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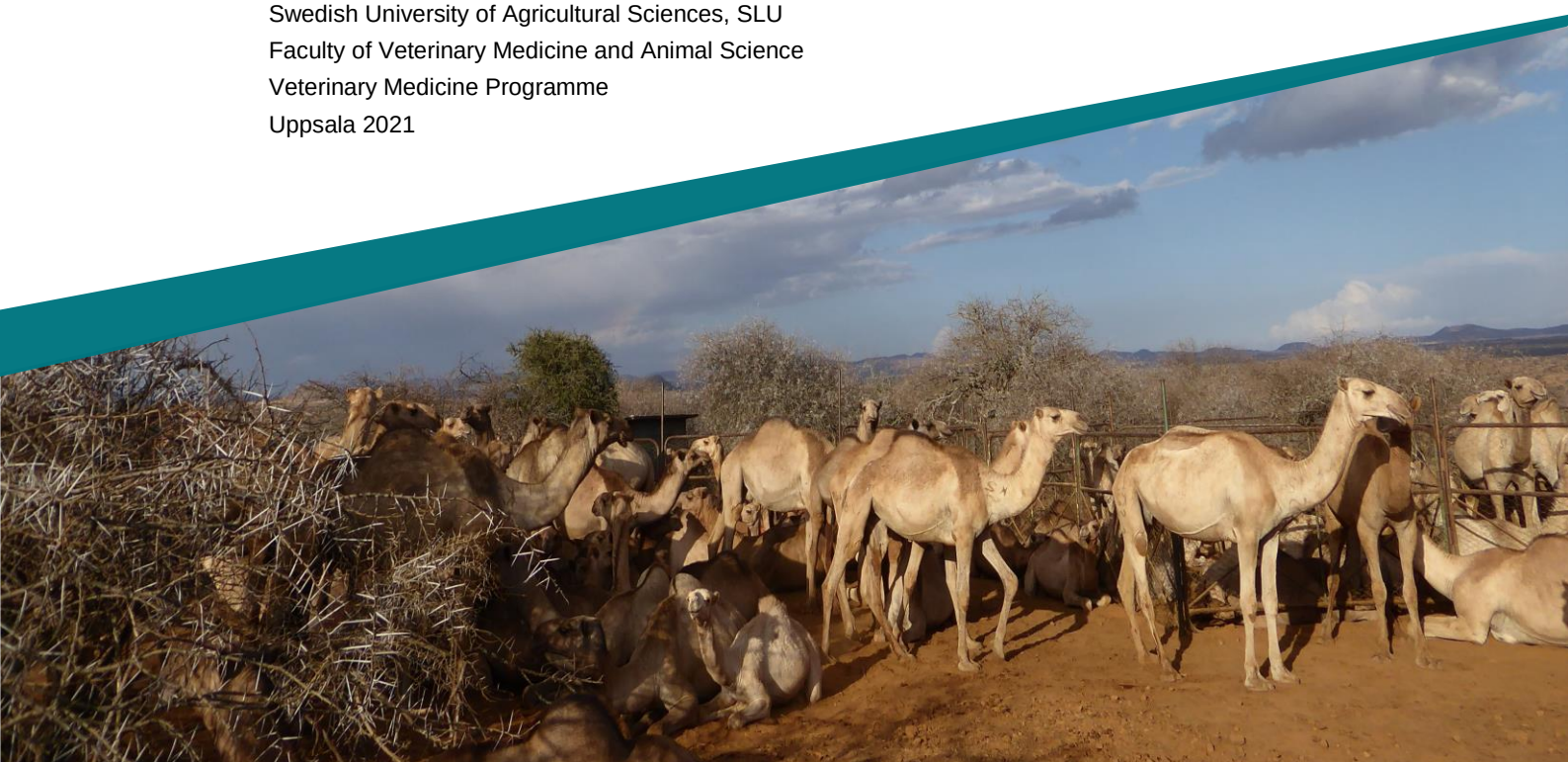

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Abstract

Camels (*Camelus dromedarius*) are the most valuable livestock species in the arid and semi-arid lands in the Horn of Africa where the majority of the human population adopts a pastoralist lifestyle with extensive animal husbandry. Camels provide an important food source, milk, which is considered to be the most important commodity, and a source of income for the pastoralists. The health of the camel, and especially the udder health, is therefore very important for the pastoralist communities living in the arid areas in Kenya that do not support other livestock.

Streptococcus agalactiae (SRA) is considered to be a zoonotic bacterium that can cause disease in both humans and animals but its zoonotic potential is not entirely clarified. It is a highly contagious udder-bound pathogen most known as a cause of mastitis, particularly in cattle, but also in camels. A few studies have also isolated SRA in apparently healthy camels, suggesting the bacteria could be a commensal.

Antibiotic resistance (ABR) is a serious health threat to both humans and animals as well as a threat to food security, global development and economies worldwide. Antibiotic resistance in bacteria causing disease in camels can potentially be transmitted to people consuming camel products or handling these animals. The two antibiotic classes that are most frequently used in livestock production are tetracyclines and β-lactams. Tetracycline resistance has been observed in SRA isolates derived from camels in Kenya, but little resistance to β-lactams has been found.

The prevalence of resistant and possibly multi-resistant bacteria in camels in Kenya is not known to a great extent. The aim of this study was to investigate if phenotypic ABR and possibly multi-resistance is present in SRA isolates from dairy camels in Laikipia County, Kenya. The overall objective was to increase the knowledge of ABR among SRA in dairy camels in order to prevent further resistance development.

In this study, six camel herds were selected for sampling; ranches (n=3), pastoralist (n=1) and smallholders (n=2). From each herd all lactating dams and their respective calves were sampled. In total, 179 individuals were sampled; 89 lactating camels and 90 calves. In lactating camels, milk samples were collected from lactating dams with a California Mastitis Test score of 2 or higher and swabs were taken from the nasal and vaginal mucosa; in their respective suckling calves from the nasal, oral and rectal mucosa. Primary identification of SRA in milk and swab samples was performed by bacterial culturing and confirmed by Matrix assisted laser desorption ionization-time of flight (MALDI-TOF). Antimicrobial susceptibility testing was performed by broth microdilution method to determine antimicrobial susceptibility in SRA isolates.

In this study, SRA was isolated from 27% of the sampled individuals. The bacterium was found at all sampling sites, except for vaginal swabs, in both healthy and CMT-positive lactating dams as well as in apparently healthy calves, supporting the suggestion that SRA is a commensal and an opportunistic pathogen in camels. The overall prevalence of tetracycline-resistant SRA isolates was high (57%) in the six herds, especially in the pastoral and ranch managed systems. No resistance to penicillin was detected. Tetracycline-resistant SRA isolates were found at all SRA-positive sampling sites. All SRA isolates from milk samples were resistant to tetracycline. A few camels and calves were SRA-positive in more than one sampling site and resistance to tetracycline could be found in one, two or none of these isolates. The results in this study, in combination with earlier results from Kenya, shows that a shift from the use of tetracycline to penicillin when treating diseases in camels would be favourable as there is a risk that tetracycline would be ineffective. Bacteria that already have acquired resistance genes will however continue to spread within and between herds. Hence, finding a resistant bacterial isolate in a camel does not per se mean that the bacterium has become resistant due to antibiotic treatment. To avoid further development of resistant bacteria, prevention of disease is the most important objective. Healthy animals do not require antibiotic treatment.

Keywords: GBS, *Camelus dromedarius*, tetracycline resistance, mastitis, milk, pastoralist

Sammanfattning

Kameler (*Camelus dromedarius*) är de mest värdefulla produktionsdjuren i öken- och torrområden i Afrikas horn. Övervägande delen av människorna som bor i dessa områden är pastoralister (herdefamiljer) och djuren hålls genom extensiv drift. Kamelerna bidrar både till pastoralisternas huvudsakliga livsmedelsförsörjning, där mjölken är den viktigaste produkten, men även som en inkomstkälla. Kamelernas hälsa, och då framför allt deras juverhälsa, är därför väldigt viktig för pastoralisterna som lever i dessa torrområden i Kenya vilka saknar förutsättningar för att hålla andra typer av produktionsdjur.

Streptococcus agalactiae (SRA) anses vara en zoonotisk bakterie vilken kan orsaka sjukdom hos både människor och djur, men dess zoonotiska potential är inte helt utredd. Det är en mycket smittsam juverbunden patogen som främst förknippas med mastit, framförallt hos kor, men även hos kameler. Ett fåtal studier har även isolerat SRA från tillsynes friska kameler vilket kan tala för att bakterien skulle kunna vara en kommensal.

Antibiotikaresistens (ABR) är ett allvarligt hot mot både människors och djurs hälsa, men även i aspekter som livsmedelsförsörjning, global utveckling och för ekonomier i hela världen. Antibiotikaresistens i bakterier som orsakar sjukdom hos kameler kan potentiellt överföras till människor som konsumerar produkter från kameler eller via närkontakt med kameler. De två antibiotikaklasser som används mest till produktionsdjur är tetracykliner och β-laktamer. Tetracyklinresistens har setts i SRA-isolat från kameler i Kenya, men förekomsten av resistens mot β-laktamer verkar vara låg.

Förekomsten av resistenta och potentiellt multi-resistenta bakterier hos kameler i Kenya är ännu inte känd i någon större utsträckning. Syftet med studien var att undersöka om det förekommer fenotypisk ABR och eventuellt även multiresistens bland SRA-isolat från mjölkkameler i Laikipia County, Kenya. Det övergripande målet med studien var att bidra med mer kunskap om ABR bland SRA hos mjölkkameler för att förhindra ytterligare resistensutveckling.

I studien ingick sex kamelbesättningar; rancher (n=3), pastoralist (n=1) och småbrukare (n=2). Från varje besättning provtogs alla lakterande djur och deras kalvar. Totalt provtogs 179 individer; 89 lakterande djur och 90 kalvar. Bland de lakterande kamelerna togs mjölkprov från kameler vilka fick värde 2 eller högre på California Mastitis Test och svabbprov togs från nos- och vaginalslemhinnan; från deras respektive kalvar togs svabbprover från nos-, oral-, och rektalslemhinnan. Primär identifiering av SRA i mjölk- och svabbprover gjordes genom bakterieodling; identifiering av bakterien bekräftades sedan med Matrix assisted laser desorption ionization-time of flight (MALDI-TOF). För att ta reda på den antimikrobiella känsligheten hos SRA-isolaten genomfördes antibiotikakänslighetsbestämning med hjälp av dilutionsmetoden.

I denna studie isolerades SRA från 27 % av de provtagna kamelerna. Bakterien påvisades från samtliga provtagningsställen, förutom från vaginalslemhinnan, från både friska och CMT-positiva lakterande kameler samt tillsynes frisk kalvar, vilket kan tala för att SRA är en kommensal och en opportunistisk patogen hos kameler. Den totala förekomsten av tetracyklinresistenta SRA-isolat i de sex besättningarna var hög (57 %), framförallt i pastoralistbesättningen och rancherna. Ingen resistens mot penicillin påvisades. Tetracyklinresistenta SRA-isolat påvisades från samtliga provtagningsställen där bakterien påvisats. Samtliga SRA-isolat från mjölkprover var resistenta mot tetracyklin. Bland några kameler och kalvar kunde SRA isoleras från mer än ett provtagningsställe; resistens mot tetracyklin kunde sedan påvisas från det ena, båda eller ingetdera av provtagningsställena. Resultaten i denna studie, i kombination med tidigare resultat från Kenya, visar att det hade varit fördelaktigt att behandla sjukdom hos kameler med penicillin istället för tetracyklin då det finns en risk att tetracyklin inte fungerar effektivt. Bakterier som redan innehar förvärvade resistensgener kommer dock kunna spridas inom och mellan besättningar; isolering av ett resistent bakterieisolat hos en kamel måste därför inte betyda att bakterien utvecklat resistens på grund av antibiotikabehandling. För att undvika ytterligare utveckling av resistenta bakterier är det viktigaste målet att djuren håller sig friska. Om djuren är friska behöver de inte behandlas med antibiotika.

