the udder rarely comes into contact with the ground even when the camel is lying down. Second, camel herds are mobile and continuously move in search of pasture. During the day, they are kept in a mostly very dry environment and are only herded back to the boma at night. This means that the bacterial load in the environment is likely to be of less concern than for cattle. In contrast, there has been an increase in environmental pathogens involved in mastitis in dairy cattle in industrialised countries, partly explained by changes in housing and management, increasing the risk of faecal contamination in the barn environment (Klaas and Zadoks, 2018). Similarly, in dairy cows in Rwanda, Ndahetuye et al. (2020) found that cows kept in zero-grazing systems are more at risk of mastitis, and there is an association between dirty hind legs and SCM. Nonetheless, also in pasture-based systems, such as the dairy industry in New Zealand, environmental pathogens, such as *Str. uberis* and *E. coli* are of concern (Compton et al., 2007).

In Paper I, we found that an older age and later lactation stage were both associated with SCM and IMI, as has previously been shown for camels, and can be explained by a longer exposure to pathogens (Ahmad et al., 2012b; Aljumaah et al., 2011) that would increase the risk of IMI and SCM. Furthermore, skin lesions on the teats or udder were risk factors for SCM, similar to previous reports in dairy camels (Abera et al., 2012; Ahmad et al.,

2012b), and are also conditions that can accumulate over time. In cattle, lesions on teats and udders can serve as reservoirs for udder pathogens, and injuries to the teat ends may result in an impaired sphincter mechanism and ability to form a keratin plug during dry-off, and subsequently a weakened barrier against invasive bacteria (Agger and Willerberg, 1986; Dingwell et al., 2004). In dairy camels in the Horn of Africa, the use of teat-tying has been widely reported and suggested to increase the risk of SCM (Abdurahman et al., 1995; Ahmad et al., 2012b; Obied et al., 1996). In Paper I, teat tying was not a significant risk factor for SCM but the recorded number of observations of teat-tying was low in comparison to other studies (Abdurahman et al., 1995; Regassa et al., 2013). The low number of observations might be an underrepresentation due to the rapid milking process applied in many of the herds, with one camel still being milked whilst the next one is being prepared for milking, with the risk of missing teat tying before removal. Furthermore, infestation of ticks on the teats and udder has been proposed as a risk factor for SCM in camels (Obied et al., 1996), but in Paper I, a recorded presence of ticks on the teats or udder was not a risk factor for SCM, similar to findings by Regassa et al (2013).

Moreover, the presence of blind teats, induration of udder tissue and anecdotal information about previous episodes of mastitis were associated with isolation of SRA from milk in the present study. Altogether, these findings point to the possibility of SRA IMI developing into a chronic condition in camels, as has previously been suggested (Younan, 2002). In cattle, chronic carriers of SRA are the main reservoirs for the maintenance and spread of the pathogen within the herd (Keefe, 1997). Longitudinal data are needed to elucidate whether camels can become chronic carriers.

6.1.3 Molecular epidemiology of SRA in camel herds

In Paper III and Paper IV, the genotypic diversity of SRA from camels was largely in agreement with earlier findings by Fischer et al. (2013), with all isolates belonging to previously defined camel-associated complexes (with the exception of one isolate that belonged to ST1/CC1).

The findings of SRA at camel level differed between Field Studies 1 and 3 with regard to prevalence and strain diversity. In Paper III, ST616 was the dominant ST in milk, corroborating previous genotypic studies on SRA from camel milk (Fischer et al., 2013). Moreover, the majority of the SRA isolates in this study harboured the Lac.2 operon, which is widely regarded as an

adaptation to the mammary gland (Richards et al., 2019), and this proportion was higher for isolates belonging to ST616. Lac.2 was found in all SRA isolates collected from bovine milk in a study conducted in northern Europe (Lyhs et al., 2016). Thus, lactose fermentation in SRA infected camel udders seems to be an evolutionary advantage, but not an absolute pre-requisite for establishment of infection, as has been described for Gram-negative udder pathogens (Holt et al., 2015). The phylogenetic differences observed for the SRA genomes in Papers III and IV could be partly attributed to the fact that the herds belonged to various management systems, likely affecting the possible between-herd transmission routes. Almost all herds in Paper IV, belonged to ranches or sedentary smallholders, characterised by being relatively closed herds with less interaction, in contrast to the peri-urban herds in Paper III, where hired labour or extended family frequently move between herds. These frequent movements were also reflected in the results from the phylogenetic analysis, where similar levels of heterogeneity of SRA genomes were demonstrated within and between herds (Paper III). In traditional pastoralist systems, recruitment of new animals to the herd is rare due to the widespread belief that lactating females are only to be sold if they have hidden faults (Noor et al., 2013). Thus, herd recruitment was based on inheritance rather than the purchase of new animals. In contrast, expansion of peri-urban herds around Isiolo town (Papers I and III) was more likely to occur by the acquisition of new animals (Issack et al., 2012) and thus, new pathogens are more likely to be introduced to these herds. Purchase of infected animals is the main route of introduction of SRA in cattle herds in industrialised countries (Agger et al., 1994). Localised geographical differences in the strain diversity of SRA in cattle dairy herds have been reported from Colombia (Cobo-Ángel et al., 2018).

