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Udder Health in Dairy Cows in Tajikistan – Prevalence of Mastitis and Bacteriological Findings

Juverhälsa hos mjölkkor i Tadzjikistan – mastitprevalens och bakteriologiska fynd

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SUMMARY

Agriculture, including dairy production, is one of the most important industries in Tajikistan, occupying more than 50% of the country's population. Consequently, milk is an important source of income, but it also contains essential nutritional components. In Tajikistan, the majority of milk production is confined to small household farms, but large commercial (enterprise or dekhan) farms are multiplying as the country is becoming more and more self-suppliant of dairy products.

To increase milk production and maintain the nutritional value of the milk, it is of great importance to prevent diseases. Mastitis, inflammation or infection of the udder, is the most costly disease in dairy cattle worldwide. It leads to great economic losses and impaired milk quality, becoming a health and welfare problem to both humans and dairy cows. To prevent mastitis, measures should be implemented - different depending on what causative agent is creating the disease.

The present study was executed in the urban and peri-urban area of Dushanbe, Tajikistan, where 351 dairy cows were sampled and the milk was evaluated with California Mastitis Test (CMT). The study was executed on both large and small household farms. Out of the 351 cows, 209 (59.5%) showed indications of an elevated SCC in one or more udder quarters. Of the 209, 26 cows (12.4%) where classified as clinical cases of mastitis, while the remaining 183 cows (87.6%) were classified as subclinical cases of mastitis. The results showed that production type (large or small household farm) was not a significant risk factor for mastitis, however, manner of milking (by hand or machine) was. The odds were 1.69 times higher that cows milked by hand were suffering from mastitis.

From cows classified with mastitis (subclinical or clinical) an aseptic milk sampled was taken, cultivated and evaluated after 24 and 48 h. In 24% of the cases, negative growth was found. The most frequently found bacterial species was *Corynebacterium* spp. (18%), followed by mixed growth (14%) and coagulase negative staphylococci (CNS) (13%). Overall results showed a prevalence of 37% contagious bacteria (including *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae* och *Corynebacterium* spp.). This was compared to environmental bacteria (coliform bacteria and *Streptococcus uberis*) that composed 9% of the findings. CNS can be transmitted both from cow-to-cow and via the environment, hence is classified on its own and was responsible for 13% of the cases of mastitis. To minimise the transmission of the most common group of bacteria, contagious bacteria, strict hygiene routines should be implemented during milking.

A comparable distribution of bacteria was found when evaluating the results based on milking manner. When evaluating the results based on farm size, large farms also had a similar distribution of bacteria. In small household farms, however, the results deviated prominently. Here CNS was the most common type of bacteria with a prevalence of 37%, followed by *Staphylococcus aureus* (11%) and *Corynebacterium* spp. (9%). A theory for this deviation is the difference in the keeping of cows – small household farmers keep fewer cows and therefore there are different principal means of transmission.

SAMMANFATTNING

Jordbruk och mjölkindustri är bland de viktigaste näringarna i Tadzjikistan, och sysselsätter mer än 50 % av befolkningen. Mjölk är därmed en viktig inkomstkälla, men även betydelsefull ur nutritionssynpunkt. I Tadzjikistan sker majoriteten av mjölkproduktionen på små gårdar med produktion för självförsörjning, men de större, kommersiella gårdarna växer i antal i takt med att landet blir mer och mer självförsörjande på mejeriprodukter.

För att öka produktionen och säkerställa mjölkens nutritionella värde är det av yttersta vikt att förhindra sjukdomar. Mastit, inflammation eller infektion i juvret, är den mest kostsamma sjukdomen hos mjölkkor världen över, och leder till försämrad mjölkkvalitet samt minskad mjölkavkastning. Konsekvenserna är ekonomiska förluster och en hälsorisk för både djur och människor. För att förhindra mastit krävs förebyggande åtgärder, olika beroende på vilken eller vilka patogener som orsakar sjukdomen.

I denna studie har, i närområdet till Tadzjikistans huvudstad Dushanbe, mjölk från 351 kor undersökts med California Mastitis Test (CMT). Av dessa hade 209 kor (59,5 %) indikationer på förhöjda celltal i mjölken från en eller flera juverdelar. Undersökningarna gjordes på både kommersiella gårdar och gårdar med mjölkproduktion för självförsörjning. Av 209 kor bedömdes 26 stycken (12,4 %) ha klinisk mastit, då de hade visuella förändringar i mjölk, tecken på inflammation i juver eller förhöjd kroppstemperatur (>39,2^{o} C). Resterande 183 kor (87,6 %) klassificerades istället som subklinisk mastit. Studien visar att produktionstyp (kommersiell eller självhushåll) inte var en signifikant riskfaktor för mastit, medan mjölkningsmetod (hand- eller maskinmjölkning) var det; oddsen var 1,69 gånger högre att kor som mjölkades för hand led av mastit.

Från kor med indikation på förhöjda celltal togs ett aseptiskt mjölkprov som odlades ut på tre olika medier: nötblodagar, mannitolsaltagar och McConkeyagar. Efter 24 och 48 h gjordes en bedömning av vilka sjukdomsframkallande agens som vuxit fram. På 24 % av odlingarna återfanns negativ växt. Den mest frekventa bakteriearten var *Corynebacterium* spp. (18 %), följt av blandflora (14 %) och koagulasnegativa stafylokocker (13 %). Sammantagna resultat visade att juverbundna bakterier utgjorde 37 % av de framodlade patogenerna (inkluderande *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae* och *Corynebacterium* spp.). Detta jämfördes med miljöbundna patogener vilka utgjorde 9 % (inkluderande koliformer och *Streptococcus uberis*). Koagulasnegativa streptokocker, som kan spridas både via miljö och från juver till juver, klassificerades för sig och låg bakom 13 % av mastitfallen. För att minimera infektioner med juverbundna patogener krävs striktare hygienrutiner vid mjölkning, vilket är deras huvudsakliga spridningsväg.

Liknande fördelning av bakterier kunde ses vid analys av resultaten från handmjölkade och maskinmjölkade kor, liksom på kommersiella gårdar. På gårdar med mjölkproduktion för självförsörjning var distributionen däremot annorlunda. Koagulasnegativa stafylokocker var på dessa gårdar den i särklass vanligaste bakteriearten (37 %), följt av *Staphylococcus aureus* (11 %) och *Corynebacterium* spp. (9 %). En teori varför ett så annorlunda bakteriemönster återfanns på denna typ av gård är ett lägre smittryck på grund av färre mjölkkor per gård.

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INTRODUCTION AND AIM OF THE STUDY

Livestock is an important and continuously growing sector worldwide (Von Braun, 2010). It serves as a source of high-quality dietary components, as well as a possible generation of capital – and, in addition, may also have a positive influence on other agricultural sectors (Randolph *et al*., 2007). In low-income countries, the livestock industry is a considerable part of improving livelihood, especially the keeping of dairy cattle (Von Braun, 2010), and milk is an animal-source food rich in multiple essential vitamins, minerals and amino acids (Pereira, 2014). Since dairy products are valuable by both nutritive and economical means, prevention of diseases that affect quality and quantity of milk is of great importance.

Mastitis, inflammation of the udder, is such a disease. It has a complex aetiology and several factors can affect the course of illness. It is omnipresent and is said to be the most costly disease in dairy cattle worldwide (Radostits *et al*., 2006). Economic losses are not only confined to veterinary services, but is also caused by e.g. decreased milk yield and increased labour (Halasa *et al*., 2007). Mastitic milk have an altered composition of nutrients, impaired quality and, additionally, if not pasteurized it may potentially become a source of human infection (Muehlhoff *et al*., 2013).

Bacteria is the most common aetiology of mastitis, and the bacterial species can have different pathogenicity, patterns of transmission and be more or less prone to create a clinical versus subclinical infection (Hogeveen *et al*., 2011, b). It is important to know what bacteria is creating the disease, because dependent on bacterial characteristics, different measures are implemented to reduce the prevalence of infection – both regarding management and treatment.

In Tajikistan, a majority of the population is working within agriculture (Landguiden, 2016) and dairy production is one of the main agricultural activities (Sattorov, 2016). The average milk yield/cow during one lactation is 780 litres – which is significantly less than adjacent countries, and ten times less than the average Swedish dairy cow (Sattorov 2016; VÄXA, 2017). There are multiple suggestions to increase outputs including an improvement of hygiene standards, which, among others, could prevent mastitis (Sattorov, 2016).

The aim of this study is to evaluate the prevalence of mastitis, both clinical and subclinical, in the population of cows in the urban and peri-urban area of Dushanbe, Tajikistan. It will also consider which pathogens are the most common aetiology behind these infections to be able to prevent genesis of disease.