Nyckelord: GBS, *Camelus dromedarius*, tetracyklinresistens, mastit, mjölk, pastoralist

Contents

Abbreviations

ABR	Antibiotic resistance
AST	Antimicrobial susceptibility testing
ASAL	Arid and semi-arid land
CMT	California Mastitis Test
ECOFF	Epidemiological cut-off value
MIC	Minimum inhibitory concentration
LMIC	Low- and middle-income countries
SRA	*Streptococcus agalactiae*

1. Introduction

In the arid and semi-arid lands (ASALs) of Kenya, where approximately 30% of the Kenyan population lives, the majority adhere to a pastoralist lifestyle with extensive animal husbandry (Amwata *et al.* 2016; Nyariki & Amwata 2019). Camels (*Camelus dromedarius*) are considered high value animals in Kenya and the Horn of Africa providing an important food source, milk being the most important commodity, and a source of income for the pastoralists (Hesse & MacGregor 2006; Elhadi *et al.* 2015; Watson *et al.* 2016). Some of the ethnic groups in Kenya, residing in the ASALs, have a long tradition of keeping camels (Musinga *et al.* 2008) and Kenya now has one of the largest camel populations in the world with over 3 million camels (FAO 2020).

Antibiotic resistance (ABR) is a serious health threat to both humans and animals worldwide as well as a threat to food security and global development (Levy & Marshall 2004; Essack *et al.* 2017; WHO 2020). When the effectiveness of antibiotics decline, bacterial infections become more difficult and costly to treat (Essack *et al.* 2017) or may even become fatal (WHO 2020). Antibiotic resistance can be a naturally occurring phenomenon in the environment (Holmes *et al.* 2016). Antibiotic resistance can also occur as a consequence of the use of antibiotics resulting in selection for resistance (Holmes *et al.* 2016), regardless of whether the usage is necessary and justified or not (Ayukekbong *et al.* 2017). The reason for the widespread extent of resistance we are faced with today is believed to be mainly due to imprudent use (i.e. unnecessary use, excessive use, use in subtherapeutic dose) of antibiotics in humans, animals and the agricultural sector (Holmes *et al.* 2016; WHO 2020).

Streptococcus agalactiae (SRA) is a zoonotic bacterium that can cause disease in both humans and animals (Hood *et al.* 1961; McDonald & McDonald 1976; Haenni *et al.* 2018). Strains of SRA have been isolated from both diseased and healthy camels suggesting the bacteria could be a commensal causing opportunistic infection (Younan & Bornstein 2007; Mutua *et al.* 2017). In camels, the pathogen is one of the most prevalent causes of mastitis (Obied *et al.* 1996; Gitao *et al.* 2014; Seligsohn *et al.* 2020), but can also for example cause respiratory disease and abscesses (Younan & Bornstein 2007). In Kenya, most of the camel milk is consumed raw, thus being a potential health risk to consumers if bacteria are present

(Matofari *et al.* 2007; Musinga *et al.* 2008; Gitao *et al.* 2014). Antibiotics are commonly used by pastoralists to treat disease in their livestock (Gitao *et al.* 2014; Lamuka *et al.* 2017); β-lactams and tetracyclines are the two families of antibiotics most frequently used (Lamuka *et al.* 2017; OIE 2020). Studies have shown that ABR is present in SRA isolated from camels in Kenya (Fischer *et al.* 2013; Seligsohn *et al.* 2020). A shift in use to other classes of antibiotics is recommended due to high resistance to tetracycline (Fischer *et al.* 2013).

Resistant bacteria and resistance genes from bacteria can be transmitted between humans and animals (Holmes *et al.* 2016). Therefore ABR in bacteria that cause disease in camels can potentially be transmitted to people consuming camel products or handling these animals. There is still a lack of evidence of the prevalence of resistant and multi-resistant bacteria in camels in Kenya today. By expanding the knowledge of ABR patterns in different bacteria and preventing overuse and inadequate use of antibiotics we can better ensure successful treatment in the future. Thus, reducing the use of antibiotics as well as preventing further resistance development.

The aim of this study is to determine the presence of phenotypic ABR and potential multi-resistance in SRA isolates from dairy camels in Laikipia County in Kenya. The overall objective of this study is to increase the knowledge of ABR among SRA in dairy camels in order to prevent further development of resistance.

2. Literature review

2.1. Camels and pastoralism in Kenya

The ASALs make up around 80% of the total landmass in Kenya (Nyariki & Amwata 2019) and are home to 30% of the population (Amwata *et al.* 2016). In these drylands, the agricultural use is limited and the majority of the inhabitants adhere to a pastoralist lifestyle with extensive animal husbandry (Amwata *et al.* 2016). The pastoralists and their livestock contribute greatly to the country's food production (Nyariki & Amwata 2019).

Some of the ethnic groups residing in the Kenyan ASALs have a long tradition of keeping camels (*Camelus dromedarius*) (Musinga *et al.* 2008). The number of camels in Kenya started to increase from around 700 000 in year 2000 to 3.3 million in the latest census from 2018 (FAO 2020). Kenya now has one of the largest camel populations in the world (FAO 2020). The majority of the camel population is managed by nomadic pastoralists (Guliye *et al.* 2007), while a few husbandry systems are semi-stationary by small households (Georgiadis *et al.* 2007) or private commercial ranches (Bornstein & Younan 2013).

In Kenya, the wet seasons occur in March to May and in November and December (Guliye *et al.* 2007). In the north of the country annual rainfall rarely exceed 400 mm. Camels are browsers (Guliye *et al.* 2007) and their adaptation to harsh environmental conditions makes them able to survive and still produce milk throughout the year, where other types of livestock perish (Schwartz & Schwartz 1985; Musinga *et al.* 2008; Bekele *et al.* 2011; Kagunyu & Wanjohi 2014). As a consequence to climate change and changing weather conditions with longer dry periods and reduced rain fall in the region, people are expanding their existing camel herd or shifting from rearing other livestock species, such as cattle and small ruminants, to camels (Kagunyu & Wanjohi 2014; Watson *et al.* 2016). Camels are considered to be the most suitable livestock to farm in this setting (Guliye *et al.* 2007).

2.1.1. Camel milk industry in Kenya

Camels are considered high value animals in the Horn of Africa functioning as an important food source and a source of income for the pastoralists (Guliye *et al.* 2007; Elhadi *et al.* 2015; Watson *et al.* 2016). Camels provide milk and meat as well as transportation (Musinga *et al.* 2008; Watson *et al.* 2016). They also play an important role in the economic security of the pastoralists and are of socio-cultural importance (Guliye *et al.* 2007). Camels can for instance be used as dowry, gifts or economic compensation.

Milk is considered the most important commodity of camels (Musinga *et al.* 2008) and is an important nutritional source for the pastoralist population; especially in the dry season (Elhadi *et al.* 2015). The milk contains protein of high quality, high levels of iron, non-saturated fatty acids and vitamin B (Al haj & Al Kanhal 2010). The level of vitamin C is three times higher compared to the level in cow's milk.

The milk yield of camels may vary depending on several factors such as age, lactation period, water and feed intake (Bekele *et al.* 2011), breed, management (Nagy & Juhasz 2016), season (Musaad *et al.* 2013), dry or wet climate (Bekele *et al.* 2002; Muloi *et al.* 2018) and manifestation of udder infection (Harmon 1994; Saleh & Faye 2011). In Somali camels (n=61) in Ethiopia, Bekele *et al.* (2002) recorded a mean milk yield of 4.14 ± 0.04 kg per day. In a later study by Bekele *et al.* (2011), the daily milk yield of eight camels ranged from 1.7 to 3.6 L. In Saudi Arabia, Musaad *et al.* (2013) reported a higher daily milk yield mean of 5.5 ± 2.2 L in 47 camels. In a questionnaire study, there are claims of daily milk yields of up to 35 L in some camel breeds in central Asia; however, these reports are difficult to assess (Aujla *et al.* 1998). In contrast to cows where feed intake and milk yield may decline already after one day of dehydration (Steiger Burgos *et al.* 2001), camels can cope with dehydration for one week before these parameters decline (Bekele *et al.* 2011).

While some traditional pastoralists still mainly use the milk for household consumption, others have adapted to commercial milk production resulting in a considerable increase of camel milk in the country in the last decades (Musinga *et al.* 2008). In 2018, almost 50% of the milk produced in Kenya was camel milk (FAO 2020) and in the east part of the country over 60% of milk consumed by the human population was obtained from camels (Elhadi *et al.* 2015). Consumers demand for milk is estimated to increase in Kenya until 2030, especially in rural areas and in the city of Nairobi (Robinson & Pozzi 2011). The price of camel milk sold in supermarkets can exceed the price of cow’s milk over four times (Musinga *et al.* 2008).

Milk let-down from lactating camels is initiated by stimulation from their suckling calf (Bekele *et al.* 2011). The camels are usually milked by hand under poor sanitary

conditions without prior cleaning of the hands, udder or milk containers (Musinga *et al.* 2008; Odongo *et al.* 2017). The milk is therefore usually contaminated with debris and microorganisms; improper storage may further spoil the quality (Brown *et al.* 2020). Significant knowledge gaps regarding food hygiene and safety have been demonstrated among most actors along the camel milk value chain in central Kenya (Odongo *et al.* 2017). Noor *et al.* (2013) reported that a majority (85.7%) of the pastoral and all (100%) of the peri-urban (i. e. camel herds kept in close proxymity to urban market) producers and milk traders in Isiolo County, Kenya, determined the hygiene and quality of milk based on subjective assessments, such as, taste and sight. The presence of potentially zoonotic pathogens in camel milk in combination with the absence of a pasteurization step prior to human consumption is a public health hazard (Musinga *et al.* 2008; Gitao *et al.* 2014; Nyokabi *et al.* 2018). If antibiotic resistant bacterial strains or antibiotic residues are present in milk, this could lead to increased ABR spread (Brown *et al.* 2020).

Several factors such as high demand for camel milk in the country, lack of concern by consumers on hygiene and food safety, cultural beliefs of medicinal properties in raw camel milk, insufficient access of clean water during the milking process and lack of tests available to determine milk quality have led to a continuous tolerance of, and therefore supply of, low quality camel milk in Kenya (Musinga *et al.* 2008). Evidently, there is a need for improved hygiene and quality for camel milk to be sold on a market beyond traditional camel-keeping communities (Musinga *et al.* 2008; Noor *et al.* 2013).

2.2. Streptococcus agalactiae

2.2.1. Characteristics

Streptococcus agalactiae is a Gram-positive β-hemolytic Lancefield Group B streptococci (GBS) (Haenni *et al.* 2018). The microorganism is considered to be a zoonotic bacterium that can cause disease in both humans and animals (Hood *et al.* 1961; McDonald & McDonald 1976; Haenni *et al.* 2018). It is a robust and versatile pathogen that has been known to infect a multitude of animal species, for example cows, horses, camels, fish and dolphins (Haenni *et al.* 2018). In animal livestock production, SRA is most known as a cause of mastitis, particularly in cattle (Keefe 2012; Haenni *et al.* 2018). The infected mammary gland is the main reservoir (Sørensen *et al.* 2019) for this highly contagious pathogen (Keefe 2012).

2.2.2. Disease in humans

In humans, SRA is an important pathogen. People can be asymptomatic carriers of SRA and it has been found in the normal bacterial flora in the intestinal- and urogenital tract (Brochet *et al.* 2006; Haenni *et al.* 2018) in up to 30% of the human population, with higher prevalence in pregnant women (DiPersio & DiPersio 2006; Simoes *et al.* 2007). Opportunistic infection can for example occur in the urinary tract and lungs (Farley 2001). In neonates, the pathogen can cause severe sepsis, pneumonia and meningitis (Schrag *et al.* 2016). Infection with SRA seems to increase in adults with pre-existing chronic medical conditions and in the elderly (Farley 2001).