The development of the camel milk market in and around Isiolo town represents an unprecedented intensification of the camel milk market in Kenya (Issack et al., 2012). For milk to be delivered from the camel herds to the town, herds stay closer to the existing infrastructure, creating higher densities of camels around these crucial transport routes. This intensification of milk production could drive the spread of infectious diseases and explain the high levels of SRA seen at herd, camel and quarter level. Intensified animal production can lead to overcrowding, resulting in stress and subsequent higher susceptibility to infections, coupled with an increased infection pressure as well as a greater exchange of pathogens among animals.

Intensification of livestock systems has also been associated with the emergence and spread of zoonotic diseases and AMR (Gilbert et al., 2021). For example, in tilapia fish, intensified production is thought to have resulted in the emergence of a particularly virulent clone of SRA, causing lethal meningoencephalitis in otherwise healthy adult humans (Barkham et al., 2019). As pastoralists live close to their livestock and consume fresh milk without prior heat-treatment, they have a high exposure to zoonotic pathogens. To ensure sustainable intensification, the continued development and optimisation of production potential in camels in northern Kenya should be closely monitored and possible threats to public health mitigated.

To explore the potential public health threat of SRA in camel milk, the presence of virulence genes *scp* and *lmd,* closely associated with human disease (Sørensen et al., 2010), was investigated in all SRA genomes included in Paper III. The absence of these virulence genes in all but one genome implies that based on our finding, SRA in camel milk is not likely to pose an urgent threat to public health at the present time, although more studies are needed to confirm these results. Nevertheless, the finding of an isolate belonging to ST1 originating from camel milk is highly indicative of transmission from humans to camels. In humans, both in Kenya and other parts of the world, ST1 is commonly found in the gastrointestinal or reproductive tract of healthy carriers and interspecies transmission between humans and cattle has been reported (Cobo-Angel et al., 2019; Huber et al., 2011; Seale et al., 2016; Sørensen et al., 2019). The difficult sanitary conditions under which camel herders operate could facilitate human-to-camel transmission. Experimental udder infections in cows with human SRA strains result in a more severe inflammation but with a shorter duration (Jensen, 1982). Nonetheless, genotypic studies in SRA isolated from humans and cattle show that some genotypes are shared, and that genotypes previously only found in humans have adapted to the bovine mammary gland (Lyhs et al., 2016). Introduction of human-derived strains in cattle herds previously free from SRA could be one explanation for the rise of SRA in countries with well-developed control programmes (Lyhs et al., 2016; Sørensen et al., 2019).

The findings of extramammary isolates of SRA in healthy camels in Paper IV illustrates that the epidemiology of SRA in camel herds is complex and transmission probably extends beyond the previously accepted udder-to-udder transmission paradigm. In all herds, multiple STs were found in

multiple sample sites, demonstrating a lack of organ specificity in the majority of strains. The prevalence was exceptionally high in the nasal mucosa of both adult and calves, similar to the findings by Younan and Bornstein (2007). Nasopharyngeal carriage has been reported to be around 10% in humans (Foster-Nyarko et al., 2016); however, a comparably high nasal prevalence has not been demonstrated in any other mammal previously and suggests that SRA could be a part of the normal nasopharyngeal flora of healthy camels.

Although some of the isolates from milk in Paper IV belonged to the udder-adapted ST616 harbouring the Lac.2 operon, the majority of isolates found in milk in this study belonged to STs that were also found in nasal, oral and rectal samples. Thus, the importance of extramammary reservoirs in SRA IMI in camels cannot be ignored. The epidemiology of SRA in camels appears more similar to the situation in humans, with a high carriage in healthy individuals (nasal prevalence over 20%), resulting in occasional opportunistic infections. On a sliding scale between contagious and environmental pathogens, SRA in camels is positioned somewhere in between, similar to *Str. uberis* in cattle, which can display a large within-herd strain diversity with varying virulence potential (Klaas and Zadoks, 2018).