LITTERATURE REVIEW

Tajikistan

Tajikistan is located in central Asia, crossing borders with Afghanistan, Uzbekistan, Kirgizstan and China. It is a land-locked country, mainly consisting of mountainous areas. Only around 7 % of the country is arable land, however agriculture is still occupying more than half of the population (Landguiden, 2016). It is the poorest of all Commonwealth of Independent States (Sedik and Lerman, 2009). According to Asadov (2013), the poverty is decreasing, but in 2011 around 41% were still living below the poverty rate[1].

The history of the country has had a great impact on the agricultural sector. Tajikistan was a part of the Soviet Union, and during this period the agricultural system was strictly organised and regulated regarding everything from supplies to production and prices. After the Soviet collapse in 1991, the system disintegrated and major declines in production and wages were inevitable, just as the incline in debts and farm losses. Civil war followed and lasted from 1992-97, which intensified the economic crisis. To reverse these effects, land reforms were implemented, with goals to reorganize the Soviet state-owned enterprise farms into individually owned farms, so called dekhan ("peasant") farms. In 2007 the Soviet type of collective farms constituted only 30% of farm land, in comparison to 99% during the Soviet era. In 2007, the production of agricultural products were back to the levels preceding the Soviet collapse (Sedik and Lerman, 2009).

The trends in livestock ownership has followed the pattern of the rest of the agricultural sector, in terms of collective farms turning into dekhan farms. The main share of livestock has however always been kept in the private sector and small household farms. Even though this was the case also during Soviet times, the number of small household farms more than doubled in the time period of 1991-2008, making the livestock ownership even more confined to the private sector (Sedik and Lerman, 2009).

Dairy production in Tajikistan

Livestock husbandry is widespread across Tajikistan and is one of the main agricultural activities. Production of meat and dairy are some of the major businesses within livestock keeping (Asadov, 2013). In January 2016, with a rising trend, 95% of the milk was produced in small household farms. A cow in a private household produces on average 780 L milk/260 days in Tajikistan, which is a lot less compared to neighbouring countries (Sattorov, 2016). In further comparison, Swedish average milk yield/cow during 2016 was 9759 L/305 days (VÄXA, 2017). Except for the domestic dairy production, there is also an import of dairy products from countries in the region, such as Russia, Kyrgyzstan, Iran, Pakistan – however the import is decreasing (Sattorov, 2016).

Within the small households keeping dairy cows, there is a lack of knowledge regarding optimal feeding, breeding and health of the animals. There is also a gap in information about accurate management of the milk, both during milking and throughout treatment, storage and transportation. There is a problem with bacterial contamination of milk – mainly due to the milking process, but there is also a lack of mastitis control. Improved hygiene is a key factor

[1] Based on international standards of 2011 (US$2.15/day), purchasing power parity (Asadov, 2013)

that could lead to both a higher quality standard of the end-product, and an increased income for the individual farmer, since a higher price is paid by the retailer for high-quality milk (Sattorov, 2016).

Livestock-keeping in development countries

Livestock and its inputs are a growing economic sector all over the world – as a whole, livestock constitutes up to 40% of the total revenues within the agriculture sector (Magnusson, 2016). In development countries, it is one of the most important overall sources of income, and especially the dairy industry has a great impact on the livelihood of poor people (Von Braun, 2010). The keeping of livestock in low-income areas are generally small scale and low-to-no-input, creating an inexpensive management of a valuable resource (Randolph *et al*., 2007).

The effect of livestock on human wellbeing does not only include the obvious contribution of animal-source foods, it also has other positive properties. Sale of excess product (or if full purpose of livestock keeping is sales) produces an income for the family. It is also common to keep livestock as a saving scheme. Other positive aspects of livestock husbandry are animal traction power, use of manure as soil fertilizer or fuel, or (depending on culture) it could be an enhancer of social status (Randolph *et al*., 2007). Livestock do in this matter contribute to other agricultural yields and are conducive to development of sustainable farming systems (Smith *et al*., 2013).

Properties of milk

Milk is an animal-source food that holds many valuable macro- and micronutrients. It contains high-quality proteins, including essential amino acids, and a wide range of different fat components. It is also a source of several important minerals, vitamins and antioxidants such as calcium, vitamin B12 and selenium (Haug *et al*., 2007). It has been shown that the nutritional components in milk helps to improve nutritional status, especially for children - components that play a key role in e.g. development of cognitive abilities and motoric functions. The high-quality proteins are of such that potentiate maximal growth and improve overall dietary quality (Muehlhoff *et al*., 2013).

Since milk and other dairy products have the above mentioned purposes in the lives of people, a decrease in milk yield can be of significant importance. Losses in milk production can be due to an abundance of different reasons: restricted food and water intake, environmental factors, diseases etc. A common disease causing a decrease in milk production is mastitis (Radostits *et al*., 2006).

Mastitis

General description

Mastitis is an inflammation of the mammary gland. The inflammation can occur due to multiple different aetiologies, but most commonly it is an infection with bacteria and/or other pathogens (Radostits *et al*., 2006; Sharma *et al*., 2012). Mastitis alters the milk composition both chemically and physically and may lead to destruction of glandular tissue - consequently this leads to a decrease in milk yield and impaired milk quality (Sharma *et al*., 2012). Mastitis is the most common disease in adult dairy cattle (Andrews *et al*., 2008.).

Clinical, subclinical and chronic mastitis

The severity of the disease is categorized depending on whether the changes only can be seen in the milk, if the udder is affected or if the cow is systemically ill. Changes in milk can be for example discoloration, clots or pus, and one or more quarters can be affected (Radostits *et al*., 2006). An infection affecting the udder may express signs of inflammation such as heat, swelling, pain or redness in affected quarter(s) (Andrews *et al*., 2008; Radostits *et al*., 2006). A systemic response to mastitis may be toxaemia, fever or depression, amongst others (Radostits *et al*., 2006). When symptoms are recognised, no laboratory tests are required for the diagnosis clinical mastitis (CM) (Sandholm *et al.*, 1995). The severity of the disease is dependent on multiple factors, such as virulence of the pathogen, and age, breed and immunological status of the cow (Viguier *et al*., 2009).

However, the lack of clinical signs of mastitis on either milk, udder or cow cannot exclude mastitis. If the cow suffers from subclinical mastitis (SCM), the infectious agents are still excreted from affected quarters, the milk composition is altered and milk yield is lowered - this despite the lack of visible indications of disease (Radostits *et al*., 2006; Sandholm et al. et al., 1995). The subclinical infection, if untreated, can persist for a long period of time, or may develop into a clinical mastitis (Andrews *et al*., 2008). Detection of SCM may be challenging due to its subtle appearance (Viguier *et al*., 2009).

Mastitis can also be categorized based on the duration of disease, either acute or chronic. The chronic state is a rarer form that indicates that the mammary gland is unable to get rid of the infection and one or more quarters permanently contains bacterial herds. The ultimate state of chronic mastitis is atrophy of the udder parenchyma and consequently a non-producing, so called blind quarter (Radostits *et al*., 2006; Viguier *et al*., 2009).

How to diagnose mastitis

Somatic Cell Count

Mastitis is most often indicated by an elevated somatic cell count (SCC) in the milk. It is the most significant deviation between normal milk and SCM milk (Radostits *et al*., 2006). Evaluation of SCC, with direct or indirect methods, has therefore become the most common way to assess udder health and evaluate the milk and milk quality (Radostits *et al*., 2006; Schukken *et al*., 2003; Sharma *et al*., 2011).

All milk contains a certain concentration, a baseline, of somatic cells, regardless of inflammation status (Sharma *et al*., 2011). Natural variation occurs, both between individuals, but it may also vary within the individual's milk composition. A few reasons for fluctuations were summarized by Sharma *et al*. (2011):

- Age/parity: with increased age and parity, a higher concentration of SCC is regularly found.
- Breed: high-producing producing breeds have been reported to more commonly have an increased SCC
- Stage of lactation: biologically, higher concentrations of SCC occurs post-partum and by the end of lactation
- Season: generally, a higher SCC has been recorded during summer compared to winter

- Diurnal variation: a difference of SCC with up to 40% has been recorded within the same individual during one day, without any change in the above-mentioned motives

A marked elevation of SCC depends on either one of two things: 1) It could be caused by a change in dilution– a lessened production of milk but the same baseline SCC makes the concentration of SCC appear higher. This is the case at a sudden drop in milk yield e.g. during systemic disease. 2) It might also be a true increase in concentration of somatic cells, e.g. due to an intra-mammary infection (IMI) (Sandholm *et al.*, 1995).

When an IMI occurs, the SCC will rise. This increase of somatic cells is generally a composition of leukocytes (mainly neutrophils) that works as a protective mechanism of the mammary gland to fight the infection, but also epithelial cells from the damaged glandular tissue (Radostits *et al*., 2006; Sharma *et al*., 2011). When the infection is defeated the SCC-levels generally goes down to normal levels within a few weeks (Schukken *et al*., 2003).