2.2.3. Potential cross-species transmission

Even though SRA can be found in both humans and animals, the potential zoonotic aspect is not entirely clarified. Brochet *et al.* (2006) reported that some strains of SRA seem to be species-specific and infect either humans or animals. However, Sørensen *et al.* (2019) found lack of host-specificity for some strains and isolated genetically identical SRA from cattle and herdspersons from the same farm. Lyhs *et al.* (2016) identified a subpopulation of SRA that was shared between humans and cattle in Sweden and Finland, suggesting that horizontal transmission between humans and cattle is probable. Singapore was affected by an outbreak of infection in the human population caused by SRA in 2015 and a strong association to consumption of raw fish was made (Barkham *et al.* 2019). Fish death has been documented in experimental infection of fish by human derived isolates (Evans *et al.* 2009). Transmission of SRA between camels and humans has not yet been proven. Fischer *et al.* (2013) reported that SRA in camels are genetically distant to SRA-strains found in humans, but further investigation on possible transmission between camels and humans is needed.

2.2.4. *Streptococcus agalactiae* mastitis in cattle and camels

Mastitis, inflammation of the udder, is one of the most prevalent diseases affecting dairy herds around the world, resulting in major economic losses (Seegers *et al.* 2003) and affecting animal welfare (Keefe 2012). The disease is often caused by bacterial infection; streptococci, staphylococci and coliforms are the main causative bacterial genera, but may vary depending on management system (Persson *et al.* 2011). Mastitis can be presented either clinically, where the animal show symptoms of the disease, palpable pathological findings are present and changes of the milk are apparent, or in a subclinical form where no clinical signs are present. Detection of changes in milk composition in the case of subclinical mastitis can be confirmed by animal-side tests, such as California Mastitis Test (CMT) and laboratory analyses such as somatic cell count (SCC) (Saleh & Faye 2011). Detection of intra-

mammary infection is usually done through bacterial culturing. Presence of inflammation leads to a decrease in milk production and the quality of milk declines as the somatic cell count increases (Harmon 1994; Saleh & Faye 2011).

Streptococcus agalactiae has been a rare cause of bovine mastitis in Sweden (Persson *et al.* 2011), but the occurrence has increased in the last decade and now accounts for a prevalence of 3% (VÄXA Sverige 2020). The bacterium can be a substantial problem in other countries: 7% in Denmark (Katholm *et al.* 2012) and 34.4% in Colombia (Ramírez *et al.* 2014). In African countries, the pathogen is frequently detected as a cause of mastitis in livestock, both in cattle and especially in camels. Several studies have isolated SRA in cases of clinical or subclinical bovine mastitis in African countries. The prevalence ranges between 4-11% with 4.4% in Uganda (Abrahmsén *et al.* 2014), 5.8% in Rwanda (Mpatswenumugabo *et al.* 2017), 7.7% in Kenya (Gitau *et al.* 2014), 8.0% in South Africa (Blignaut *et al.* 2018) and 11.4% in Ethiopia (Abera *et al.* 2012).

In camels, SRA is one of the most prevalent causes of mastitis (Younan *et al.* 2001; Gitao *et al.* 2014; Seligsohn *et al.* 2020), particularly subclinical mastitis (Obied *et al.* 1996). In Kenya, studies have reported a prevalence of SRA in dairy camels at quarter level of 19.3% (Seligsohn *et al.* 2020) and 9.6% (Toroitich *et al.* 2017) and at udder level of 22.7% (Gitao *et al.* 2014) and 12% (Younan *et al.* 2001). In other East African countries, researchers have observed a prevalence at quarter level of 27% in Eastern Sudan (Obied *et al.* 1996) and at 3.8% in Eastern Ethiopia (Abera *et al.* 2010).

2.2.5. *Streptococcus agalactiae* in extramammary reservoirs and in the environment

Streptococcus agalactiae is considered to be a primarily udder-bound pathogen in livestock (Keefe 2012; Haenni *et al.* 2018), but there is evidence that points in other directions. In a study in dairy cows in Colombia, Cobo-Ángel *et al.* (2018) isolated SRA from rectal swabs from cows and environmental samples, indicating faecal shedding. In Norway, similar findings were made in dairy cows by Jørgensen *et al.* (2016). SRA was isolated from rectal and vaginal swabs from adult cows and additionally from throat swabs from calves; the pathogen was also retrieved from environmental samples. These findings suggest that SRA might not always be an obligate intramammary pathogen in bovine herds. Jørgensen *et al.* (2016) proposes an oro-fecal transmission cycle, including drinking water, in addition to the transmission cycle between infected udders and milking equipment.

In camels, SRA has for instance been isolated as a pathogen from respiratory tract infections, vaginal infections, abscesses and joint infections (Younan *et al.* 2000; Younan & Bornstein 2007). Isolation of SRA has also been reported from healthy

individuals in two studies conducted in Kenya and Somalia (Younan & Bornstein 2007; Mutua *et al.* 2017). Mutua *et al.* (2017) isolated SRA from the nasal cavity; Younan & Bornstein (2007) isolated SRA from the vaginal mucosa and the nasopharynx proposing that in camels, SRA can be a common commensal and an opportunistic pathogen in these isolation sites.

2.3. Antibiotic resistance and antibiotic use

2.3.1. Antibiotic resistance – a global challenge

Antibiotic resistance is a serious health threat to both humans and animals as well as a threat to food security, global development and economies worldwide (Levy & Marshall 2004; Essack *et al.* 2017; WHO 2020). When the effectiveness of antibiotics decline, bacterial infections become more difficult and costly to treat (Essack *et al.* 2017) and may become fatal (WHO 2020). In cases of multi-resistant bacteria, the choices of antibiotic treatment are limited and the infection may even be incurable (Levy & Marshall 2004). In developing countries, where there may be a more pronounced frequency of infectious diseases and economic constraints to obtain more suitable treatment for infections, this problem may be even more evident (Okeke *et al.* 2005a). Prudent use of antibiotics is therefore crucial to retain their indispensable properties (Levy & Marshall 2004; Prescott 2018). This crisis necessitates urgent action and a non-negotiable multisectoral One Health approach to prevent a post-antibiotic era where bacterial infections may no longer be treatable (WHO 2015).

2.3.2. How bacteria can acquire resistance to antibiotics

Any use of antibiotics in humans, animals or agriculture will build up a selection pressure leading to the survival of some bacteria and thus promote ABR (Schwarz *et al.* 2001; Prescott 2018). This selection for resistance will occur in all bacteria exposed to the antibiotic agent, not only the pathogen targeted (O'Brien 2002). If the bacteria possess resistance genes against the antibiotic used, a rapid overgrowth of these bacteria may occur (Schwarz & Chaslus-Dancla 2001). The pattern of resistance that bacteria display differs depending on the bacterium itself, the mechanisms of resistance and the antibiotic substance (van Duijkeren *et al.* 2018). In this manner, commensal bacteria and environmental bacteria can serve as reservoirs of resistance genes (O'Brien 2002). Antibiotic treatment with a single agent can result in reduced susceptibility to multiple agents if several antibiotic classes have the same target site on the bacterium (Schwarz *et al.* 2001). If a bacterium displays resistance towards several antibiotic agents, it is described as multi-resistant (Levy & Marshall 2004).

Antibiotic resistance can be either intrinsic - a naturally occurring phenomenon within the bacterium, or acquired - a response to the exposure of antibiotic agents (Holmes *et al.* 2016; van Duijkeren *et al.* 2018). Acquired resistance can be manifested through three types of mechanisms: (1) destruction or modification of the antibiotic agent by enzymatic inactivation; (2) increased efflux and/or decreased influx of the antibiotic agent in cells; and (3) modification of the antibiotic agent target structure which can develop through mutation (van Duijkeren *et al.* 2018). Resistance is usually transmitted between bacteria through acquisition of antibiotic resistance genes which can be achieved by: (1) conjugation where plasmids (circular extrachromosomal DNA) are transferred between bacteria; (2) transduction where bacteriophages transfer DNA; and (3) transformation where bacteria take up naked DNA from their surroundings (Boerlin & White 2013). This horizontal transfer of mobile genetic elements carrying resistance genes can occur in all susceptible bacteria regardless of bacterial species and genera (Schwarz & Chaslus-Dancla 2001). Vertical transmission of resistance genes in bacteria occurs by cell division.

2.3.3. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing (AST) is performed prior initiation of antibiotic therapy, when the antimicrobial susceptibility of a bacterial pathogen cannot be determined solely from its identification, if antibiotic resistance is suspected due to poor clinical response or for the purpose of surveillance (CLSI 2017). The results from ASTs can provide important information regarding selection of the most appropriate antimicrobial and is considered an essential component for responsible use of antibiotics (Watts *et al.* 2018).

Clinical breakpoints are established based on minimum inhibitory concentration (MIC) distributions, pharmacokinetic-pharmacodynamic data and clinical outcome data, and are used to categorize bacteria as susceptible, intermediate or resistant to an antibiotic agent (CLSI 2017). A bacterium is defined as being resistant to an antibiotic agent if the recommended antibiotic concentration fails to inhibit growth or kill the bacterium at the infection site (CLSI 2017). The MIC is specific for each combination of bacterial species and antibiotic agents (Watts *et al.* 2018). When epidemiological cut-off (ECOFF) values are determined, the bacterium is distinguished by distribution of MIC as “wild type” or “non-wild type” to the antibiotic tested and the ECOFF value represents the upper MIC of the “wild type” distribution (European Committee on Antimicrobial Susceptibility Testing 2020). The term “wild type” of a bacterium is defined as the intrinsic or natural resistance of that bacterium and these isolates have MICs that falls within the range that are considered to be normal for “unchanged” isolates of that particular bacterial species (European Committee on Antimicrobial Susceptibility Testing 2020). The term

"non-wild type" is used to classify bacterial isolates with phenotypic acquired resistance mechanisms and their MIC fall outside the normal range of susceptible bacteria displaying a reduced susceptibility to one or several antibiotics. The ECOFF value is not always equivalent to the clinical resistance breakpoint (Swedres-Swarm 2019) since the value is only based on in vitro data using MIC distributions (CLSI 2017).

2.3.4. Antibiotic therapy and emergence of resistance

Antimicrobial therapy, antibiotics included, has been used to treat infections in humans, animals and plants for over 60 years (van Duijkeren *et al.* 2018) and thus made it possible to cure infectious diseases and save lives (Boerlin & White 2013). The use of antimicrobial drugs in agriculture and for the treatment of animal disease was initiated shortly after the Second Word War (Prescott 2018). Since the introducetion of antibiotic drugs, the development of resistant bacteria has been evident (Levy & Marshall 2004). New antibiotic classes and analogues were developed in the mid to late 20th century to meet the increased resistance (Prescott 2018); hundreds of new antibiotics have been developed since the discovery of penicillin in the 1920s (Boerlin & White 2013). Today, however, few new antibiotic agents are developed due to lack of investments (WHO 2015).

Every year, millions of kilograms of antimicrobials are used to treat and prevent infections in humans, animals and the agricultural sector (Levy & Marshall 2004). According to the "ECDC/EFSA/EMA second joint report on the integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals", the consumption of antimicrobials was lower in food-producing animals compared to humans in 50% of the 28 European countries included in the analysis (ECDC, EFSA and EMA 2017). However, in the whole of the EU, the consumption in mg/kg biomass was higher for animals. In Africa, the reported quantity of antimicrobial substances intended for use in animals in 2016 was lower than in the other OIE regions (OIE 2020).

High levels of antibiotics kill susceptible strains of bacteria and emergence of resistant strains may occur (Levy & Marshall 2004). It has been argued that the main reason for the widespread extent of ABR we are faced with today is due to imprudent use (i.e. unnecessary use, excessive use, use in subtherapeutic dose) of antibiotics in humans, animals and the agricultural sector (Holmes *et al.* 2016; WHO 2020). A positive association between consumption of antibiotic agents and ABR in both humans and animals has been described; however, other factors than consumption may well affect the level of ABR due to its complex epidemiology (ECDC, EFSA and EMA 2017). Although unnecessary and extensive use of anti-

biotics may increase the development of resistance to a greater degree, justified use of suitable agents will also contribute (Ayukekbong *et al.* 2017).