Calves have been suggested as a source of contamination of the udder in camels (Noor et al., 2013) and this study showed that SRA can be present in the mucous membranes of the oral and nasal cavity of camel calves. This suggests a possible transmission cycle between the oral and nasal mucosa of the calf and the udder of the mother. In cattle, calves have been shown to act as reservoirs of SRA that could infect pre-parturient heifers (Schalm, 1942). In addition, in contrast to a previous report for camels (Packer et al., 1992), cross-suckling was observed on multiple occasions in different herds. Thus, it cannot be ruled out that camel calves might be involved in contagious transmission between udders, similar to the suggested route of transmission of *S. aureus* in sheep flocks (Mørk et al., 2012). Nonetheless, it has also been argued that suckling calves could be beneficial for udder health. Ndahetuye et al. (2020) found that cows being regularly suckled by their calves were less at risk of contracting SCM. The benefit of suckling calves has been attributed to the calves' ability to empty the udder more efficiently than hand milking, minimising the amount of residual milk that could otherwise function as a substrate for bacterial growth. Furthermore, frequent milkings

could flush out bacterial colonisation of the teat canal and antibacterial enzymes in the saliva could inhibit the growth of udder pathogens (González-Sedano et al., 2010). Attempts to restrict the suckling of the camel calf, by the application of anti-suckling devices (teat-tying) may thus prevent these potential positive effects of suckling. The role of the calf as a potential transmitter of SRA in camels should be further explored.

6.1.4 Antimicrobial susceptibility of udder pathogens and extramammary SRA

Decreased susceptibility to penicillin was found in both *S. aureus* and NAS, at similar or much lower levels than in other reports from East Africa (Getahun et al., 2008; Mahlangu et al., 2018; Ndahetuye et al., 2020; Shitandi and Sternesjö, 2004), but higher than in industrialised countries (Bengtsson et al., 2009; Persson et al., 2011). In Field Study 1, all herds reportedly used a combination of benzylpenicillin and streptomycin to treat disease. Tetracycline was reportedly used in 60% of these herds for treatment of different types of diseases, similar to previous reports (Lamuka et al., 2017). Subsequently, the observed decreased susceptibility in *S. aureus* and NAS is likely to depend on selection pressure and the expansion of resistant clones (Bengtsson et al., 2009). In contrast, the susceptibility pattern detected in SRA was the inverse to that found in staphylococci, as all SRA isolates in Paper I were susceptible to penicillin, although for tetracycline, most were phenotypically classified as non-wild type. The high levels of decreased susceptibility to tetracycline in SRA isolates from milk could be attributed to a combination of selection pressure, horizontal gene transfer and spread of resistant clones within the herd. All phenotypically resistant (non-wild type) isolates subjected to genomic analysis also harboured the *tet*(M) gene, one of the most common genes coding for resistance to tetracycline (Burdett, 1991). The presence of the *tet*(M) gene has been described in SRA from humans, fish, cattle and camels (Barkham et al., 2019; Da Cunha et al., 2014; Duarte et al., 2005; Fischer et al., 2013; Richards et al., 2019). Furthermore, the *tet*(L) gene, another gene coding for tetracycline resistance which has not previously been reported in camels, was detected in four nasal isolates all originating from the same herd, suggesting within-herd spread of this resistance gene. In Paper IV, the susceptibility patterns differed markedly between herds, and furthermore, non-wild types were restricted to only a few of the detected STs. Interestingly, isolates originating from milk were more

likely to be non-wild type than extramammary isolates. The high genomic plasticity in SRA affects the acquisition of mobile genetic elements carrying, for example AMR genes (Richards et al., 2019). In SRA in camels, transfer of the *tet*(M) gene is likely linked to a mobile genetic element, the Tn*619* (Fischer et al., 2013). Scornec et al. (2017) showed that exposure to subtherapeutic levels of ribosome-targeting antibiotics, such as tetracyclines, macrolides and lincosamides, could induce the expression and transfer of Tn*619* in *Enterococcus faecalis in vitro*. In cattle, parenteral treatment with tetracycline is not commonly used to treat mastitis as the distribution in the udder leads to subtherapeutic concentrations and subsequent delayed bacteriological cure, which also promotes the survival of resistant pathogens in the udder (Gruet et al., 2001; Lents et al., 2002).

There is a knowledge gap regarding optimal antibiotic treatment of mastitis in camels, and there is a lack of evidence-based dosage regimens based on pharmacological studies (Ali et al., 1996). In Kenya, the distribution, sale and use of antibiotic drugs is largely unmonitored, and veterinary services targeting camels are rare (Heffernan and Misturelli, 2000; Higham et al., 2016; Lamuka et al., 2017). These factors may contribute to the development of AMR together with the common use of substandard antibiotic drugs (Koech et al., 2020; Muthiani, 2012). To curb the development of tetracycline resistance in SRA in camels, Fischer et al (2013) suggest a shift from using tetracycline to other drugs with less widespread resistance. However, preventive measures to reduce the incidence and prevalence of mastitis as well as antibiotic use, are also urgently required.

6.1.5 Ways of improving udder health in pastoralist dairy camels

When discussing a possible mastitis control strategy for dairy cattle, Dodd et al. (1969) state:

> "To be accepted, a control must cost much less than the losses caused by the disease, it must be relatively simple to carry out, there should be good experimental evidence that the control works under a range of conditions, and it must be obvious to the farmers who adopt the method that [clinical] mastitis is much reduced."