A few recorded cases have shown that IMI may occur without the occurrence of an elevated SCC (Schukken *et al*., 2003).

How to measure SCC

SCC can be measured on quarter level, cow level or herd level (Sandholm *et al.,* 1995). The most precise way to measure SCC is on quarter level, since all four quarters are separate units and may differ in infection status (Schukken *et al*., 2003). Studies have shown that uninfected quarters have a mean SCC of below 100 000 cells/ml, but variation occurs naturally, and therefore the threshold for disease has been established to be 200 000 cells/ml (Schukken *et al*., 2003; Sharma *et al*., 2011). This does not mean that a SCC of 200 000 cells/ml is the ultimate SCC-level to strive for, but rather the optimal threshold for diagnostic of disease, keeping diagnostic error at a minimum (Schukken *et al*., 2003).

A precise SCC can be measured with cell counters such as the Fossomatic (Foss Electric, Hillerød, Denmark) or DeLaval cell counter (DeLaval International AB, Tumba, Sweden), which both are well correlated with the reference microscopic method of counting SCC (Gonzalo *et al*., 2006). Indications of an elevated SCC on quarter level can easily be done with on-site tests like the California Mastitis Test (CMT) (Grace *et al*., 2017; Sharma *et al*., 2011). Other means of detecting mastitis is with e. g. immunoassays, nucleic acid testing or measuring milk conductivity (Viguier *et al*., 2009).

California Mastitis Test as an indication of SCC

California Mastitis Test (CMT) is a cow-side method to indirectly estimate SCC. It works by determining the amount of DNA in the milk, which stands in direct correlation to the number of cells in the milk (Rebhun *et al*., 2008). CMT-solution is added to milk and mixed, cell membranes and nuclei deteriorates in contact with the solution, leading to a release of DNA into the milk (Sandholm *et al.*, 1995). The higher the amount of DNA, the more viscous the mixture becomes, and the level of viscosity is thereafter graded (Viguier *et al*., 2009). Nordic countries have agreed to grade on a scale of 1-5. The interpretation of the test works well, provided that a single, experienced person evaluates the result, to avoid inconsistency of the readings (Sandholm *et al*., 1995).

An on-site test like CMT is only indicative of disease and will not present the definite infection status of the udder (Viguier *et al*., 2009). It may, however, work as a guideline for what quarters should be sampled for bacterial culture through detection of an elevated SCC (Sargeant *et al*., 2001). Caution should always be taken when evaluating CMT scores in cows with a biologically elevated SCC (post-partum, end-lactation, etc.) (Rebhun *et al*., 2008), but even so, it has been shown that CMT can be used for indication of disease even during these scenarios (Bhutto *et al*., 2012; Sargeant *et al*., 2001).

It has repeatedly been shown that a disadvantage of CMT is the relatively low specificity for mastitis, and hence gives a high amount of false positive results (Bhutto *et al*., 2012; Sandholm *et al.*, 1995). Despite this, CMT is considered a helpful source in detecting mastitis, both clinical and subclinical (Rebhun *et al*., 2008). CMT is inexpensive, easy to use and recommended for variable farm conditions (Grace *et al*., 2017).

Underlying causative pathogens

Most commonly, mastitis is caused by a bacterial invasion, even though algae and fungi (including yeast) are other possible pathogens (Hogeveen *et al*., 2011, b). The bacteria are generally divided into contagious and environmental, although some can be classified as both (Radostits *et al*., 2006).

Contagious bacteria

Contagious bacteria are transmitted from cow to cow, for example during the milking procedure when the same cleaning cloth is used on multiple cows, or via milking machines. To eradicate such bacteria, recommendations are improvements in hygiene, especially during milking, in combination with antimicrobial therapy and possible culling of chronically infected animals. Some common contagious bacteria are *Staphylococcus aureus* and *Streptococcus agalactiae* (Radostits *et al*., 2006).

Staphylococcus aureus is an important causative agent of mastitis in most countries. It can cause everything in between SCM to acute, severe clinical mastitis. It has features that makes some strains more resistant to phagocytosis, thus treatment is made more difficult (Hogeveen *et al*., 2011, b). Some strains have also developed resistance towards penicillin, which otherwise should be the first choice of antibiotics. To prevent the disease from spreading, chronically infected or penicillin-resistant cows are in Sweden recommended to be culled (SVA, 2017 a; SVS, 2015), and, furthermore, improved milking hygiene and implementation of milking order, where *S. aureus*-infected cows are milked last, are suggested actions (SVA, 2017 a). Even though *S. aureus* is infectious mainly via the milking procedure (SVA, 2017 a), studies have also shown that environmental reservoirs contributes to another dimension of contamination (Capurro *et al*., 2010).

Streptococcus agalactiae is, unlike other streptococci, considered to be a highly contagious agent. It is an obligate udder pathogen, renown to create chronic and/or recurrent infections. Cases of acute CM does however occur (Sandholm *et al.*, 1995). When chronically infected, antibiotic treatment does not have desired effect (SVA, 2017 b). It is possible to eliminate *S. agalactiae* infections in farms, and it has been accomplished with mastitis control efforts. It does however remain an isolated problem in farms without an appropriate eradication plan (Watts, 1988).

Streptococcus dysgalactiae is less contagious, but is more prone to generate acute CM. Altogether, it can give rise to both clinical, subclinical, acute and chronic mastitis (SVA, 2017 c). It is also a cow-bound bacteria, but not strictly defined to the udder (Sandholm *et al.*, 1995). In a study by Calvinho (1998), *S. dysgalactiae* is classified as an environmental bacterium, while Lundberg *et al.* (2016) reports that it can use either route of transmission. It is a part of the pathogenesis of "summer mastitis", and therefore also suspected to be spread through flies (SVA, 2017 c).

Corynebacterium spp., with *C. bovis* being the most common subtype, is another infectious agent, regularly found on the udder. It is however not assumed to cause acute clinical mastitis, but rather cause an infection with less profound features. Post-milking dip is supposedly a preventive measure (Lindhagen and Waller, 2006). In Sweden it is rarely classified as a mastitis pathogen but a part of the normal flora (Persson *et al*., 2011), but in studies in Finland (Pitkälä *et al*., 2004) and Switzerland (Busato *et al*., 2000) it has been found as one of the main causative agents, especially concerning SCM.

Environmental pathogens

Environmental pathogens are more commonly associated with inadequate pre-milking routines and environmental factors such as bedding and housing. Common environmental bacteria are *Streptococcus uberis* and coliforms (Radostits *et al*., 2006). Mastitis caused by environmental bacteria are generally prevented through good housing environment, clean cows and a good immunologic status (SVA 2017, d, e).

The nature of *Streptococcus uberis* have been debated. A study by Zadoks et al. (2003) concludes it most likely spreads with environmental transmission arguing that it appears in heifers and dry cows, which equals no cow-to-cow contact. Also, different strains of the bacteria can be found within one herd – which should be compared to cow-to-cow transmission where the same strain spreads throughout the herd. Some molecular patterns of the bacterial structure are however indicative of infectious features. Lundberg *et al.* (2016) reports, in agreement with other studies, that *S. uberis* mainly causes SCM.

Escherichia coli (*E. coli*) is found in faeces and therefore an unescapable environmental pathogen. It is known for its fast onset with acute clinical mastitis and sometimes endotoxemia, however, development into sub-clinical or chronic infections do occur (SVA, 2017 d). *Klebsiella* spp. is another environmental gram-negative bacteria, generally associated with severe clinical mastitis. It originates from dirt, and is generally associated with saw dust. Treatment of *Klebsiella* spp. is often difficult, and the mortality rate is high (Sandholm *et al.*, 1995).

Coagulase negative staphylococci (CNS) is a collective name for a group of Staphylococci with variating virulence and means of transmission (SVA, 2017 f). It generally leads to SCM or mild CM (Taponen *et al*., 2006). The awareness of this bacteria has risen in recent years, since studies have reported an increasing prevalence all over the world (Thorberg *et al*., 2009). Due to its mild appearance the infection can persist for a long period of time, leading to increased SCC and a drop in milk quality and milk yield, and hence leading to great economic impact (Sandholm *et al.*, 1995).

Impact of mastitis

Mastitis in the world

As previously stated, mastitis is a global disease. As a first step in preventing mastitis, prevalence of disease and causative agents needs to be evaluated for every area, to be able to assess what control measurement plan is appropriate to apply (Karimaribu, cited in FAO, 2014).