2.3.5. The use of antibiotics in livestock production

In livestock production systems, antibiotics are used to treat diseases and maintain productivity (Van Boeckel *et al.* 2015). The use of antibiotics in nontherapeutic concentrations as a feed additive for growth promotion and/or as prophylaxis of disease has historically been a strategy in intensive production systems (Schar *et al.* 2018). This repeated use of antibiotics in low or subtherapeutic doses promote the development and spread of drug-resistant pathogens (You & Silbergeld 2014). Bacteria resistant to antibiotics may be transmitted from animals to humans by consumption of animal-based food products, handling these products or animals and through the environment (EFSA & ECDC 2020).

In Sweden, the use of antibiotics as growth promoters was discontinued in 1986 by the Feedingstuffs Act (SFS 1985:295) and the European Union followed in 2006 (European Medicines Agency, European Surveillance of Veterinary Antimicrobial Consumption 2020). The proportion of OIE Member Countries banning the use of antibiotic agents as growth promoters has increased from approximately 50% in 2012 to 74% in 2015 (Moulin *et al.* 2016). In Kenya, the use of antibiotics for growth promotion in animal production is still not regulated by law (WHO 2018). In 31 European countries, overall sales of veterinary antibiotic agents to food producing animals, including horses, have declined by over 34% in the last decade, including classes of antibiotics that are considered to be of critical use in humans (i. e. 3rd- and 4th-generation cephalosporins, polymyxins, fluoroquinolones and other quinolones) (European Medicines Agency, European Surveillance of Veterinary Antimicrobial Consumption 2020).

In developing countries, the demand for animal-based diet is growing as the world population and incomes are increasing (Robinson & Pozzi 2011). The livestock sector is, therefore, growing rapidly. With a world population growth of 40% by 2050, it is estimated that an increase of up to 100% in agricultural production is needed in developing countries (Robinson & Pozzi 2011). Van Boeckel *et al.* (2015) have estimated a global increase in the use of antimicrobials in livestock animals by 67% from 2010 to 2030. This estimate is calculated on the basis that a shift from extensive to intensive farming is expected to evolve in middle income countries to meet a greater demand for animal-based food products in low- and middle income countries (LMIC) and that intensive production systems more often use antimicrobials in subtherapeutic doses (Van Boeckel *et al.* 2015). The statement thus presumes that the overall use of antibiotics to food-producing animals in the world would not decline. However, several expert organizations are predicting

different future scenarios in aspects of antibiotic use to food-producing animals (ECDC, EFSA and EMA 2017).

In animals, both the narrow-spectrum β-lactam penicillins, and the broad-spectrum antibiotics tetracyclines are considered first hand choice for treatment of bacterial disease caused by susceptible bacteria (Haenni *et al.* 2018; Prescott 2018). Tetracyclines were the most frequently used antibiotics in animal food production worldwide in 2016; with penicillins being the second most frequently used class (OIE 2020). The use on a global scale of these antibiotic classes corresponded to the use in 21 African countries (OIE 2020).

2.3.6. Factors affecting antibiotic resistance in low- and middle income countries

The extent of antibiotic resistance and imprudent use of antibiotics in LMIC is due to several factors. In high income countries, quantification and agent specification on antibiotic use for animals in food production are frequently available (Prescott 2018), such data is scarce in LMIC, and monitoring the use and implementing regulations in LMIC may be more difficult (Schar *et al.* 2018). Just over 60% of the 44 African countries responding to a questionnaire survey by OIE in 2018-2019 could provide quantitative data on usage of antibiotics in animals (OIE 2020). However, only two countries were able to provide detailed information regarding the use in categories of animals. Lack of regulations for the use of veterinary drugs may contribute to antibiotic residues in food products (Brown *et al.* 2020). As a consequence, antibiotic resistance is likely to increase (Van Boeckel *et al.* 2015) where surveillance is lacking (Okeke *et al.* 2005b). According to the World Health Organization, the Kenyan government has approved a national antimicrobial resistance action plan that reflects the Global Action Plan objectives on antimicrobial resistance approved at the World Health Assembly in 2015, but it is not yet fully implemented (WHO 2015, 2018).

When an animal acquire a bacterial infection that requires antibiotic treatment, individual therapy is desirable (Schwarz *et al.* 2001). The animal should preferably be examined and a resistance pattern of the bacterium should be established to assure that the most suitable antibiotic agent is administered in an adequate dose and in appropriate intervals. Laboratory services are non-existent in many rural areas inhabited by pastoralists (Gitao *et al.* 2014). In rural areas of East African countries where laboratory services are available, these health care centres are seldom equipped to perform susceptibility testing of bacteria (Kakai & Wamola 2002). Treatment is therefore rarely preceded by bacteriological analysis and susceptibility testing (Gitao *et al.* 2014) and antibiotics are usually prescribed based on empirical experience (Kakai & Wamola 2002).

To minimize imprudent use of antibiotics, the therapy agent should be carefully selected and administered under veterinary supervision (Schwarz & Chaslus-Dancla 2001). The sparse accessibility to veterinary services make them easier for larger ranches to employ (Mugunieri *et al.* 2002) but is limited for pastoralist communities (Schwartz & Schwartz 1985; Mutua *et al.* 2017), as such it is not uncommon for pastoralists to administer drugs themselves (Lamuka *et al.* 2017; Toroitich *et al.* 2017; Caudell *et al.* 2020). The high economic and food security value of livestock for farmers in LMIC may increase the use of antibiotics as an insurance to prevent losses due to disease (Brown *et al.* 2020).

Even though medical prescription is desirable and recommended by the World Health Organisation, it is not uncommon for antibiotics to be sold without a prescripttion in LMIC (Muloi *et al.* 2019; Caudell *et al.* 2020). Many livestock households buy antibiotics at "agrovets", i. e. shops selling animal health products (Lamuka *et al.* 2017). Personnel working in shops selling veterinary drugs may not have sufficient knowledge about veterinary services and accurate treatment regimen in animals (Lamuka *et al.* 2017). Antibiotics can also be of substandard quality due to passing of expiration date, improper storage or being counterfeit drugs (Okeke *et al.* 2005b). The World Health Organization state that laws and regulations about antibiotic prescription and sale of antibiotics to humans and animals are implemented in Kenya (WHO 2018). However, in a study by Muloi *et al.* (2019), none of the antibiotics in the 19 veterinary drug stores in Nairobi visited were sold on prescription, and consumers were often free to purchase antibiotics according to preference. Uncontrolled sales of veterinary drugs, including antibiotics, may result in imprudent use, i.e. substances used without correct indication, incorrect administration and/or dosing regimen (Lamuka *et al.* 2017).

An important strategy to reduce the use of antibiotics is to prevent the spread of infectious disease (Ayukekbong *et al.* 2017) and for this to be achieved maintaining a high standard of hygiene and sanitation is crucial (WHO 2015). In developing countries, maintaining a good hygiene and sanitation may not be possible due to lack of clean water (Gitao *et al.* 2014), poor infrastructure (Lamuka *et al.* 2017) and economic constraints (Okeke *et al.* 2005a).

2.4. Antibiotic use and antibiotic resistance in *Streptococcus agalactiae* in camels in Kenya

2.4.1. Disease and antibiotic use in camels in Kenya

A wide spectrum of diseases affecting camels has been described in Kenya. The most common diseases reported by camel keepers in Kenya are mastitis and

Trypanosomasis (Musinga *et al.* 2008; Lamuka *et al.* 2017). Other diseases are: brucellosis, worm infestation, diarrhoea, tuberculosis and camel pox (Lamuka *et al.* 2017). Application of anti-sucking devices to the udder (i. e. tying off the teats with fabrics or plant fibres) to prevent calves from drinking milk, cauterization of udder skin (Obied *et al.* 1996; Abdurahman 2006), heavy infestation of ticks (Abera *et al.* 2010) and camel pox (Younan *et al.* 2001) can predispose for mastitis. Disease is a limiting factor in camel milk production (Farah *et al.* 2007; Bornstein & Younan 2013). Many of these diseases can affect milk quality and have a direct negative effect on production (Kashongwe *et al.* 2017). In camel rearing in Kenya, herds may manage camels and other livestock species together and movement of female camels between different herds for reproduction may also occur (Seligsohn *et al.* 2020); pathogens can then be transmitted to camels from other livestock species and between camel herds (Keefe 2012; Browne *et al.* 2017; Lamuka *et al.* 2018). Several of the diseases affecting camels are caused by zoonotic pathogens which can cause disease in both animals and humans (Browne *et al.* 2017; Lamuka *et al.* 2018; Nyokabi *et al.* 2018; Hughes & Anderson 2020; Njenga *et al.* 2020). Zoonotic pathogens can be transmitted and infect humans by livestock contact or consumption of animal based products (Nyokabi *et al.* 2018) and have been reported in pastoralists (Njenga *et al.* 2020).

Conventional treatment of disease in camels consists of antibiotics (Lamuka *et al.* 2017) trypanocides, removal of parasites such as ticks and the use of de-wormers (Musinga *et al.* 2008). It has been suggested that both the use of acaricides (Abera *et al.* 2010) and removal of ticks by hand can reduce udder health problems and mastitis in camels (Abdurahman 2006). Many herdsmen and farmers also use traditional medicine (Heffernan & Misturelli 2002), such as medicinal plants and cauterization of udder skin in cases of mastitis (Abera *et al.* 2010). Antibiotic treatment in camels is common among pastoralists in Kenya (Lamuka *et al.* 2017). When interviewing herdsmen in 20 dairy camel herds in central Kenya, everyone stated that antibiotics had been used within the last year (Seligsohn *et al.* 2020). The most frequently used antibiotics are β-lactams and tetracyclines (Younan *et al.* 2000; Lamuka *et al.* 2017; Seligsohn *et al.* 2020).

Antibiotics are widely used in cases of mastitis in camels (Gitao *et al.* 2014), but pastoralist camel herdsmen are more likely to consider treatment of mastitis when the infection is acute (Younan 2002). In 20 camel herds in central Kenya, 85% of the respondents stated that they would use antibiotics to treat camels with clinical mastitis (Seligsohn *et al.* 2020). In a study in Northern Kenya by Younan (2002), camel herdsmen were familiar with parenteral administration of antibiotics, but dose regimens that require several administrations per day may not be followed through. Antibiotic therapy administered via intramammary tubes was not well recognised as a treatment option (Younan 2002).

Some pastoralists are aware of the need for milk-withdrawal periods after treating their camels with antibiotics, but veterinary advice on the length of the withholding period has differed between 1 and 5 days (Lamuka *et al.* 2017). In a study by Odongo *et al.* (2017), knowledge among herdsmen regarding milk-withdrawal period was poor, similar to the situation in extensive smallholder livestock farming systems in Ethiopia (Gemeda *et al.* 2020). Intramammary tubes intended for cows are seldom used in camels as the anatomy of their teats makes the administration unsuitable (Saleh *et al.* 1971) and there is little information about the milk-withdrawal times in camels for these tubes (Elemam *et al.* 2010).

Veterinary services are rarely used by small-scale camel milk producers in Kenya (Musinga *et al.* 2008). In a study by Heffernan & Misturelli (2002), only 28% of livestock households in six districts in Kenya would consult the government veterinary services. Constrains in purchasing veterinary drugs for pastoralists may depend on expensive prices (Lamuka *et al.* 2017) or lack of access to veterinary services (Heffernan & Misturelli 2002). In a study by Lamuka *et al.* (2017) in central Kenya, almost all (80%) of the pastoralists replied that they purchased drugs from "agrovet" shops; many (45.8%) of them stated that they administered drugs to camels without advice from veterinary or community-based animal health workers. Veterinary drug use may often be based on experience and earlier recommenddations by veterinary services (Lamuka *et al.* 2017).