Given the monumental differences in management systems, resources and sociocultural significance of animals between dairy cattle farms in industrialised countries and camel herds kept under pastoralist conditions in Kenya, direct extrapolation of existing control programmes is not feasible, but interventions should be adapted and tailored to the local context. For

example, regular monitoring of SCC at individual level, culling of chronic carriers or bacteriological testing of milk before introducing a new animal to the herd are interventions commonly practised in industrialised countries that are not applicable in the Kenyan pastoralist context due to their dependence on advanced testing equipment or elimination of a valuable breeding female. Nevertheless, the same *principles* as stated by Dodd et al. (1969) could still function as a fundamental framework for the development of a control strategy in the camel pastoralist setting.

Since pastoralists live close to their animals and are dependent on them for food security, their knowledge about the clinical manifestation of different animal diseases is usually good (Mochabo et al., 2005) and disease syndromes can be categorised in great detail (Amenu et al., 2017). However, pastoralist systems are deeply rooted in tradition and local perceptions are rarely based on scientific knowledge, resulting in potentially risky behaviour (Kamau et al., 2021; Muga et al., 2021). In a study by Odongo et al. (2016), pastoralists reported no perceived health risks from drinking raw camel milk and believed that the camel udder is always clean, despite the fact that raw camel milk can transmit a number of zoonotic diseases (Esmaeili et al., 2019; Ibrahim et al., 2021; Wainaina et al., 2020). When conducting a study on camel calf health in northern Kenya, Kaufmann (2003) concluded that knowledge of the disease perceptions among the target group is crucial when designing and delivering animal health advice so that animal owners can understand and act on it, and thus ensure implementation of the interventions. In Field Study 1, mastitis was only mentioned as a common disease in the herd by one of the respondents, despite more than half of the camels (Paper III) being affected by some form of mastitis. Exploring the concept, perceptions and different understandings of mastitis among pastoralist groups is a necessary first step towards developing a control strategy (Chenais and Fischer, 2018; Ebata et al., 2020; Tasker, 2020). Greater awareness among pastoralists of the disease, its related negative impacts and the possible benefits of improved udder health would be fundamental prerequisites for introducing prevention or control measures.

A cornerstone in mastitis prevention is hygienic milking practices (Philpot, 1979). Acceptable milking hygiene in terms of hand-washing prior to milking, cleaning of the udder and/or teats prior to milking and post-milking teat disinfection was lacking in most herds, as in previous reports from comparable settings (Kashongwe et al., 2017; Odongo et al., 2016).

This is likely to contribute to the spread of udder pathogens within herds and is an important issue to target for prevention and control. When comparing the total viable (bacterial) count (TVC), number of coliforms and *S. aureus* in swabs taken from camel udders and milkers' hands, the overall result indicated that the bacterial load on the milkers' hands was higher than on the udder (Odongo et al., 2016). Camel pastoralists often live in remote areas where a lack of infrastructure and restricted access to clean water are common obstacles, making cleaning of hands and udder difficult. Furthermore, given the sensitive process of milk let-down in camels, it can be hypothesised that pre-milking cleaning of udder and/or teats, after the calf has started to suckle, could interrupt the milk-let down (Nagy and Juhasz, 2016). In cattle, post-milking teat disinfection is a common element of a hygienic milking routine and efficiently prevents entry of both contagious and environmental pathogens through the teat canal (Hogan et al., 1987). This is a possible disease-reducing intervention that could be evaluated for use in dairy camel herds as the procedure of post-milking teat disinfection is simple and there are cheap but efficient products available. Moreover, a milking order was commonly employed in the studied herds. Adhering to a milking order based on the camel's udder status could prevent the spread of contagious udder pathogens, as has been shown for cows (Nielsen and Emanuelson, 2013). Although teat-tying was not a statistically verified risk factor (Paper I), it is likely to contribute to the development of teat lesions, impaired teat function and possibly negatively impact efficient udder emptying, all factors that could increase the risk of mastitis (Abdurahman et al., 1995; Agger and Willerberg, 1986; Dingwell et al., 2004; González-Sedano et al., 2010). The efficacy of various interventions should be studied in camel herds before recommending control measures to pastoralists. It is also possible that an increased consumer demand for hygienic milk could create an economic incentive that would motivate improved udder and milk hygiene.

6.2 Methodological considerations

The Kenyan camel population is estimated to be one of the largest in the world (FAOSTAT, 2019; Faye, 2020). However, in this thesis only a small subset of the total population was sampled. There are no formal registers of camel keepers in Kenya, and in view of the difficulties of obtaining access

to herds, the widespread distribution of camel herds over remote and inaccessible areas and their frequent movements, a convenience sampling technique had to be applied. Based on our small sample size and the geographical limitations, our findings cannot be said to be representative of all camel herds in Kenya. Nonetheless, the results from Field Study 1, with regards to management strategies and risk factors, are in agreement with previous work conducted in the peri-urban camel milk cluster around Isiolo town (Kashongwe et al., 2017; Wayua et al., 2012) strengthening the evidence of the associations between these factors and SCM/IMI in camels.