Multiple studies have been executed all over the world. In Costa Rica one study found a prevalence of mastitis of 24.2% on cow-level (De Graaf and Dwinger, 1996). In Sub-Saharan Africa multiple studies have been executed; results from two Ethiopian farms shows a prevalence of 80% respectively 30% (Workineh *et al*., 2002), and in Tanzania a study was made on both smallholder and large farms, showing overall SCM prevalence on 64%, and overall CM prevalence of 11% (Shem *et al*., 2001). In both African studies, *Staphylococcus* spp. (especially *S. aureus*) was the most frequently isolated bacteria, followed by *Streptococcus* spp. This is in alignment with the results from other African studies summarized in the report by FAO (2014).

Also studies from Asia shows the same patterns of contagious agents, with staphylococci most commonly being reported, followed by streptococci. Traditionally, *S. aureus* is the most prevalent bacteria, with *S. agalactiae* also being a common pathogen, however, in later years a declining one. There are differences in exact distribution and prevalence between studies, farms and countries – all summarised in the review by Sharma *et al.* (2012). The review also describes CNS as an emerging group of pathogens, as it in recent years have been recorded to be the most prevalent mastitic aetiology in multiple countries, especially regarding SCM. Similar trends are described by Pyörälä and Taponen (2009).

Economical aspects

Mastitis is repeatedly reported to be the most costly disease in the dairy sector all over the world (Halasa *et al*., 2007; Radostits *et al*., 2006; Viguier *et al*., 2009). The extent of the economic impact is hard to calculate, since losses are made in several steps of the milk production. In the review articles by Halasa et al. (2007) and Viguier et al. (2009), the following aspects are discussed:

- Mastitis leads to expenditures on veterinary care, diagnostics and drugs
- It leads to increased labour, or redistributes resources that could have been used elsewhere
- Mastitic milk may be discarded both due to quality and antibiotic residues, which will be a loss of income due to less milk for sale.
- Mastitis has repeatedly been reported to cause a decrease in milk yield. Even after the infection has been treated, lowered yield can remain for weeks. Some studies claim that the milk production never fully returns (Halasa *et al*., 2007).
- More than that, the elevated SCC might never return to initial levels - and since milk quality payments are based on SCC-levels in some countries, a loss of income might be a direct consequence
- It is also a “loss” of resources in the production of milk (eg feed and water for the cow).
- A cow with mastitis is a risk factor for transmission of bacteria to other animals, and may, consequently, generate other cases of mastitis or diseases of other sorts

- If mastitis will lead to culling of the cow – to minimize the spread or due to the severity of the disease – there is a cost of culling and replacing the cow. However, preventing the risk of spreading disease must also be taken into consideration. The actual cost for culling is therefore difficult to estimate.

The economical loss due to mastitis will differ between countries, regions and farms, but the principles behind the economic losses are undeniably the same (Huijps *et al*., 2008). The many aspects of economic loss make the assessment of magnitude intricate. A Dutch study showed that the majority of farmers (72%) underestimated the economic impact of mastitis. Particularly difficult was the evaluation of production losses due to a high SCC – which, as previously mentioned, is an indicator of SCM (Huijps *et al*., 2008).

Effect on milk quality and potential human health risks

The presence of both CM and SCM also have an impact on the compositional standards of the milk (Ogola *et al*., 2007). High levels of SCC have been reported to lead to a decreased shelf life. It also unfavourably alters the organoleptic features of the milk, making it more rancid and bitter. The negative effects are caused by an increase in enzymatic activity, in combination with a decreased synthetization of milk components (proteins, fats and lactose). Subsequently, an alteration of milk composition is present (Barbano *et al*., 2006). This also have a significant, negative effect on properties in yoghurt fermentation and cheese production (Le Maréchal *et al*., 2011).

Dependant on the causative agent infecting the udder, it might also become a direct threat to humans if it is a zoonotic pathogen. Pasteurization is an important part of prevention of this transmission route of disease, however, toxins produced by mastitic bacteria are unaffected by heat treatment and may still be a health hazard (Le Maréchal *et al*., 2011). Proper management of milk after treatment is also needed to reduce the risk of re-contamination of the milk (Muehlhoff *et al*., 2013). Pasteurization and cooling chains are not always available worldwide, and in Tajikistan a lack of knowledge and money makes people both sell, buy and drink unpasteurized milk (Sattorov, 2016; Lindahl *et al*., 2015).

Animal welfare

Not only decreased production numbers and compromised economy are consequences of mastitis. It is a painful disease, and pain should always be considered an animal welfare problem (Hogeveen *et al*., 2011, a). Cows with induced mastitis have been witnessed to experience discomfort, especially during the pre-clinical and acute phase of the disease, thus pain relief medication should always be considered to cows with mastitis (de Boyer des Roches *et al*., 2017). Animal welfare is perceived differently depending on country and culture (Magnusson, 2016), but in high-income countries it is a matter of increasing importance to costumers to keep ethical standards on an acceptable level (Bradley, 2002). A Swedish research programme finished in 2012 discusses that a decreased reproduction rate is an indication of impaired animal welfare, and that one of the key factors compromising the reproduction rate is a decreased milk yield and elevated SCC (Gustafsson, 2012).

MATERIAL AND METHODS

Study area

The selected area for the study was the urban and peri-urban area of Dushanbe, including a radius of up to 40 km from the city centre (see fig 1). In this area five districts were visited: Sharinav, Hisor, Varzob, Rudaki and Vahdat. Both enterprise and dekhan farms (from now on collectively termed large farms) and small household farms (SHH farms) were subjects of examination. The large farms kept cows for commercial use, unlike SHH farms where cows were kept for family use.

In each district, the aim was to visit two larger farms, blindly chosen from a list provided by the state veterinary service. If the number of milking cows in one large farm was <20, all cows were included in the study. The number of cows sampled on remaining large farms were restricted by time. No more than 40 cows were examined in a single farm due to work load and timescale.

Also, three jamoats[2] in every district were randomly chosen, wherein each three SHH farms were visited. All cows in each SHH farm were examined, regardless of the number.

All field work was executed in collaboration with the local veterinary services.

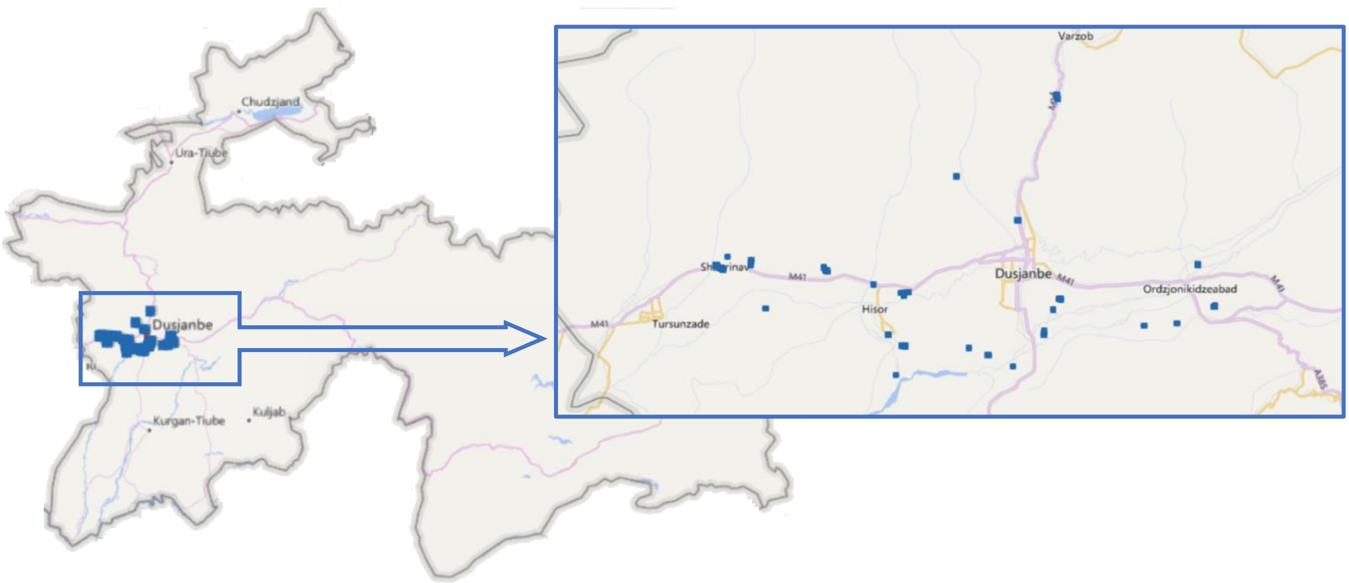

Fig 1. To the left a map of Tajikistan, and to the right a zoom of the study area. The blue dots are the coordinates of sampled farms. Both maps are created in Microsoft Excel by author.