Improved hygienic conditions during handling and milking are desirable in disease prevention, but are complicated by lack of clean water (Gitao *et al.* 2014). To avoid transmission of contagious mastitis pathogens, such as SRA, between infected and susceptible camels and to minimize contamination in the milk for human consumption, hygienic milking practices are an important factor (Ahmad *et al.* 2012; Ramírez *et al.* 2014). Protective variables of infection are cleaning of the udder, teat disinfection pre- and post-milking (Keefe 2012; Ramírez *et al.* 2014) and washing of the milkers' hands prior to and after milking (Ahmad *et al.* 2012). Among pastoralist camel herdsmen in central Kenya, few maintained basic hygiene; hands and udders were rarely cleaned before milking and teats were not disinfected after the milking procedure (Seligsohn *et al.* 2020). On the positive side, many herds employed a milking order (based on newly-calved camels or age), which is commonly practiced and recommended in cases of mastitis in cattle dairy production systems globally (Keefe 2012). In a study by Toroitich *et al.* (2017), over 80% of the milkers responded that they would wash their hands before milking the camels, but a majority would not clean the udders.

Awareness among pastoralists in regard to transmission between animals and humans with zoonotic pathogens and infection-preventive measures is lacking (Lamuka *et al.* 2018; Nyokabi *et al.* 2018; Njenga *et al.* 2020).

2.4.2. Antibiotic resistance in *Streptococcus agalactiae* in camels in Kenya

Information about prevalence of resistance in bacterial species is vital to prevent imprudent use of antibiotics in locations where resistance is endemic (Levy & Marshall 2004) but data on antimicrobial susceptibility in SRA from camels in Kenya is still limited. In cows, Abrahmsén *et al.* (2014) reported that all isolates of SRA retrieved from subclinical mastitis milk samples in Uganda were resistant to tetracycline but susceptible to benzylpenicillin. Gitau *et al.* (2014) reported a sensitivity of 50% and 40% for ampicillin and tetracycline, respectively, in isolates of SRA from cows with subclinical mastitis in Kenya.

Some studies have, however, shown that tetracycline resistance is present also in SRA isolated from camels in Kenya and that resistance towards penicillin is absent or low (Table 1; Younan *et al.* 2000, 2001; Fischer *et al.* 2013; Seligsohn *et al.* 2020). Younan *et al.* (2001) found a resistance frequency of 40% for tetracycline in milk samples, but all isolates were sensitive for ampicillin and penicillin G. In an earlier study, Younan *et al.* (2000) observed that tetracycline resistance in non-milk samples from camels and calves with respiratory and joint infection was 50%; no resistance to ampicillin or penicillin G was detected. Fischer *et al.* (2013) reported a prevalence of 34% resistance to tetracycline in camel SRA isolates from milk and wound infections/abscesses in northern Kenya and recommended a shift in use to other classes of antibiotics. They found a high sensitivity to β-lactam antibiotics; only one isolate displayed reduced susceptibility. In a study by Seligsohn *et al.* (2020) conducted in central Kenya, the tetracycline resistance in SRA isolates collected from pastoralist dairy camel herds was remarkably high. Only 4.9% of the isolates were sensitive to tetracycline. In contrast, none of the isolates exhibited resistance to penicillin. In a study where susceptibility testing was performed on bacteria isolated from nasal swabs from camels in Kenya, susceptibility towards tetracycline and ampicillin was generally high for streptococcus isolates, but resistance patterns were not presented on species level for SRA (Mutua *et al.* 2017).

Table 1. Resistance towards β-lactams and tetracyclines in Streptococcus agalactiae isolates from camels in Kenya.

Type of isolates	**Resistance to β-lactams**	**Resistance to tetracyclines**	**Source**
Nose swab, joint aspirate	0%	50%	Younan *et al.* 2000
Milk sample	0%	40%	Younan *et al.* 2001
Milk sample, wound infection/abscesses	1%	34%	Fischer *et al.* 2013
Milk sample	0%	95.1%	Seligsohn *et al.* 2020

Antibiotic resistance among other bacteria than SRA found in milk samples have also been investigated in studies in East Africa (Befekadu *et al.* 2016; Ngaywa *et al.* 2019; Omwenga *et al.* 2020). In Ethiopia, raw camel milk samples were collected from pastoralist and semi-pastoralist households to investigate the prevalence of antibiotic resistance in *Staphylococcus aureus* (Befekadu *et al.* 2016). The pathogen was isolated in 6.5% of the samples and the resistance pattern for these isolates was 25% to penicillin G and amoxicillin and 50% to tetracycline. Antimicrobial-resistant *Escherichia coli* have been isolated in pooled raw milk of livestock in pastoral areas of Northern Kenya (Ngaywa *et al.* 2019). Raw milk samples from several livestock species from pastoral communities in Northern Kenya were investigated by Omwenga *et al.* (2020). They found that the isolates were contaminated with antimicrobial-resistant *S. aureus*; multi-drug resistant MRSA strains were also obtained.

The presence of antibiotic residues in household and commercially available camel milk products have been reported in Kenya (Kang'ethea *et al.* 2005; Brown *et al.* 2020). Residues of antimicrobial agents above recommended minimum residues level (MRL) in marketed milk in Kenya have been investigated by Kang'ethea *et al.* (2005). They found a prevalence of antimicrobial residues in up to 16% of the samples, with higher prevalence in samples from rural consumers in contrast to samples from urban areas or pasteurized milk products. In Nairobi, Kenya, antibiotic residues were found in 10.5% of milk samples analysed with a higher prevalence in unpasteurized milk (Brown *et al.* 2020). Of these samples, residues from β-lactams and tetracycline were found in 7.4% and 3.2%, respectively, of the samples.

Since camels are potential reservoirs for bacteria harbouring antibiotic resistance, they could be a contributor to the spread of ABR which pose a risk to public health and food safety (Lamuka *et al.* 2017). The presence of antibiotic residues in milk can increase the spread of ABR when bacteria are exposed to low levels of antibiotics (Brown *et al.* 2020). Knowledge about risk factors associated with potential transmission of resistant bacteria and residues of veterinary drugs in animal-based products have been shown low among Kenyan pastoralists in a study by Lamuka *et al.* (2017). In a cross-sectional survey of practices and knowledge among retailers of antibiotics in Nairobi, Kenya, by Muloi *et al.* (2019), the majority of the respondents acknowledged antimicrobial resistance as a threat to human health and that a reduction in use for livestock animals is a step in the right direction. None of the antibiotics in the 19 veterinary drug stores were sold on prescription and consumers' preference was taken into account when supplying the drugs. Some of the pharmacists did however not see how they could help stop antimicrobial resistance (Muloi *et al.*2019).

3. Material and Methods

3.1. Study area and population

Material for this study was collected in Laikipia County located in central Kenya by the main supervisor. The semi-arid land of Laikipia covers 9 666 m^2 and presents a large biodiversity. Rainy seasons occur in April-May, August and November, with an annual rainfall of 639 mm (Georgiadis *et al.* 2007). The population of camels has increased, presumably due to longer periods of drought, and measured approximately 9, 800 in 2018 (Kenya National Bureau of Statistics & County Government of Laikipia 2019). Land use is typically group ranches in the north and permanent agriculture by smallholders in the south (Georgiadis *et al.* 2007). Camels are mainly ranched and semi-stationary. Sampling took place during the wet season in November, 2019. This study was approved by the National Commission for Science, Technology and Innovation, Nairobi, Kenya (Permit number: NACOSTI/P/19/84995/13088). Camel owners included in the study gave their oral permission to participate.

3.2. Sample selection

For this study, six herds were selected for sampling. The herds were categorised as ranches (*n*=3; herd A, B and D), pastoralist (*n*=1; herd C) and smallholders (*n*=2; herd E and F). From each herd all lactating dams and their respective calves were selected for sampling. Herds were selected on the basis of prior knowledge of subclinical mastitis being prevalent within the herds (Tinggren 2019). Other factors taken into consideration were accessibility to the herds and cooperation and willingness to participate among the camel owners.

The ranches were managed and run by land-owners and employed herdsmen were in charge of the camels. In the ranches, the camels were kept in enclosed fenced areas overnight and during the milking process. The pastoralist herd was owned by a Somali woman with a tradition of camel keeping and employed herdsmen managed the camels. Milk from the ranches and the pastoralist herd was sold for commer-

cial use. In the smallholder herds, camels were managed by Maasai groups on communal land. Camels were kept in bomas (traditional enclosures constructed from branches and bushes) at night and during milking. Other animal species, for example sheep and goats, were also kept on the grounds. Some milk was sold commercially, but the majority was consumed by the households.

All camels were hand-milked. Calves were released to initiate milk let-down during the milking procedure. Camels were milked once a day in herd A, twice a day in herd B, C, E and F, and four times a day in herd D. None of the herds cleaned the udders or the teats before milking. No teat disinfection was practiced pre- or post-milking in any of the herds. In herd A and B, milkers would wash their hands after milking the whole herd.

3.3. Sample collection

Prior to sampling, animal owners and herders received oral or written information about the purpose of the study and the sampling procedures, and consent to take part was obtained.

Lactating camels and their calves were sampled in connection with the early morning milking at 4-8 am. Composite milk samples were collected aseptically from lactating dams with a CMT score of 2 or higher (Nordic scale 1-5) in at least one udder quarter using CMT (Schalm & Noorlander, 1957); no clinical evaluation of the udder was performed. Milk samples were kept cold during transport and were frozen at -20°C within 4 h from the time of collection. Swab samples were collected using sterile flocked nylon swabs (e-swab, Coopan diagnostics Ltd. Murieta, CA, US). In lactating camels swabs were taken from the nasal and vaginal mucosa and in their respective suckling calves from the nasal, oral and rectal mucosa. All sampling sites were assessed clinically according to the following criteria, nasal tract: presence/absence of respiratory disease (nasal discharge, cough, breathing difficulties); vaginal mucosa: presence/absence of vaginal discharge or lesions, rectal mucosa: presence/absence of diarrhoea and wounds in the rectum area. Absence of the above listed symptoms was classified as “apparently healthy”. The localized assessment of clinical health status was performed by the samplers directly prior sampling by following the criteria set for each sampling site. Swab samples were kept refrigerated at 4-8°C and cultured within 1-10 days from collection.

3.4. Bacterial culturing

Initial laboratory analyses were performed at the Department of Public Health Pharmacology and Toxicology, College of Agriculture and Veterinary Sciences, University of Nairobi, Nairobi, Kenya and continued at the Swedish National Veterinary Institute (SVA, Uppsala, Sweden).

Primary identification of SRA in milk and swab samples was based on morphology on the selective culture medium Edwards agar (Oxoid, CM0027) and catalase test. After 18-48 hours of aerobic incubation at 37°C, colonies of blue pigmented β-hemolytic catalase-negative streptococci were identified and subjected to Christie, Atkins, Munch-Petersen-test (Christie *et al.* 1944) and slide latex agglutination test (Streptex Latex Agglutination Test, ThermoFisher Scientific Inc., Waltham, MA, USA). Positive isolates were frozen and transported to SVA. Matrix assisted laser desorption ionization-time of flight mass spectrometry analysis (MALDI-TOF MS) (Bizzini *et al.* 2010) was used to confirm bacterial strains. Criteria for species identification in the MALDI-TOF were as follows: a score of ≥2 indicated identification at species level, 1.80 to 1.99 at genus level, and <1.80 no identification. Species identification was performed using a custom-made database including the Bruker databases no. 5627 and no. 5989.

3.5. Antimicrobial susceptibility testing

Isolates of SRA kept at -80°C at SVA, were tested for presence of phenotypic antimicrobial resistance by determination of MIC by the author.