The definition of CM and its subcategories used in Papers I and III might not be comparable to other studies as cases were classified as acute or chronic, based on the clinical presentation, despite the absence of longitudinal data to underpin these decisions. In cases of chronic clinical mastitis in cattle, loss of milk epithelial cells and a proliferation of fibrotic tissue are often seen, resulting in nodular induration of the udder tissue or occasionally fibrotic atrophy of the quarter (Sandholm, 2008). As SRA commonly causes chronic clinical or subclinical mastitis in dairy cows (Holmøy et al., 2019; Keefe, 1997), and a similar pathophysiology has been suggested for camels (Younan, 2002), this classification was made to further explore this hypothesis. Subsequently, the classifications "chronic clinical mastitis" and "acute clinical mastitis" were used to make a distinction between cases displaying symptoms in agreement with a long-lasting process and those with symptoms indicative of a more recent inflammatory response.

A CMT score of 3 or higher was classified as SCM and according to the results in Paper II, this cut-off corresponded to a mean SCC of 811,000 cells/mL. For cattle, SCC >200,000 cells/mL is widely recognised as indicative of IMI (Bradley and Green, 2005). Since overall SCC values for camels in Paper II were similar to the levels seen in cows, it cannot be ruled out that a lower CMT score should be used as cut-off for IMI in camels. However, as CMT is a subjective measure and distinguishing between CMT 1 and CMT 2 can be difficult for inexperienced users, a cut-off set at CMT ≥3 would be more practical for camel owners. To increase the sensitivity of CMT, CMT 2 was used as an inclusion criterion for milk sampling in Paper IV. However, SRA can be shed from quarters with SCC <200,000 cells/mL in cattle (Mahmmod et al., 2015) and subsequently, there might have been a failure to sample camels with SRA IMI. Nonetheless, this study design was

necessary to ensure compliance with sampling protocol by the camel keepers.

Despite the claimed interdependence of udder quarters, it has been reported that indicators of inflammation, such as SCC, NAGase and LDH, can increase in healthy quarters adjacent to quarters with CM or SCM in dairy cows (Bansal et al., 2005; Paixão et al., 2017). These associations have not been studied for camel udders. Nonetheless, it cannot be ruled out that the study design in Paper II, using matched quarters in the same camel for assessment of inflammatory markers, may have influenced the results.

A methodological consideration that might have influenced culture results is that due to short and easily disrupted milk let-down in camels and a subsequent rapid milking process, aseptic milk sampling can be challenging, as reflected in the high proportion of contaminated milk samples in Paper I. Some of these contaminated samples may have harboured IMI-causing environmental pathogens. However, these samples were excluded from analysis since mixed growth cannot be interpreted consistently.

For logistical reasons, all milk samples were frozen prior to culturing. Studies on the recovery of bacteria from milk samples from cows have shown that freezing may have a negative influence on the recovery of *E. coli* and *T. pyogenes*, a positive effect on NAS species and no significant effect on *Streptococcus* spp. and *S. aureus* (Schukken et al., 1989). The absence of *T. pyogenes* and coliforms, specifically *E. coli,* reported in Paper I could be attributed to the pre-culture freezing of samples, although, according to other reports on the bacterial aetiology of mastitis in camels, these pathogens are less common in camels than in dairy cows (Abeer et al., 2016; Ahmad et al., 2012b; Gruet et al., 2001). Furthermore, negative growth after culturing could be attributed to IMI in remission, no shedding of bacteria at the time of sampling, or undetectable levels in the milk (Taponen et al., 2009). Some bacterial species, such as *Mycoplasma* spp., require special culture conditions and are slow growing, and would not have been detected using standard protocols (Sachse et al., 1993). Furthermore, it is possible that a streptococcal selective supplement to the TH broth, inhibiting non-streptococcal growth, could have increased the recovery rate of SRA from the swab samples in Paper IV.

7. General conclusions

- There was a high prevalence of SCM in lactating dairy camels in the sampled herds in Isiolo and Laikipia Counties; the udder pathogens SRA, NAS and *S. aureus,* were the most common causes of IMI.
- The main risk factors identified for SCM were a later stage of lactation, increasing age and lesions on the udder or teats. For IMI, the risk was higher with increasing age and parity, whereas risk factors for isolation of SRA in milk were increasing age and parity, a previous history of CM, induration of the udder and the presence of blind teats.
- The inflammatory markers SCC, NAGase and LDH were all associated with SCM in camels.
- Decreased susceptibility to tetracycline was widespread among SRA isolated from milk, but in *S. aureus* and NAS decreased susceptibility to benzylpenicillin was more common.
- Most mastitis-causing SRA belonged to a common genotype that shows signs of niche adaption to the mammary gland; phylogenetic analysis of SRA from milk collected in the Isiolo region revealed similar heterogeneity within and between herds.
- The prevalence of nasal carriage of SRA in healthy camels was higher than described for other mammals. Camel calves and extramammary body sites can be involved in the transmission or serve as reservoirs for udder infection of SRA.