Study group

To get a representative picture of the occurrence of mastitis in the study area, 384 cows were to be examined. This was assuming the prevalence of mastitis was 50%, and using a confidence level of 0.95 and Z of 1.96. It was based on a dairy cow population of 45 000 individuals, data received from a study in the same area performed in 2011 (Lindahl *et al*., 2014). The 384 cows examined were to be evenly distributed between the districts, 76/district.

[2] The Tajik equivalence of a commune

Examination of cow and milk

If the cow population in one farm exceeded 20 cows, examined individuals were randomly selected. Cows were excluded from the study if entering dry period, or if currently showing symptoms of other diseases than mastitis, alternatively under treatment for such diseases. During each examination, the udder was thoroughly palpated before milking to detect possible signs of inflammation of the udder. Next, two ml of milk were collected from each quarter into individual sample cups on a CMT paddle. To each cup, equal amounts of CMT fluid was added, and with a circular movement, the CMT solution and the milk was mixed. The result was evaluated after 10-20 seconds, and graded on a scale of 1-5, where 1 was normal viscosity and 5 was greatly increased viscosity of the milk. If graded 3 or higher, an aseptic milk sample was collected after careful disinfection of the teat, sampled with the teat held horizontally (according to recommendations by Sandholm et al. (1995)). Due to restricted numbers of cultivation media, only one quarter was sampled/cow – generally the quarter that scored highest on the CMT. Body temperature was also measured, and specific questions were asked about cows with a CMT-score of ≥3 (see questions 20-24 in appendix 1).

Cows that scored a CMT ≥3 on one or more quarters and also had deviant milk (e.g. discoloured milk or milk containing clots), deviant udder parenchyma (warm, firm) and/or an elevated body temperature (>39,2^{o} C) were classified as cases of CM. If scored a CMT ≥3, with no changes in neither milk, nor udder or body temperature, they were classified as cases of SCM. The term "mastitis" incorporates both CM and SCM, unless more specifically defined.

Cultivation of and interpretation of bacteria

The milk samples were immediately refrigerated after collection, and kept so until cultivated within 24 h on **sel**ective **ma**stitis medium (SELMA® manufactured by SVA, Swedish National Veterinary Institute) in order to make an elementary identification of pathogenic agents. All three fields of the SELMA plate were used; bovine blood agar with aesculine, mannitol salt agar and McConkey agar. Single-use plastic loops containing 10 µl were used to apply the milk on the agar to guarantee the same amount of milk was cultivated each time.

The SELMA plates were incubated at 37^{o} C ± 1^{o} C and bacterial growth was evaluated after 24 and 48 h. The bacterial examination was based on pattern of growth and evaluation of macro morphology of the colony forming units (CFU), including haemolysis. If the CFU appearance deviated from known macro-morphology or growth patterns, potassium hydroxide and hydrogen peroxide were used to distinguish gram negative from gram positive bacteria, respectively differentiating staphylococci from streptococci. Such bacterial colonies were also gram stained and micromorphology was evaluated under a microscope.

The bacterial growth was classified as following: to be considered as positive bacterial growth, 3 or more CFUs needed to be present after incubation. In a positive growth where one type of CFU was found, the bacterial finding was noted. In the event of a growth with two different types of CFU, both bacterial species were noted, as long as both species presented with ≥3 CFUs of each type. However, in the event of three or more types of CFU – irrespective of number of CFUs of each bacterial specie – the growth was described as ”mixed growth”.

Notably, in clinical practise, if bacteria in pure culture is found in a milk sample with elevated CMT, the bacterial species is regarded as causative (and the cow treated accordingly). However, despite this empirical clinical evidence, in the current study we refrain to use the wording “causative” as we didn’t perform challenge studies of the found bacteria.

Questionnaire

Specific questions were asked concerning cows with a CMT scored ≥3. In addition, comprehensive questions were queried in every farm, regarding e.g. cow management, milking routines and milk production - this to get overall information about the farm. With help of an interpreter (the same on all farms), the questions were translated into Tajik, and asked to the owner or main caretaker of the animals.

Since this study was executed simultaneously with one evaluating milking routines, some information acquired from the questionnaire is not presented in this report. The full questionnaire is found in appendix 1.

Statistical analysis

Regarding the analysis of prevalence of mastitis, the CMT-positive cows (CMT ≤3) were evaluated in relation to exposure of environmental factors. In this study, those environmental factors were: type of milking practice and size of farm. Calculations were made in a single-variable χ^2-test to find whether or not exposure to such factors were a significant risk of mastitis.

Ethical statement

The study protocol included non-invasive collection of milk samples, done by traditional hand-stripping milking technique. The sampling was approved by Chief Veterinary Officer at the State Veterinary Inspection in Tajikistan, as well as by the Council for Animals in Research and Education at SLU, Uppsala; registration number SLU.ua.2017.1.1.1-2557.

RESULTS

Description of farms

During September and October in 2017, 11 large and 45 SHH farms were visited and sampled. The aim was to visit two to three large farms in each district, however, due to disease outbreaks and long distances, no farms were visited in Varzob. Instead four farms were visited in Hisor to reach a sufficient number of sampled cows. The number of cattle on the large farms ranged between 9 and 500, with six farms having <100 milking cows, of which two had <20 cows. The number of cattle in SHH farms varied between 1 and 8, where 1-2 cows/farm being the most common setup. Due to the reasons mentioned above, 351 cows were sampled and not the 384 that was aimed for.

Three of the large farms operated with machine milking in milking parlours, in contrast to hand milking which was the other method used in both large and small farms. Only one SHH farmer (6 cows) had adapted to machine milking. The milk yield/cow/day were generally higher in the large farms (table 1).

Table 1. A presentation of the results from the answers on question 10 in the questionnaire (see appendix 1), "How much do your cows milk/day, on average?"

Milk yield (L/cow/day)	**0-5 litres (%)**	**5-10 litres (%)**	**10< litres (%)**
Large farms	0/11 (0)	2/11 (18)	9/11 (82)
SHH farms	18/45 (40)	22/45 (49)	5/45 (11)

Overall prevalence of mastitis

The number of animals examined was 351, whereof 281 were kept in large farms and 70 in SHH farms. A number of 209 out of 351 cows (59.5%) presented with milk interpreted as CMT 3 or more on at least one quarter – these were classified as cows with mastitis. Described on quarter level, 477 out of 1378 quarters (34.6%) were affected. In 26 out of 209 cases (12.4%) changes in milk, udder or body temperature were detected and therefore classified as cases of CM. Remaining 183 cases were classified as SCM. All collected information from the sampling is summarised in table 2 and 3.

Table 2. A summary of the findings from the large commercial (dekhan or enterprise) farms in the urban and peri-urban area of Dushanbe. It presents what milking practice was used on each farm, the total number of cows on each farm, the number of sampled cows on each farm and the number of cows with a CMT score ≥3 (presented as "number of cows with mastitis") on each farm. The table also contains information about the prevalence of mastitis on quarter level, number of blind quarters and cases of clinical mastitis (CM) on every farm

District	Type of farm	Milking practice	Number of milking cows	Number of examined cows	Number of cows with mastitis	Number of Quarters	Blind quarters	Cases of CM
Sharinav	Dekhan	By hand	53	16	9 (56.3 %)	25/62 (40.3 %)	2	4
Sharinav	Dekhan	By hand	25	16	6 (37.5 %)	15/63 (23.8 %)	1	0
Hisor	Enterprise	By hand	400	40	37 (92.5 %)	93/158 (58.8 %)	2	4
Hisor	Dekhan	By hand	120	24	9 (37.5 %)	15/95 (15.8 %)	1	1
Rudaki	Enterprise	By hand	9	9	5 (55.6 %)	7/36 (19.4 %)	0	0
Rudaki	Enterprise	By hand	23	18	12 (66.7 %)	31/70 (44.3 %)	2	0
Rudaki	Dekhan	By hand	13	13	11 (84.6 %)	24/50 (48.0 %)	2	0
Vahdat	Dekhan	By hand	200	30	16 (53.3 %)	36/119 (30.3 %)	1	4
Vahdat	Dekhan	Machine	60	35	17 (48.5 %)	32/139 (23.0 %)	1	7
Hisor	Enterprise	Machine	400	40	21 (52.5 %)	58/154 (37.7 %)	6	3
Hisor	Enterprise	Machine	500	40	22 (55.0 %)	51/157 (32.5 %)	3	0
		Total:	**1803**	**281**	**165 (58.7 %)**	**387/1103 (35.1 %)**	**21**	**23**

Table 3. A summary of the study results from the small household farms (SHH farms) in the urban and peri-urban area of Dushanbe. The table shows the number of SHH farms visited in each district and the amount of cows sampled in each district (all cows on every visited farm were sampled). It describes the number of cows with a CMT score ≥3 (presented as "number of cows with mastitis") in each district. The table also contains information about the prevalence of mastitis on quarter level, the number of blind quarters, cases of clinical mastitis (CM) in and what milking practice was used in each district