The isolates were re-cultured on 5% horse blood agar plates (SVA, Uppsala). Antimicrobial susceptibility testing was performed via broth microdilution method using cation-adjusted Mueller-Hinton broth, Sensititre™ STAFSTR panels (TREK diagnostic system ltd, UK) and Sensititre™ NLD1GNS panels (TREK diagnostic system ltd, UK) according to standards by the Clinical and Laboratory Standards Institute (CLSI 2017). A quality-control strain, *Staphylococcus aureus* ATCC 15019, was tested in parallel with the isolates; results were within acceptable ranges. Antimicrobial substances inoculated to determine MIC were: cephalotin, clindamycin, enrofloxacin, erytromycin, gentamicin, nitrofurantoin, penicillin, tetracycline and trimetoprim-sulfametoxazol. Isolates were classified as "susceptible" or "resistant" to each antimicrobial agent tested based on species-specific ECOFF values issued by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, clindamycin. erythromycin, nitrofurantoin, penicillin and tetracycline) or by clinical cut-off values from SVA (cephalotin and trimetoprim-sulfametoxazol). *Streptococcus* species have a low inherent susceptibility to quino-

lones and an ECOFF value is not defined by EUCAST. Minimum inhibitory concentration for enrofloxacin has been defined in this study, but has not been interpreted with any breakpoint. In this study, the ECOFF value for gentamicin was defined as a MIC of 32 µg/mL, since it was the highest range of concentration that could be tested in the chosen panel, although 64 µg/mL is defined by EUCAST.

Out of the 58 isolates inoculated on the Sensititre^{TM} STAFSTR panel, 17 isolates showed growth at the highest concentration of gentamicin provided at 8 µg/mL. These isolates were retested on the Sensititre^{TM} NLD1GNS panel with a wider range for gentamicin (up to 32 µg/mL) and the gentamicin MIC from this panel was used for these isolates.

3.6. Statistical analyses

Data editing, descriptive statistics and frequency tables were generated using Excel (Microsoft Corp., Redmond, WA). Associations between categorical variables were conducted using chi-square test and other suitable statistical analyses. All statistical analyses were performed in STATA (Stata Statistical Software, release 13.1; StataCorp LP, College Station, TX).

4. Results

4.1. Descriptive and statistic data

4.1.1. Study population and herd management

The six herds selected for sampling are shown in Table 2. In the selected herds, the number of camels ranged from 53 to 180 individuals. A total of 179 individuals were sampled from the six selected herds; 89 lactating camels and 90 calves. The proportions of sampled individuals from each herd ranged from 21 to 63%, and in total, 34% of the individuals in the six herds were sampled. The majority of the camels were of Somali breed; followed by Turkana breed, crossbreeds and other breeds.

Table 2. Presentation of study population: herd id, management system, herd size, number and percentage of sampled lactating camels and calves from each selected herd in Laikipia County, Kenya, 2019.

Herd	Management system	Herd size	Sampled camels	Sampled calves	Percentage sampled individuals
A	Ranch	76	22	26	63%
B	Ranch	75	23	23	61%
C	Pastoralist	180	19	19	21%
D	Ranch	86	11	9	23%
E	Smallholder	53	8	8	30%
F	Smallholder	53	6	5	21%

4.1.2. Prevalence of *Streptococcus agalactiae* isolates

In all, 58 out of 465 (12.5%) isolates were positive for SRA after bacteriological culturing and analysis (Table 3 and Figure 1). *Streptococcus agalactiae* was isolated from all sampling sites in both age categories, except for vaginal swabs. In total, SRA was isolated from 20.0% (10/50) of milk samples, 27.3% (24/88) of nasal swabs from adults, 22.2% (14/63) of nasal swabs from calves, 7.9% (7/89) of oral swabs and 3.4% (3/87) of rectal swabs from calves. In herd A, no nasal swabs

from calves were collected. Herd prevalence of SRA was highest in the two small-holder herds F (41.4%) and E (22.5%); followed by herd D (13.6%), B (9.4%), A (8.3%) and C (7.8%).

Among sampled individuals from all six herds, SRA was isolated from 29 out of 89 (33%) lactating camels and 19 out of 90 (21%) calves; and from 27% (48/179) of the total number of sampled individuals. Out of the 58 SRA isolates, 34 isolates came from lactating camels and 24 from calves. In five lactating camels, SRA was isolated from both milk samples and nasal swabs; three out of these individuals belonged to herd D. In five calves, SRA was isolated from both nasal swabs and oral swabs; three out of these individuals belonged to herd F. In 31% (9/29) of the cases, both lactating dam and the suckling calf were positive for SRA (herd B=3, E=2, F=4).

Table 3. Distribution of positive Streptococcus agalactiae isolates (n=58) including herd level and sampling site from lactating camels and calves in Laikipia County, Kenya, 2019.

	Adult			Calf			
Herd	**Milk isolates**	**Vaginal isolates**	**Nasal isolates**	**Nasal isolates**	**Oral isolates**	**Rectal isolates**	**Total**
A	16.7% (2/12)	0% (0/22)	22.7% (5/22)	n/a	0% (0/26)	7.7% (2/26)	8.3% (9/108)
B	28.6% (4/14)	0% (0/23)	13.0% (3/23)	13.6% (3/22)	4.5% (1/22)	4.3% (1/23)	9.4% (12/127)
C	11.1% (1/9)	0% (0/19)	15.8% (3/19)	21.1% (4/19)	0% (0/19)	0% (0/17)	7.8% (8/102)
D	30.0% (3/10)	0% (0/11)	36.4% (4/11)	11.1% (1/9)	0% (0/9)	0% (0/9)	13.6% (8/59)
E	0% (0/3)	0% (0/7)	57.1% (4/7)	37.5% (3/8)	25.0% (2/8)	0% (0/7)	22.5% (9/40)
F	0% (0/2)	0% (0/6)	83.3% (5/6)	60.0% (3/5)	80.0% (4/5)	0% (0/5)	41.4% (12/29)
Total	20.0% (10/50)	0% (0/88)	27.3% (24/88)	22.2% (14/63)	7.9% (7/89)	3.4% (3/87)	12.5% (58/465)

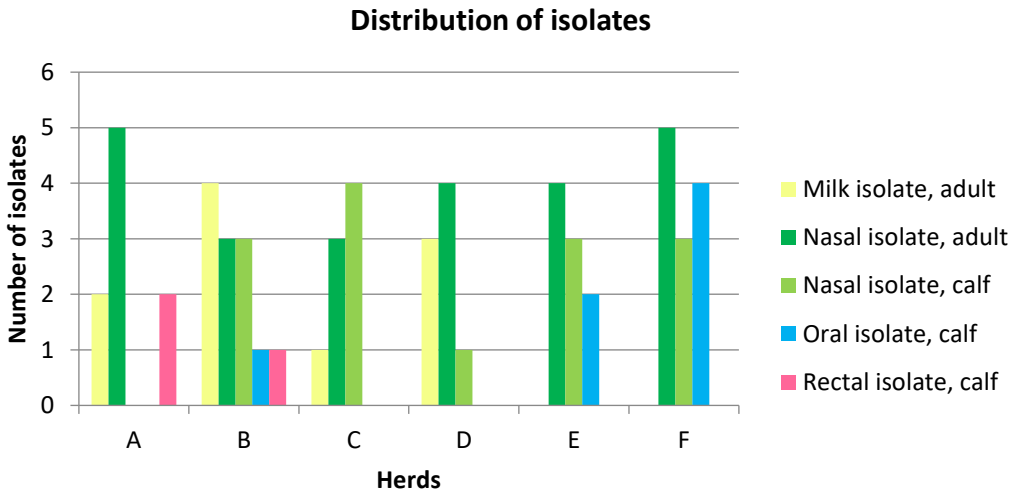

Figure 1. Distribution of positive Streptococcus agalactiae isolates (n=58) including herd level and sampling site.

4.1.3. Antimicrobial susceptibility testing – Prevalence of resistance in *Streptococcus agalactiae* isolates

In all, 58 SRA isolates were tested for antimicrobial susceptibility. The MIC for cephalotin, clindamycin, enrofloxacin, erytromycin, gentamicin, nitrofurantoin, penicillin, tetracycline and trimetoprim-sulfametoxazol and the results of the susceptibility test are shown in Table 4.

Among the SRA isolates, the only antibiotic where resistance was present was tetracycline. A bimodal distribution of MIC of tetracycline was observed, with a wild type population with MICs ranging from ≤0.25 to 0.5 µg/mL. A total of 57% (33/58) SRA isolates were classified as resistant towards tetracycline since they displayed growth at MIC above the given ECOFF. All of the tested isolates were sensitive to penicillin since they grew at MIC well below the given ECOFF. All the SRA isolates were also sensitive to cephalotin, clindamycin, erytromycin, gentamicin, nitrofurantoin and trimetoprim-sulfametoxazol as they had MICs below the ECOFF. For enrofloxacin, no ECOFF or clinical breakpoints were available for comparison. Multi-resistant SRA isolates were not detected.

Table 4. Distribution (percentage) of MIC and prevalence of resistance (percentage) for Streptococcus agalactiae (n=58) isolated from lactating camels and calves in Laikipia County, Kenya, 2019[1].

		Distribution (%) of MICs (µg/mL)											
Test agent	**% R**	**≤0.03**	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**
Cephalotin	0						100						
Clindamycin	0					100							
Enrofloxacin	NA					70.7	29.3						
Erytromycin	0					100							
Gentamicin	0							5.2	65.5	29.3			
Nitrofurantoin	0										100		
Penicillin	0	27.6	72.4										
Tetracycline	57				39.7	3.4			1.7	55.2			
Trimethoprim-Sulfametoxazol	0				100								

[1]Unshaded cells indicate the range of concentrations tested for each antimicrobial agent. Shaded cells indicate concentrations outside the range tested for each substance. Minimum inhibitory concentration (MIC) equal to or lower than the lowest concentration tested for an antibiotic substance (≤Y µg/mL), is given as a percentage at the lowest tested concentration. Blank unshaded cells indicate lack of isolates with that MIC. Bold vertical lines indicate epidemiological cut-off values retrieved from the European Committee on Antimicrobial Susceptibility Testing or clinical cut-off values from SVA.

Camel keepers from each herd were asked if the sampled camels had been treated with antibiotics in the last two weeks before visits; however, class of antibiotic was not specified. Antibiotics had only been given to two of the sampled camels in herd B. In one of these two camels, SRA was isolated from a milk sample, and in the other camel from a milk sample and a nasal swab. All three isolates showed resistance to tetracycline.

4.1.4. Distribution of tetracycline resistance at herd level

The overall herd prevalence of tetracycline resistant SRA isolates was 57% (33/58); all herds but one yielded tetracycline resistant SRA isolates, as presented in Table 5.

Table 5. Distribution (number of isolates) of MIC and prevalence of tetracycline resistance (percentage) at herd level for Streptococcus agalactiae (n=58) isolated from lactating camels and calves in Laikipia County, Kenya, 2019.

		Distribution (number of isolates) of MICs (µg/mL)					
Herd	**% R**	**<= 0.25**	**0.5**	**1**	**2**	**4**	**>4**
A	56	3	1				5
B	100						12
C	100						8
D	88	1				1	6
E	0	8	1				
F	8	11					1
Total	57	23	2			1	32

Ordinary logistic regression showed a significant association between management system and occurrence of tetracycline resistance, with ranches being more at risk (OR=2.76, *P*=0.004) compared to the other management types (pastoralist and smallholders).

4.1.5. Distribution of tetracycline resistance at sampling sites

Resistance towards tetracycline was found in SRA isolated from all sampling sites, except vaginal swabs (Table 6, Table 7 and Figure 2).

For isolates from lactating camels, the prevalence of tetracycline resistance was 62% (21/34); and in calves, tetracycline resistance was found in half of the isolates (12/24).

Table 6. Distribution (number of isolates) of MIC and prevalence of tetracycline resistance (percentage) at sampling site for Streptococcus agalactiae (n=58) isolated from lactating camels and calves in Laikipia County, Kenya, 2019.