8. Future perspectives

Hunger is still a persistent problem in many parts of the world, and the anticipated population increase in the coming decades means that the public demand for animal products will increase (Robinson and Pozzi, 2011). In the Horn of Africa, extreme climate events have challenged food production for as long as it has been inhabited. The effects of climate change, leading to prolonged droughts and erratic rainfall are resulting in the situation becoming even more pressing for both human and animal populations (Faye et al., 2012). As camels are crucial for food security in the arid and semi-arid lands of East Africa, their production potential needs to be unlocked for the benefit of public health and poverty reduction. Curbing disease transmission of contagious udder pathogens such as SRA and improving udder health are likely to have positive effects on food supply and household income for people who sell milk. To gain insights into the transmission routes and dynamics of SRA in these settings, longitudinal studies in camel pastoralist herds are needed. Studies of dairy cattle herds have revealed that humans tending the animals can be involved in the transmission cycle, and that strains originating from humans can be introduced to cattle herds and infect cows (Cobo-Angel et al., 2019; Lyhs et al., 2016; Sørensen et al., 2019). In Kenya, SRA collections from humans, presented in scientific publications, are based on isolates from the urban areas of large cities, such as Nairobi and Mombasa (Huber et al., 2011; Jisuvei et al., 2020). Thus, they are not representative of camel-keeping communities living in the remote northern parts of the country. To elucidate potential interspecies transmission between camels and humans, samples from people living in close contact with camels should be included in genomic analysis.

Antibiotic resistance is a global threat to human and animal health potentially making some bacterial diseases incurable (Levy and Marshall,

2004). There is a knowledge gap regarding efficient mastitis treatments in camels, and routine treatment with parenteral antibiotics of differing quality without appropriate dosing regimens is likely to promote the development of AMR. More detailed studies in camels are needed to optimise treatment with antibiotics, but there is also an urgent requirement to use other approaches to prevent and control mastitis. Interventions aimed at reducing mastitis should focus on biosecurity, milking hygiene and mastitis detection. For the research community to address the human dimensions of disease transmission more effectively and increase awareness of the disease among animal owners, knowledge of how pastoralists perceive and understand mastitis needs to be improved. This field of future research should also include an evaluation of the adaptation and implementation of possible mastitis prevention and control interventions in pastoralist camel herds.

In summary, the following knowledge gaps should be addressed:

- Knowledge of transmission routes and dynamics of SRA in camel herds should be enhanced through the collection of longitudinal data.
- Potential interspecies transmission of SRA between camels and humans living in close contact with camels and other types of livestock should be further explored
- The efficacy of different mastitis treatment in camels should be studied.
- The perceptions and understanding of mastitis among camel pastoralists should be investigated.
- Possible mastitis-reducing interventions and their efficient implementation in pastoralist camel herds should be considered.

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Popular science summary

Globally there are 200 million pastoralists (nomadic people who keep livestock) who are directly dependent on their animals for food supply. The availability of good grazing grounds is fundamental for this kind of animal keeping, making pastoralist systems particularly vulnerable to weather challenges. Ongoing climate change has made this factor a pressing concern. In the drylands of the Horn of Africa, droughts are becoming longer and rainfall more difficult to predict. These changes affect both human and animal populations. In these regions, many communities have long-standing traditions of keeping one-humped camels (dromedaries), due to their suitability to a harsh climate. Camels can keep producing milk without drinking water for over two weeks, and they feed on pastures not suitable for cattle. In northern Kenya, camel milk is an important food source, and apart from its nutritional value it is thought to possess medical properties.

In addition to droughts and feed shortages, camel pastoralists struggle to manage a variety of animal diseases. One of the main obstacles for optimised milk production is mastitis, an inflammation of the udder. Mastitis negatively impacts milk yield and quality, the shelf life of the milk and its nutritional value. Mastitis can be either clinical or subclinical. In the case of clinical mastitis, the affected udder quarter might become swollen, warm and painful to touch, visual changes of the milk may occur (clots, watery, colour changes, blood-tinged) and in severe cases, the whole animal may be affected, with fever and changed general demeanour. Subclinical mastitis, however, cannot be detected visually but requires milk analysis. Due to these differences in presentation, pastoralists are generally familiar with clinical mastitis, whereas their awareness of subclinical mastitis is limited. The root cause of mastitis is mostly a bacterial infection, entering the udder through the teat. Among camels, the bacterial species *Streptococcus (Str.) agalactiae* is

commonly found to be a cause of mastitis. In cows, this bacterium is regarded as contagious and udder-bound, and often results in long-lasting (chronic) infections. The mode of transmission is not entirely clear in camels. This thesis investigated the occurrence of subclinical mastitis and the associated bacterial panorama, as well as the antibiotic susceptibility of the bacteria. Furthermore, potential risk factors for subclinical mastitis were determined and inflammatory markers in the milk were evaluated. By analysing bacterial DNA from *Str. agalactiae*, possible transmission routes in camel herds were mapped, and the genetic characteristics of the bacteria were investigated.