District	Number of SHH visited	Milking practice	Number of cows examined	Number of cows with mastitis	Number of mastitic quarters	Number of blind quarters	Cases of CM
Sharinav	8	All by hand	12	9 (75.0 %)	17/48 (35.4 %)	0	0
Hisor	9	All by hand	19	9 (47.4 %)	25/75 (33.3 %)	1	2
Varzob	9	All by hand	14	10 (71.4 %)	17/55 (30.9 %)	1	1
Rudaki	10	9/10 by hand 1/10 machine	15	9 (60.0 %)	16/58 (27.6 %)	2	0
Vahdat	9	All by hand	10	7 (70.0 %)	15/39 (38.5 %)	1	0
Total:	**45**		**70**	**44 (62.9 %)**	**90/275 (32.7 %)**	**5**	**3**

Prevalence of mastitis in relation to environmental parameters

Differences in prevalence in relation to farm type

There were 281 cows sampled in the 11 large farms, of which 165 showed signs of mastitis (58.7%). The incidence of mastitis on quarter level on these farms was 387 of 1103 quarters (35.1%). In the SHH farms, 70 cows were examined of which 44 cases were classified as mastitis (62.9%). On quarter level on these farms, the incidence of mastitis was 90 out of 275 sampled quarters (32.7%). χ^2-test showed no correlation (*p=0.621*) between type of farm and the prevalence of mastitis (calculated on cow level).

Differences in prevalence in relation to manner of milking

The total number of cows milked with machines was 121 out of 351, of which 62 cases (51.2%) were classified as mastitis. On quarter level the results showed 144 out of 473 affected quarters (30.4%). This is to be compared to milking by hand, where 147 out of 230 hand milked cows (63.9%) showed signs of mastitis, and 333 of 905 quarters (36.8%) where affected. An evaluation with a single-variable χ^2-test on the cow numbers showed a significant difference between machine milked and hand milked animals (*p=0.029*), where cows milked by hand were more likely to suffer from mastitis, odds ratio 1.69.

Prevalence of mastitis in relation to cow parameters

Information was collected via the questionnaire on every animal with a positive CMT (see questions 20-24 in appendix 1). Parity (which stands in linear relation to age of the cow) and time passed since calving is presented in table 4, and neither were distinctly associated with prevalence of mastitis.

Table 4. Distribution of mastitis, described in relation to parity and time passed since the cow had her previous calf. Information of calving and interval since calving is collected from questions 20 and 21 in appendix 1. Cows were sampled in the urban and peri-urban area of Dushanbe, Tajikistan

Question 20: Time since calving?		
	Cow level (number of cows)	Quarter level (number of quarters)
1-3 months	70	151
4-6 months	63	139
6-12 months	63	149
12< months	12	32
Unknown	1	2
Question 21: How many calves has she had?		
	Cow level (number of cows)	Quarter level (number of quarters)
1	47	110
2	39	83
3	46	99
4	37	88
5 ≤	38	92
Unknown	2	2

Type of bacterial infection and risk factors for different pathogens

All bacteriological results are presented on cow level, since sampling for bacterial cultivation on quarter level was not performed.

One species of bacteria was frequently isolated from the milk samples. After consultation with a veterinary at the National Veterinary Institute of Sweden it was determined to be a subspecies of *Corynebacterium* (Fasth, 2017). Overall results showed that *Corynebacterium* spp. was the most prevalent bacteria (18%), followed by mixed growth (14%) and CNS (13%), see figure 2. After cultivation, 24% (n = 49) of the SELMA plates presented with negative bacterial growth.

The group of "undefined streptococci" includes streptococci that could not be precisely classified into species due to divergence from normal CFU appearance or haemolysis. In the category "undefined pathogen", four out of eight cases were suspected *Enterococcus* spp.

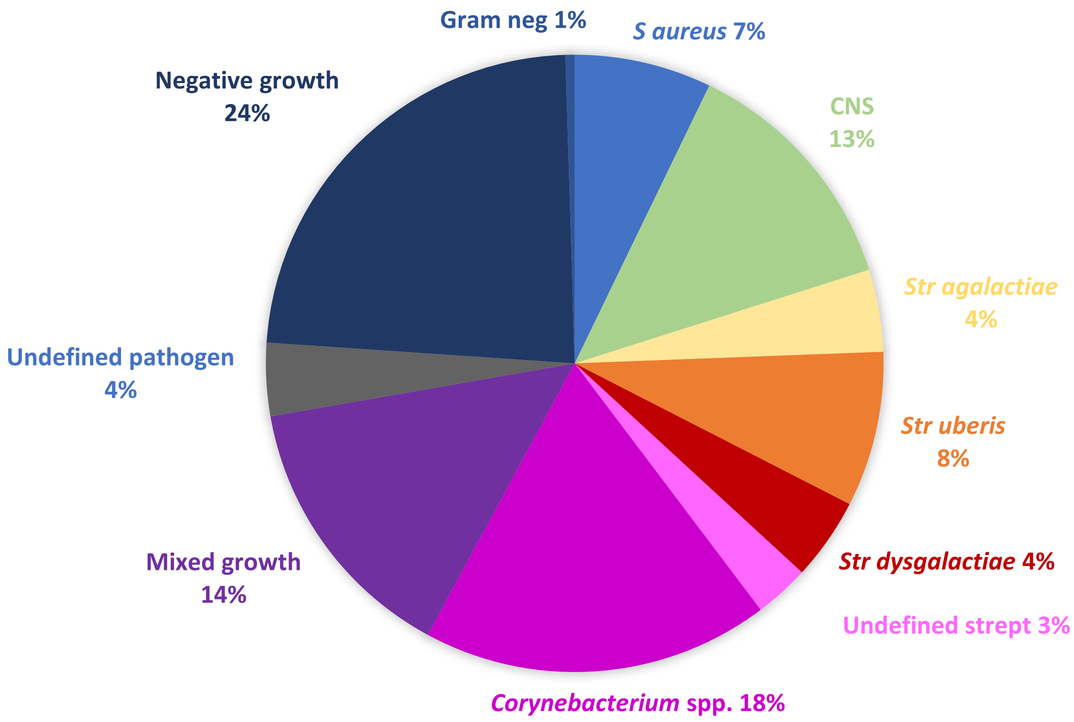

Fig 2. Overall distribution of mastitis pathogens in the urban and peri-urban area of Dushanbe. The results are acquired from bacterial cultivation of milk sampled from cows (n=209) with mastitis, as determined by CMT. The milk was sampled in both small household, dekhan and enterprise farms.

Of the 26 CM cases, a negative growth was found in 8 of 26 cases, followed by mixed growth (5/26 cases) and *Corynebacterium* spp. (4/26 cases). Bacteriological findings in the CM and SCM cases, respectively, are summarised in table 5.

Table 5. A description of which bacterial species were found in clinical mastitis (CM) versus subclinical mastitis (SCM). The results were obtained after bacterial cultivation of milk sampled from cows (n=209) with mastitis as determined by CMT in both small household, dekhan and enterprise farms

Bacterial species	**Cases of CM**	**Cases of SCM**	**Total**
S. aureus	2	13	15
CNS	2	25	27
Str agalactiae	1	8	9
Str uberis	2	15	17
Str dysgalactiae	0	9	9
Undefined str	1	5	6
Corynebacterium spp.	4	34	38
Mixed growth	5	25	30
Undefined pathogen	1	7	8
Negative growth	8	41	49
Gram neg	0	1	1
Total	**26**	**183**	**209**

Distribution of bacteria in relation to type of farm

Depending on type of farm, different distribution of pathogens was found. In larger farms, *Corynebacterium* spp. (20%) was the dominating bacteria (fig 3). Mixed growth (17%) and *S. uberis* (10%) followed in subsequent order, and thereafter a relatively even distribution between *Streptococcus* spp. and *Staphylococcus* spp. was found.

In SHH farms (fig 4), the negative bacterial growth was almost doubled the amount in large farms. The most prevalent pathogen was CNS (37%), which dominated significantly, followed by *S. aureus* (11%) and *Corynebacterium* spp. (9%).