		Distribution (number of isolates) of MICs (µg/mL)					
Samples	**% R**	**<=0.25**	**0.5**	**1**	**2**	**4**	**>4**
Milk isolates, adult	100						10
Nasal isolates, adult	46	13				1	10
Nasal isolates, calf	57	5	1				8
Oral isolates, calf	29	5					2
Rectal isolates, calf	67		1				2
Total	57	23	2			1	32

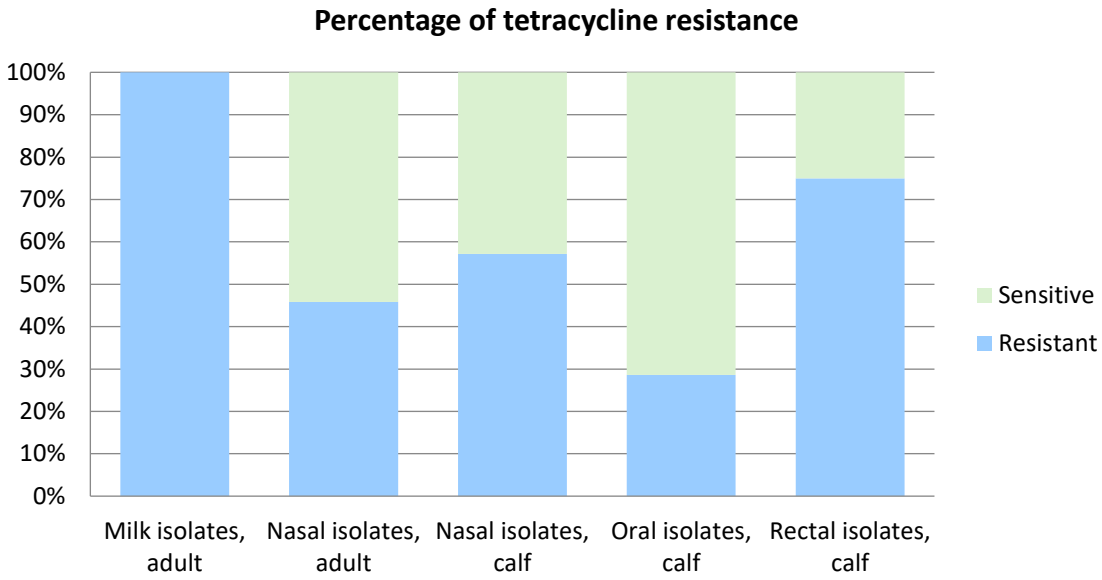

Figure 2. The proportion of tetracycline resistance (percentage) in Streptococcus agalactiae (n=58) isolates, presented according to sampling site.

Table 7. Distribution of tetracycline resistant Streptococcus agalactiae (n=58) isolates including herd level and sampling site.

	Adult		**Calf**		
Herd	**Milk isolates**	**Nasal isolates**	**Nasal isolates**	**Oral isolates**	**Rectal isolates**
A	100% (2/2)	40% (2/5)	n/a		50% (1/2)
B	100% (4/4)	100% (3/3)	100% (3/3)	100% (1/1)	100% (1/1)
C	100% (1/1)	100% (3/3)	100% (4/4)		
D	100% (3/3)	75% (3/4)	100% (1/1)		
E		0% (0/4)	0% (0/3)	0% (0/2)	
F		0% (0/5)	0% (0/3)	25% (1/4)	
Total	100% (10/10)	45.8% (11/24)	57.1% (8/14)	28.6% (2/7)	66.7% (2/3)

Out of the 29 lactating camels from which isolates of SRA were retrieved, nine of their calves were also positive for SRA. Resistance towards tetracycline was found in isolates from three lactating dams and their respective calves from herd B. The remaining six SRA-positive lactating dams and their respective calves belonged to herd E and F; among these individuals, tetracycline resistant SRA was only isolated from one calf in herd F.

4.1.6. Distribution of tetracycline resistance at individual level

Streptococcus agalactiae was isolated from 29 out of 89 (33%) lactating camels and 19 out of 90 (21%) calves. In five lactating camels, SRA was isolated from both milk samples and nasal swabs. Out of these five lactating camels, three had tetracycline resistant isolates in both milk samples and nasal swabs (one in herd B and two in herd D), while two only displayed resistance to tetracycline in SRA isolated from the milk samples (herd A and D). In five calves, SRA was isolated from both nasal swabs and oral swabs. Out of these five calves, one had SRA isolates in both sampling sites (herd B) that displayed tetracycline resistance, one only in the oral swab isolate (herd F); while the SRA isolates from the remaining three calves showed sensitivity to tetracycline (herd F).

Ordinary logistic regression showed no significant association between age category and risk for resistance when comparing calves with lactating camels (OR=0.84, P=0.77).

5. Discussion

In the ASALs in the Horn of Africa, camels are considered high value animals and their milk the most important commodity. The health of the camel, and especially the udder health, is therefore very important for the pastoralist communities living in these dry areas in Kenya, both for food security and also as a financial security. *Streptococcus agalactiae* is a common pathogen among camels in Kenya. The aim of this study was to determine the presence of phenotypic ABR and assess potential multi-resistance in SRA isolates from dairy camels in Kenya with the overall objective to increase the knowledge of ABR among SRA in dairy camels in order to prevent further development of resistance.

In this study, resistance to tetracycline was the only antibiotic resistance found in the camel SRA isolates from Laikipia County, and no multi-resistant strains were detected. The overall prevalence of tetracycline resistance in the SRA isolates derived from the six selected camel herds was 57% (33/58). It should be kept in mind that the method of the antimicrobial susceptibility testing performed has a one-step margin of error as the antibiotics are inoculated in double concentration for each step in the panels used. The MICs for the SRA isolates categorized as tetracycline resistant were however well above the EUCAST ECOFF values used which supports the results of phenotypic resistance to tetracycline in these isolates. An increased resistance to tetracycline was apparent while no resistance towards penicillin was detected. However, due to the limited number of SRA isolates, no general conclusions can be drawn regarding the resistance status of camels in Kenya.

This discussion will address how SRA as a commensal bacteria and resistant strains of SRA in apparently healthy camels potentially can be maintained and spread in camel herds in Kenya. It will also address, based on the results in this study in combination with earlier findings from Kenya, that a shift of antibiotic agents used to treat disease in camels would be favourable. Lastly, the discussion will emphasise the importance of disease prevention in order to reduce antibiotic resistance in camels.

5.1. Isolation of *Streptococcus agalactiae* in apparently healthy camels

In total, 34% (179/523) of the individuals in the selected herds were sampled. It should be noted that herd size is a sensitive question for pastoralists to state; the total number of individuals may therefore not be exact for the pastoralist herd. *Streptococcus agalactiae* was isolated from milk, in nasal and oral cavities and rectal swabs but not from vaginal swabs. The isolation of SRA in other sites than milk samples in apparently healthy camels and calves could indicate that the bacterium is a commensal in camels. The possibility that SRA could be a commensal in camels that may cause opportunistic infection in many different types of tissues has also been suggested in two previous studies (Younan & Bornstein 2007; Mutua *et al.* 2017). The nasal cavity was the most common site for SRA in both age categories in this study. A SRA-prevalence of 40% in nose swabs from healthy camels in the study by Younan & Bornstein (2007) corresponds to the prevalence found in lactating camels from the ranch herd D and in calves in the smallholder herd E in the present study. The present study did, however, observe a higher prevalence of SRA-positive nasal isolates in lactating camels in smallholder herd E and lactating camels and calves in herd F, despite not finding any SRA-positive milk isolates in these herds. The prevalence of SRA-positive oral isolates was also quite high in the smallholder herds in the present study, while SRA-positive rectal isolates only were found in two of the ranches. A possible explanation for high prevalence of SRA-positive isolates from the nasal- and oral cavity in the smallholder herds could be that these sites are colonized by the same strain.

The isolation frequency of SRA in milk samples from the pastoralist herd and ranches are in agreement with previous studies in Kenya (Younan *et al.* 2001; Gitao *et al.* 2014; Toroitich *et al.* 2017; Seligsohn *et al.* 2020). Camel keepers are often unaware of the existence of the subclinical form of mastitis as it is undetected in the absence of tests available to determine milk quality (Abera *et al.* 2010); causative pathogens may therefore persist in the herd. The finding of CMT-positive but SRA-negative milk samples in lactating camels in the smallholder herds in this study could be that the camels were affected by other udder pathogens. *Streptococcus agalactiae* was however present in the two smallholder herds in this study and was found in high prevalence in nasal and oral isolates. Another reason for not finding the bacteria in cases of mastitis, could possibly be due to intermittent shedding of the bacteria (i.e. the camel would have been CMT-positive but no viable bacteria were present in the milk) (Mahmmod *et al.* 2015). Low prevalence of SRA in milk samples from camel and cattle smallholder herds has been observed in Eastern Ethiopia and Uganda (Abera *et al.* 2010; Abrahmsén *et al.* 2014). In the smallholder herds other livestock species were also kept on the grounds. Direct interaction between other ruminants and camels, or indirect by people handling

these animals, could potentially contribute to the spread of SRA, however, according to Fischer *et al.* (2013), SRA in camels seems to be host-specific.

The epidemiology of SRA is still unknown in camels. Although no environmental samples were collected in this study, the presence of SRA isolated from rectal, nasal and oral swabs in calves and in nasal swabs from adults, could indicate faecal and respiratory shedding of the pathogen, as have been suggested for cattle in Norway and Colombia (Jørgensen *et al.* 2016; Cobo-Ángel *et al.* 2018). The harbouring of the bacteria in the gastrointestinal- and/or respiratory tract in camels and shedding of the bacteria by these extramammary sites could be one explanation for how the bacterium can persist in herds in addition to the transmission cycle between infected udders and milking equipment. A transmission cycle between calves and between calves and lactating dams other than their mother may also be plausible. Associations between calves consuming SRA-contaminated milk and transmission in cattle have been made (Schalm 1942). This could potentially pose a risk for transmission to humans, through consumption of raw camel milk or through close contact with camels. Understanding the inter-species transmission between camels and humans requires genomic analyses of sympatric and contemporaneous isolates. Whole genome sequencing could be a valuable tool in understanding the complex SRA epidemiology in camels.

5.2. Tetracycline resistance in *Streptococcus agalactiae* isolates in apparently healthy camels

Among the SRA isolates in this study, tetracycline resistance was found in isolates from all sampling sites (except for vaginal swabs) from apparently healthy lactating camels, calves and lactating camels with mastitis. Resistance patterns for SRA were not investigated in the previous two studies that identified SRA in healthy camels (Younan & Bornstein 2007; Mutua *et al.* 2017). The prevalence of tetracycline resistance in the SRA isolates investigated in this study is in agreement with earlier findings in Kenya (Younan *et al.* 2000, 2001; Fischer *et al.* 2013; Seligsohn *et al.* 2020). Carriers of tetracycline-resistant SRA were found in five out of the six herds and it was shown that tetracycline-resistant SRA isolates were more likely to be found in the ranch management system. The prevalence of SRA in the different management systems could depend on the bacterium capacity to spread in that particular setting. Theoretically, prevalence of resistant SRA isolates could be a result of a high consumption of antibiotics in the pastoralist herd and ranches in comparison to the smallholder herds; however, no long-term information about antibiotic treatment in these herds was obtained. A more detailed analysis between management system and findings of phenotypic resistance in SRA could have been made with a larger sample size. In the studies investigating resistance patterns in

SRA isolates from camels in Kenya, mentioned in the literature review, the majority of the camels were managed under pastoral conditions or management types were not defined (Younan *et al.* 2000, 2001; Fischer *et al.* 2013; Seligsohn *et al.* 2020). Only one herd in the study by Younan *et al.* (2001) was a ranch. Comparisons between studies investigating resistant bacteria in camels or cattle from other countries are problematic due to differences in management systems, information about antibiotic use, hygiene practices in studied herds and a lack of detailed presentation of results from the antimicrobial susceptibility testing. For this reason, it is difficult to draw conclusions from this study as to why the resistance profiles differ between the management systems in camel herds in Kenya.