In the first part of the project, milk samples from 804 udder quarters in 206 camels from 20 herds in Isiolo County, Kenya, were investigated. Almost half of the camels (46%) had subclinical mastitis and most (82%) of these cases were attributed to a bacterial infection. The most common bacterial species in milk was *Str. agalactiae* (72% of the isolates), followed by non-aureus staphylococci (19%) and *Staphylococcus aureus* (13%). A higher age (>9 years), later stage of lactation (12-23 months) and lesions on the udder or teats were risk factors for subclinical mastitis. Having a non-funtioning (blind) teat or quarter and/or a previous history of clinical mastitis were specific risk factors for udder infections with *Str. agalactiae*. The susceptibility to penicillin among staphylococci, ranged between 59% and 66% whilst only 5% of the *Str. agalactiae* isolates were susceptible to tetracycline.

The second part of the project focused on inflammatory markers in milk. Somatic cell count (SCC), N-acetyl-β-D-glucosaminidase (NAGase) and lactate dehydrogenase (LDH) were evaluated as markers of subclinical mastitis. For all of the markers, the levels increased in milk from udder quarters with subclinical mastitis in comparison with healthy udder quarters.

In the final two parts of the project, DNA analysis was performed of *Str. agalactiae* isolates collected from milk and other tissues (nose, mouth and rectum) from camels and their calves. Most *Str. agalactiae* from milk were genetically related and adapted to udder tissue. One of the isolates most probably originated from a human, suggesting that there might be a risk of transmission between humans and camels. Healthy camels were commonly carriers of *Str. agalactiae* in their noses and the same strains were found in milk. In most of the herds, milking hygiene was inadequate, with limited possibilities for washing hands or disinfecting the teats. This is likely to contribute to the high levels of mastitis and transmission of *Str. agalactiae*

between camel udders. Another possible transmission route is between the nose or mouth of adults and calves.

In conclusion, subclinical mastitis is common among dairy camels and mainly associated with contagious udder pathogens that exhibit resistance to the most commonly used antibiotic drugs. The genetic studies of *Str. agalactiae* revealed that some strains are specific to the udder, and also suggest that *Str. agalactiae* is part of the normal flora of healthy camels and therefore can be transmitted to the udder through interaction between camels. In addition, transmission between humans and camels might pose a risk. To improve udder health among pastoralist camels, in depth studies of the perception of mastitis among pastoralists are needed. This would aid in the development and establishment of preventative measures that could easily be accepted and incorporated into the daily care routine.

Populärvetenskaplig sammanfattning

I världen finns ungefär 200 miljoner pastoralister (djurskötande nomader) som är beroende av sina djur för att ha säker tillgång till livsmedel. Eftersom djuren ständigt hålls på bete är denna sorts djurhållning extra känslig för väderstörningar. I och med klimatförändringen har detta blivit mer aktuellt. I ökenområdena på Afrikas horn har torrperioderna blivit längre och regnperioder mer oförutsägbara än tidigare. Detta får konsekvenser för både djur och människor. Flera folkgrupper i dessa områden har en lång tradition av att hålla enpuckliga kameler (dromedarer), eftersom kamelerna är väl anpassade till ett liv i ett hårt klimat. Kameler kan exempelvis fortsätta producera mjölk utan att dricka vatten på över två veckor och föda sig på beten som inte kan nyttjas till boskap. I norra Kenya är kamelmjölk en viktig livsmedelskälla och utöver att fungera som näring, tillskrivs mjölken ofta medicinska egenskaper.

Förutom torka och betesbrist tampas kamelpastoralister ofta med olika sorters sjukdomar på sina djur. Ett av de största hindren för optimal mjölkproduktion är mastit, en inflammation i juvret. Mastit påverkar mjölkmängd, -kvalitet, hållbarhet och näringsvärde negativt. Mastit kan vara klinisk (synlig) eller subklinisk (osynlig). Vid klinisk mastit kan man se att den drabbade juverdelen blir svullen, öm och varm, mjölken kan bli synbart förändrad (klumpig, vattnig, förändrad färg, blodinslag) och ibland blir hela djuret påverkat med feber och nedsatt allmäntillstånd. Vid en subklinisk mastit kan man däremot inte upptäcka förändringarna utan att mjölken analyseras. På grund av dessa skillnader är klinisk mastit ofta ett välkänt sjukdomssyndrom bland pastoralister, medan kunskapen om subklinisk mastit är låg. Orsaken till mastit är oftast en bakteriell infektion som tagit sig in i juvret via spenen. Bland kameler är bakterien *Streptococcus (Str.) agalactiae* vanlig. Bland kor har denna bakterie framför allt hittats i och på

juvret och anses vara smittsam. Den resulterar också oftast i långdragna (kroniska) infektioner. För kameler är det inte helt klarlagt hur denna bakterie sprids mellan djuren. I den här avhandlingen undersöktes förekomsten av subklinisk mastit bland kameler i ett område i Kenya. Vi undersökte även vilka bakterier som kunde påvisas vid subklinisk mastit och om dessa bakterier var känsliga för antibiotika. Dessutom kartlade vi olika riskfaktorer för subklinisk mastit och undersökte olika inflammationsmarkörer i mjölken som kan hjälpa oss att påvisa subklinisk mastit. Genom att analysera DNA från ett urval av de *Str. agalactiae* som vi fann i mjölk, samt i andra vävnader från både mjölkande kameler och deras kalvar, kunde vi beskriva möjliga smittvägar inom kamelhjordar.