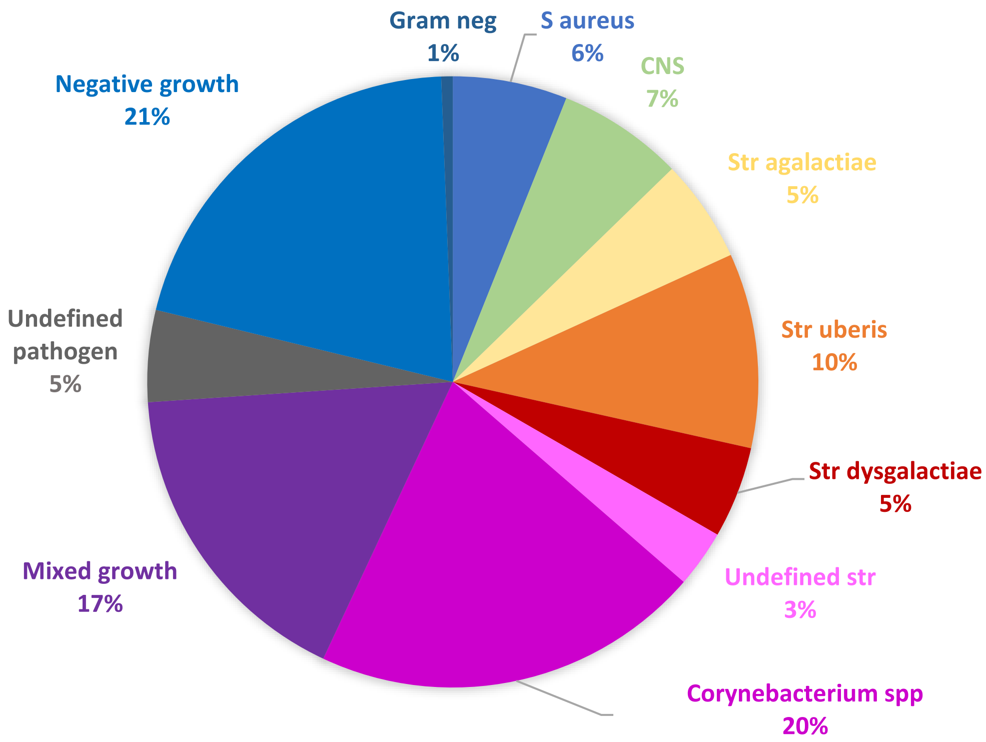

Fig 3. Distribution of pathogens in mastitis cases in the commercial large (dekhan/enterprise) farms in the urban and peri-urban area of Dushanbe. The results are acquired from bacterial cultivation of milk sampled from cows (n=165) with mastitis as determined by CMT.

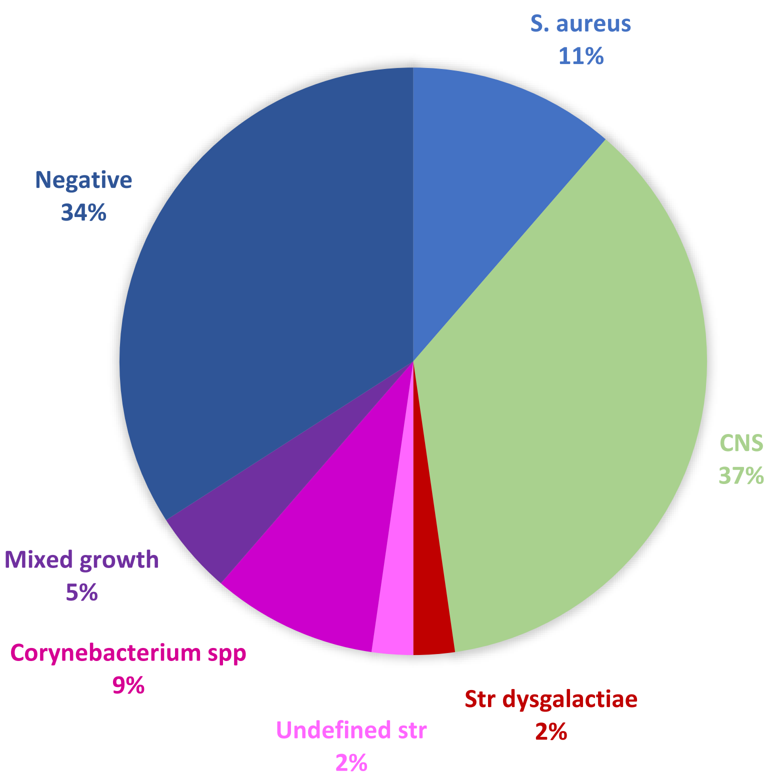

Fig 4. Distribution of pathogens in mastitis cases in the small household farms in the urban and peri-urban area of Dushanbe. The results are acquired from bacterial cultivation of milk sampled from cows (n=44) with mastitis as determined by CMT.

Distribution of bacteria in relation to method of milking

In machine milking regime, the prevalence of *Corynebacterium* spp. was almost doubled, and mixed growth was doubled, compared with the results from farms with hand milking regime (fig 5). In reverse, with hand milking there was a more than doubled percentage of CNS (fig 6).

Notably, the negative growth in machine milking was 3%, which is to be compared with hand-milking, where 28% of the SELMA plates showed negative growth.

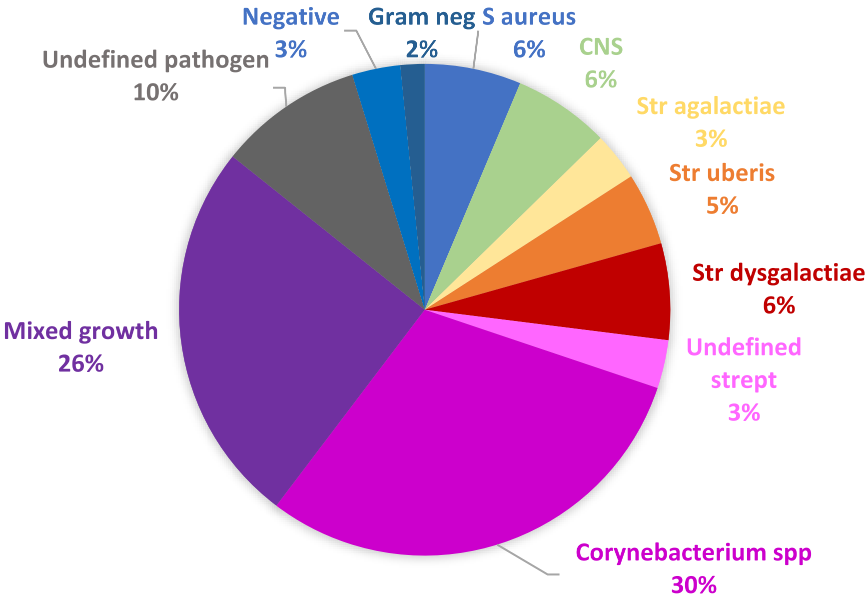

Fig 5. Distribution of mastitic pathogens in the visited farms in the urban and peri-urban area of Dushanbe that used machine milking systems. The results are acquired from bacterial cultivation of milk sampled from cows (n = 62) with mastitis as determined by CMT.

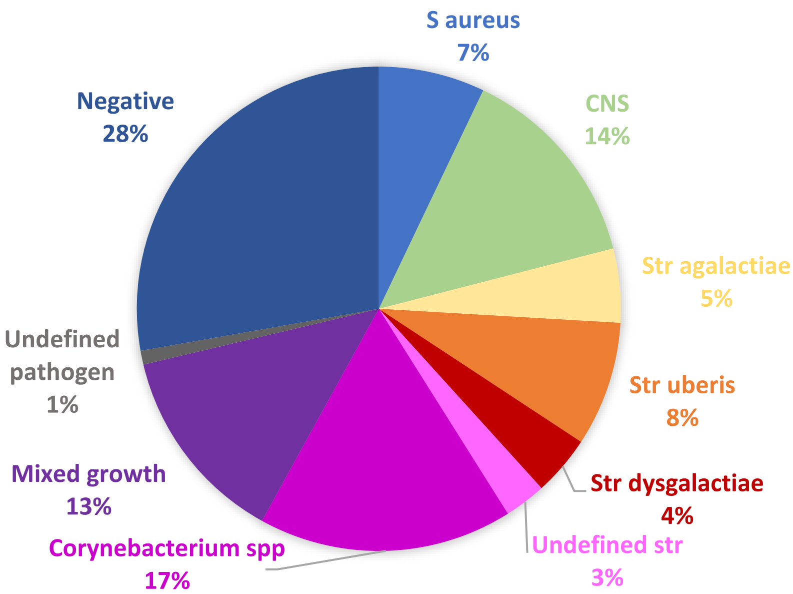

Fig 6. Distribution of mastitic pathogens in the farms in the urban and peri-urban area of Dushanbe that used hand-milking regime. The results are acquired from bacterial cultivation of milk sampled from cows (n=147) with mastitis as determined by CMT.