The presence of tetracycline-resistant SRA in nasal swabs was detected in approximately half of the lactating camels and calves sampled. Colonization of a tetracycline-resistant SRA-strain in the upper respiratory tract seems to be common in the pastoralist herd and ranches. Although only ten SRA milk isolates were found in this study, the results of their resistance pattern correspond to results found in the study by Seligsohn *et al.* (2020). In the later study, tetracycline resistance was found in 96.1% of the SRA isolates in cases of subclinical mastitis in lactating camels from 20 pastoralist herds around Isiolo town, Kenya. One potential explanation for camels and calves being more at risk in the ranch management systems in this study is an overall high resistance pattern among udder pathogens in these herds. The high prevalence of tetracycline-resistant SRA from milk samples could be due to transmission of resistance genes; from other mastitis pathogen species, environmental bacteria, commensals from the udder or teat skin, via calves or the milkers' hands. Camel keepers from the pastoralist herd and ranches in the present study did not wash their hands before milking the camels; SRA, including tetracycline-resistant strains, would then have good opportunity to spread within the herd. The only difference in milking practice hygiene observed between the herds in this study was the washing of hands by the milkers after milking the whole herd (herd A and B); however, SRA was frequently isolated from milk samples in these herds. In cases of clinical mastitis in cows, an increased milking frequency per day to enhance the removal of pathogens from the teat canal has been debated as a supportive treatment measure, but its potential positive effect on cure rates seems to depend on the causative pathogen (Roberson *et al.* 2004; Krömker *et al.* 2010; Suojala *et al.* 2010). In cows, where udder-bound SRA more often cause subclinical mastitis, dry cow therapy is recommended (Keefe 2012). In the present study, the ranch herd D had the highest prevalence of SRA in milk samples and in this herd, camels were reportedly milked four times a day in comparison to once a day in herd A and twice a day in herd B, C, E and F. Since SRA is highly contagious and the primary risk period for transmission of the bacterium between animals is during the milking procedure (Keefe 2012), increased exposure due to several milkings per day under poor sanitary conditions could potentially contribute to the higher preva-

lence of the pathogen in milk samples seen in this study, including tetracycline resistant strains. However, the association between milking frequency and SRA prevalence at herd level was not investigated in this study. In ranch D, the oldest camels were milked first and in ranch A and B, no milking order was applied. Both age (Ahmad *et al.* 2012; Seligsohn *et al.* 2020) and the absence of milking order have been shown to be predisposing factors for mastitis in camels (Keefe 2012); this may explain the higher prevalence of SRA-isolation and tetracycline-resistant SRA milk isolates in these herds. Biosecurity measures are recommended to prevent introduction of contagious mastitis pathogens in cattle herds (Keefe 2012). Another possible reason for the high prevalence of SRA milk isolates in ranch D could be that they had purchased new camels within the last year; however, no information about biosecurity measures implemented with regards to the purchase of new animals in herd D was obtained.

The finding of tetracycline-resistant isolates in one, both or neither of the sampling sites in the individuals having two SRA-positive isolates could suggest that camels in Kenya may harbour different strains of SRA, some that are tetracycline-resistant and some that are not, but further genomic studies are needed to clarify this issue. In nine cases, both lactating camels and their respective calf were SRA-positive, but in only three pairs (mother and calf) did the isolate from the calf display similar MIC to tetracycline as the isolate from the mother (all from herd B). Tetracycline-resistant isolates were found at all sampling sites in these individuals, consequently, both a milk- and gastrointestinal transmission route as well as a respiratory transmission route could be suggested, but the numbers of samples are limited. However, since all of the SRA-isolates in herd B were resistant to tetracycline, it could also be suggested that resistance genes have been spread among SRA colonizing different sites.

5.3. Transmission of tetracycline-resistant *Streptococcus agalactiae* in camels in East Africa

Resistance in bacteria can be acquired by the selection pressure built up by the use of antibiotics, but the spread and long-term persistence of bacterial resistance genes is also seen in the absence of direct antibiotic selection pressure (Holmes *et al.* 2016). This means that the resistance already present in bacteria will continue to spread in animals and in humans even if the use of antibiotics is ended. A resistant bacterial isolate found in a camel does not per se mean that the bacterium has become resistant due to antibiotic treatment. Acquisition of new genes is the most common mechanism whereby bacteria become resistant to tetracyclines (van Duijkeren *et al.* 2018); the *tet*(M) gene being one of these tetracycline-resistant genes (Haenni *et al.* 2018). In the study by Fischer *et al.* (2013), SRA isolates from

camels in East Africa were characterized using multilocus sequence typing (MLST), capsular typing and antimicrobial susceptibility testing. The *tet*(M) gene was found in all camel SRA isolates displaying phenotypic resistance towards tetracycline; a majority of these isolates came from mastitic milk samples. Since the *tet*(M) gene was linked to the Tn-916 transposase (mobile element), the authors concluded that resistance gene transfer is likely to occur via this mobile element (Fischer *et al.* 2013). The *tet*(M) gene was observed in different MLST clades, suggesting that acquisition of the tetracycline resistance gene has occurred on several occasions (Fischer *et al.* 2013). This suggests that some SRA clones that are spread among camels in East Africa today already have acquired tetracycline resistance.

5.4. A shift of antibiotic treatment in camels to reduce resistance development and ensure successful treatment

The fact that tetracyclines are one of the most frequently used antibiotic classes in pastoral camel herds in the Horn of Africa, including Kenya, (Younan *et al.* 2000; Lamuka *et al.* 2017; Seligsohn *et al.* 2020) may be one explanation for the high frequency of tetracycline-resistant SRA isolates found in this study. Other antibiotics than tetracycline should be used in treatment of SRA infection in camels in East Africa since tetracycline has a widespread risk of being ineffective and to prevent further spread of tetracycline-resistant strains, as suggested by (Fischer *et al.* 2013). The authors also raised concern about a possible emergence of β-lactam antibiotic resistance, since one camel SRA isolate displayed resistance to amoxicillin-clavulanic acid. However, resistance to penicillin was not detected in the present study and remains low in the study area. A shift from the use of tetracyclines to penicillins may therefore be favourable.

Information about antibiotic dose regimens in camels is scarce and needs further investigation (Lamuka *et al.* 2017). The stability of penicillin in the ASALs also needs to be further explored to ensure successful treatment (Younan 2002).

5.5. Prevention of disease in camels

Camels are predicted to increase in numbers in the ASALs of Kenya (Kagunyu & Wanjohi 2014; Watson *et al.* 2016). The camel dairy production has increased in the country in the last decades and is of great importance. Knowledge about diseases, transmission of pathogens, disease preventive measures and efficient treatment

in camels is still scarce in Kenya. Regulations of antibiotic purchases are also low (Muloi *et al.* 2019). Camel management and other factors such as veterinary services in aspects of prevention and control of disease, as well as diagnosis and treatment might be even more important in the future.

Prevention of disease is a cornerstone in reducing the use of antibiotics and consequently reducing the possible development of antibiotic resistance. To put it plainly; healthy animals will not require antibiotic treatment. General disease preventive measures proposed in a camel manual for pastoralist camels in Kenya are; public education and awareness of diseases, surveillance and sampling, vaccination against rift valley fever, camel pox, brucellosis, anthrax and rabies, quarantine and/or livestock movement control and to safely dispose the remains of dead animals (Younan *et al.* 2012).

It can be difficult for herdsmen to follow recommendations suggested to eradicate SRA in cattle milk production systems in high income countries due to the way camels are managed in Kenya. The recommendations for mastitis control in camels should be tailored to a pastoralist context and adapted to the local setting. Herdsmen should preferably wash their hands with water and soap prior to milking, but since water might be scarce in the pastoralist communities (Gitao *et al.* 2014), hand disinfection could potentially be applied. In a study by Toroitich *et al.* (2017) the herdsmen believed that the calf would clean the udder while suckling to initiate milk let-down before milking, but this could potentially be a risk for contamination. After the calf initiates milk let-down, the camel udder can be wiped with a clean cloth prior milking. The CMT-test has been shown to work well as a screening method for intramammary infection with *Staphylococcus aureus* and SRA in camels in Kenya (Younan *et al.* 2001) and should preferably be used. Milking order should be applied, where camels with signs of clinical mastitis or a positive CMT-score are milked last. Vaccination against SRA is not available, but would be desirable (Fischer *et al.* 2013). The recommendations above do however require investigation in camels over time to be able to evaluate their effectiveness in disease prevention. Their accuracy for camel husbandry systems in Kenya and willingness of camel keepers of implementing these recommendations also need to be further investigated.

5.6. Conclusions

A high point prevalence of tetracycline resistance was found in SRA isolates from six camel herds investigated in Laikipia County, Kenya; no other resistance was detected. There was an association between management system and the finding of phenotypic resistance in SRA, with ranches being more likely to have tetracycline-

resistant isolates. *Streptococcus agalactiae* was found at all sampling sites, except for in vaginal swabs, and was most commonly found in the nasal cavity in camels irrespective of age category, suggesting that SRA could be a commensal and an opportunistic pathogen in camels. Since administration of tetracyclines has a risk of treatment failure in camels, a shift from the use of tetracyclines to penicillins would be favourable. However, to avoid further development of antibiotic resistance and improve camel health, prevention of disease should be the highest priority. If the camels are healthy they do not require antibiotic treatment.

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Popular science summary

Camels are the most valuable livestock species in the drylands of Kenya. Here, a majority of the human population are pastoralists (herding families). Camels provide an important food source, especially the milk, which is considered to be the most important product, and a source of income for the pastoralists. The health of the camel, and especially the udder health, is therefore very important for people living in the drylands in Kenya where rearing other livestock, such as cattle, is more challenging due to the harsh environments.

The bacterium *Streptococcus agalactiae* is best known as a cause of infection in the udder, particularly in cattle, but also in camels. A few studies have also found *Streptococcus agalactiae* in healthy camels, suggesting that the bacterium could be part of the normal bacterial flora. The bacterium can also be found in humans, but the potential transmission between animals and humans is not well investigated.

Bacteria can be resistant to antibiotic treatment. This means that bacterial infection might be more difficult to cure, or in worst case scenario, it cannot be cured. The resistance in bacteria can be spread to other bacteria, and this may happen more often when much antibiotic-usage occur. A resistant bacterium found in a camel does not, however, per se mean that it has become resistant due to antibiotic treatment in that individual. *Streptococcus agalactiae* that already are resistant to an antibiotic agent are widespread among camels in East Africa. *Streptococcus agalactiae* from camels in Kenya have been shown to be resistant to the antibiotic class tetracyclines, but not to β-lactams (e.g. penicillins). The presence of resistant and possibly multi-resistant bacteria (bacteria that are resistant to more than one type of antibiotic class) in camels in Kenya is however not known to a great extent. The aim of this study was to investigate if *Streptococcus agalactiae* found in camels in Kenya is resistant to one or several classes of antibiotics. The overall objective was to increase the knowledge of antibiotic resistance among *Streptococcus agalactiae* in camels in order to ensure successful treatment and prevent further resistance development.

In this study, lactating camels and calves from six camel herds were sampled (milk sample, vaginal-, nose-, oral- and rectal swab). An antimicrobial susceptibility test

was performed to see if the bacterium was resistant to a number of selected types of antibiotics.

The results showed that resistance to tetracycline in *Streptococcus agalactiae* was common in camels in the studied herds, but no resistance to penicillin or other classes of antibiotics were found. This result is similar to previous reports from Kenya. Resistance to tetracycline in *Streptococcus agalactiae* was found in milk, nose, oral and rectal samples in both lactating camels and in apparently healthy calves, which supports the possibility of *Streptococcus agalactiae* being a part of the normal bacterial flora in camels. Resistance to tetracyclines may become more common while resistance towards penicillins seems to remain low. However, due to the limited number of SRA samples, no general conclusions can be drawn regarding the resistance status of camels in Kenya.

In conclusion, it would be better to treat diseases requiring antibiotics in camels with penicillins instead of tetracyclines, since the latter might not cure the camel and may lead to further development of resistance. However, the most important objective is prevention of disease. If the camels are healthy, they do not need to be treated with antibiotics.