I den första delen av projektet undersökte vi mjölkprover från 804 juverdelar från 206 kameler i 20 hjordar i Isiolo County, Kenya. Nästan hälften (46%) av alla kameler hade subklinisk mastit och i de flesta fall berodde det på en bakterieinfektion (82%). Den vanligaste bakterien i mjölk var *Str. agalactiae* (72% av alla bakteriella diagnoser), följt av koagulasnegativa stafylokocker (19%) och *Staphylococcus aureus* (13%). Det var större risk att drabbas av subklinisk mastit för äldre kameler (>9 år), senare i laktationen (12–23 månader) och om de hade hudskador på spenar eller juver. Specifika riskfaktorer för att ha *Str. agalactiae* i mjölken var att en eller flera juverdelar helt slutat producera mjölk och om kamelen hade haft klinisk mastit tidigare. Bland de olika stafylokockerna var endast lite fler än hälften känsliga för penicillin (59–66%) och endast 5% av de insamlade *Str. agalactiae*-isolaten var känsliga för tetracyklin.

I den andra delen av projektet undersökte vi olika inflammationsmarkörer i mjölk. Vi undersökte celltal, laktatdehydrogenas och N-acetyl-β-D-glukosaminidas som markörer för subklinisk mastit och kunde se att alla dessa markörer ökade i mjölk från juverdelar med subklinisk mastit jämfört med friska juverdelar.

I de två sista delarna analyserade vi DNA från *Str. agalactiae* som hade samlats in från mjölk och andra vävnader (noshåla, munhåla och ändtarm) från friska kameler och kamelkalvar. De flesta bakteriestammar från mjölk var besläktade och visade sig vara anpassade specifikt till juvervävnad. Ett isolat härrörde dock sannolikt från en människa och det fyndet visar på en risk för spridning mellan djur och människor. Samtidigt var det vanligt att friska kameler var bärare av olika *Str. agalactiae*-stammar framför allt i noshålan och dessa hittades också i mjölk. I de flesta av hjordarna som ingick

i studierna var mjölkningshygienen dålig, med kraftigt begränsade möjligheter till exempelvis handtvätt och spendesinficering. Detta är troligtvis en bidragande faktor till de många mastiterna och även smittspridning av *Str. agalactiae* mellan kameljuver. Det är också möjligt att kamelerna kan infektera varandra genom spridning mellan nos och/eller munhåla mellan vuxna och kalvar.

Sammantaget var subklinisk mastit vanligt bland mjölkande kameler och oftast orsakad av smittsamma juverbakterier. Dessa juverbakterier är i olika utsträckning mer eller mindre känsliga för de vanligaste sorterna av antibiotika. De genetiska studierna av *Str. agalactiae* visade att det finns juveranpassade stammar, men också att *Str. agalactiae* troligtvis ingår i den normala bakteriefloran hos friska kameler och kan överföras till juvret genom interaktion mellan kameler. Smittöverföring mellan människa och kamel kan också förekomma. För att förbättra juverhälsan hos kameler krävs fördjupade studier av hur pastoralisterna uppfattar mastit. En ökad förståelse skulle förbättra möjligheterna att utforma förebyggande åtgärder som kan accepteras och införlivas i den dagliga skötselrutinen.

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Acta Universitatis Agriculturae Sueciae

Doctoral Thesis No. 2021:40

The aim of this thesis was to increase the knowledge about mastitis in pastoralist dairy camels in Kenya. The prevalence, aetiology, antimicrobial susceptibility, risk factors for subclinical mastitis and the molecular epidemiology of *Streptococcus agalactiae* were investigated. The results show that subclinical mastitis is common, and several risk factors were identified. Decreased susceptibility to penicillin and tetracycline was found among mastitis pathogens. *Streptococcus agalactiae* is likely transmitted both contagiously and from the environment. Better milking hygiene could improve udder health.

Dinah Seligsohn received her postgraduate education at the Department of Clinical Sciences, Swedish University of Agricultural Sciences. She obtained her degree in veterinary medicine in 2014, at the Faculty of Veterinary Medicine and Animal Science at the same university.

Acta Universitatis Agriculturae Sueciae presents doctoral theses from the Swedish University of Agricultural Sciences (SLU).

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