DISCUSSION

Prevalence of mastitis

The overall prevalence of mastitis (defined as a CMT score of ≥3) in the urban and peri-urban area of Dushanbe, Tajikistan, was 59.5%, which included both clinical and subclinical mastitis. In other studies, a varying incidence of mastitis between 24 and 80% have been found (De Graaf and Dwinger, 1996; Workineh *et al*., 2002), hence results from current study are deemed to be reasonable. Of the 351 animals sampled, 26 cows (7.4%) suffered from CM, and correspondingly, 52.1% suffered from SCM. Both results are similar to previous study results worldwide (FAO, 2014; Shem *et al*., 2001)

When assessing occurrence of mastitis in the study area, it was evaluated in relation to different factors. No significant difference between the occurrence of mastitis dependent on farm size could be detected, however, a feature that significantly ($p=0.029$) seems to increase the prevalence of mastitis is manner of milking. Hand milked cows were in this study more likely to suffer from mastitis, with an odds ratio of 1.69. Reyes *et al.* (2017) described that bulk tank milk in hand milked herds had a significantly increased SCC – and since this study is basing the diagnosis of mastitis on indications of an elevated SCC, the results are in line with those from Reyes et al.

In current results, a few things need to be considered during interpretation. From certain farms (presented in table 2) the prevalence of mastitis was markedly higher, some with a prevalence of mastitis of more than 80%. Both those farms used hand milking and both were large farms, hence the results describing possible risk factors might have been influenced. Similarly, the overall results might have been altered by farm-specific results. Further studies need to be executed for a more in-depth evaluation of risk factors.

Another part of this study was composed of questions asked concerning cows with a positive CMT (score ≥ 3). Parity and time since calving have in previous studies (Pyörälä, 2008; Taponen *et al*., 2017) been shown to be parameters that could correlate to mastitis. Since only animals with suspected illness were questioned (due to time restrictions), no statistical analysis can be made due to the absence of a healthy control group. A subjective evaluation of table 4 shows that no distinct risk group can be detected. Indeed, only twelve cows had a calf more than 12 months ago, which is a markedly lower number comparatively - however, one must consider that this group might be very small since most cows enter a new lactation period within those 12 months. A study with a different design would be needed to obtain proper statistical results and possibly detect an association between mastitis and parity and/or time since calving.

Distribution of bacteria

The most common result from the growth on the selective media was negative growth (24%), followed by *Corynebacterium* spp. (18%) and mixed growth (14%). When aligning bacteria based on property of transmission, the findings are that a majority are contagious bacteria: *Corynebacterium* spp, *S. aureus*, *S. agalactiae* and *S. dysgalactiae* make up a total amount of 37% of the cases of mastitis. This is to be compared with environmental bacteria (coliform bacteria and *S. uberis*) and CNS, which were isolated from 13% and 9% of the cases respectively. CNS is an opportunistic bacterial species, with different subspecies seemingly transmitting differently, and therefore not placed in either group.

The bacteria found in the CM cases were more or less following the overall results of distribution of pathogens. *Corynebacterium* spp. was continuously the most commonly found pathogen. However, only 26 out of 209 cases were classified as CM, and it is not possible to draw significant conclusions without a larger study group.

The findings indicate that the pathogens mainly are spread via the milking practice. Consequently, to eradicate such bacteria, implementations of new milking routines with focus on hygiene should be prioritised.

Distribution of bacteria based on farm size

When evaluating bacterial prevalence and comparing results based on farm size, there was a difference between large, commercial farms and SHH farms. The large farms followed the trend from the overall results with contagious bacteria being the chief category (36%), in comparison to environmental bacteria (11%) and CNS (7%). On the SHH farms the situation was different: 37% were CNS, and 22% contagious bacteria, with no findings of purely environmental bacteria.

A plausible reason for these different results is the differences in the manner of keeping the animals. If one cow develops mastitis in a large farm, it can transmit the disease via the milking practice to cows subsequent in milking order, spreading the disease efficiently if hygiene standards are inadequate. Once introduced, the pathogen might not easily get eradicated but will rather circulate within the herd.

In the SHH farms, generally housing only one or two cows, it is less likely that the animals will catch a contagious pathogen since there are fewer potential carriers of disease. Indeed, it is contradictive that all pathogens (except CNS) found on the SHH farms generally are classified as contagious. However, as discussed above, these bacteria have been recorded to have potential environmental characteristics or reservoirs (Calvinho *et al*., 1998; Capurro *et al*., 2010), and it is not unrealistic to assume that such reservoirs can be the underlying cause of disease in SHH farms. The prominence of CNS in the SHH farms may reasonably be explained by their opportunistic features. From normal flora, they infect during a time of a weakened immune system of the cow without any means of infection. Since they often create SCM or a very mild CM (Taponen *et al*., 2006), they proceed unnoticed.

Noted should be that only 70 out of 351 sampled cows were residing in SHH farms. The aggregated results from both type of farms might very well be affected by this.

Distribution of bacteria based on manner of milking

Evaluating the results on the basis of milking practice, the trend was similar to the overall prevalence – predominantly contagious bacteria – on both machine and hand milked farms. On machine milked farms *Corynebacterium* spp. had a prevalence of 30%, and together with other contagious bacteria they constituted 45% of the total bacterial prevalence in such farms. The environmental kind of bacteria formed 7% and CNS 6% of the total bacterial distribution. The results from the hand milking farms were of 28% contagious bacteria, 14% CNS and 6% environmental bacteria.

When interpreting these results, one should keep in mind that the absolute majority of animals on SHH farms (64/70 cows) were hand milked. Since the bacterial distribution on SHH farms were deviating from the overall bacterial distribution, it might interfere in evaluation of the relationship between milking manner and bacterial prevalence. The results in hand milked cows are visibly influenced by the results of bacterial prevalence in SHH farms.

The prevalence of **Corynebacterium** *spp.*

The overall high prevalence of *Corynebacterium* spp. was not expected since they, by Swedish standards, are not considered common mastitic pathogens (Fasth, 2017). Prevalence of *C. bovis* has however been reported in other countries, in quantities that could not be overlooked (Busato *et al*., 2000; Pitkälä *et al*., 2004). In present study, *Corynebacterium* spp. was found in all types of farms and in all conditions, associated mainly with SCM but also with four cases classified as CM. In the study by Taponen et al. (2017) it was found that *C. bovis* was associated with parlour milking, which is in alignment with the results from this study – the highest percentage of *Corynebacterium* spp. (30 %) was found in machine milked farms, of which 120/126 cases was executed in milking parlours.

The incidence of negative bacterial growth

As earlier mentioned, the most frequent outcome was negative findings on the growth media. The absence of growth can have multiple different causes. Bacteria can be excreted intermittently, i.e. during chronic mastitis, where encapsulated bacterial assemblies may occur within the udder, and bacteria are only excreted sporadically. Even if bacteria were present in the milk, not all can be cultivated during the set-ups of this study (eg anaerobic bacterium, bacteria who cannot grow on selected media), which also would lead to a false negative bacterial incidence.

Another reason for negative findings can be related to elevated SCC-levels due to non-infectious reasons, such as biological fluctuations during the lactation. Even though CMT has been recorded to be useful in these scenarios (Bhutto *et al*., 2012; Sargeant *et al*., 2001), it is not unreasonable to suspect some misinterpretations have occurred. The CMT is also recorded to have a relatively low specificity for infection, creating a fairly large number of false positive results. This too is reasonable to occur in this study. The study by Sargeant *et al.* (2001) showed a specificity of CMT stretching between 54.8% and 66.7% post-partum, and another one by Rasmussen *et al.* (2005) showed that the specificity of CMT stretched between 30-73%. Rasmussen found, however, a sensitivity of 93-99%, thus correctly identifying true positive results. In conclusion, a study with repeated samplings should be executed to minimise negative findings.

The negative results were more common in SHH compared to large farms (34% and 21% respectively). The dairy production in SHH farms is less profit-driven and in general have less yield/cow/day (see table 1), for reasons that could be dependent on both economy and lack of knowledge. The positive CMT findings were interpreted as an elevated SCC, but it might well be a misinterpretation of the normal baseline SCC – this would falsely appear as elevated due to a minimal milk volume, making the somatic cells less diluted. This is all in accordance to the reasoning by Sandholm *et al.* (1995).

CONCLUSION

In this study the overall prevalence of mastitis in the urban and peri-urban of Dushanbe, Tajikistan, was found to be 59.5%. Out of these, 12.4% (n=26) were clinical cases of mastitis and consequently, 88.5% (n = 185) were subclinical. The prevalence of mastitis was significantly higher (p=0,029) in cows milked by hand instead of by machine. The most common pathogen found was *Corynebacterium* spp. (18%), and an overall pathogenic distribution showed predominantly contagious bacteria (37%). To prevent mastitis caused by such, it is important to implement hygienic milking routines to avert bacterial transmission from cow-to-cow.

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Spoken Sources

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APPENDIX 1

General questions

Question	Response	Comment
1. Type of farm	1. Enterprises 2. Breeding 3. Dekhkan farm 4 Small householder	
2. Stable type (when/if not in pasture)	1. Tied-up 2. Loose-housing 3. Combination 4. In pasture	
3. When was the cow bought?	1. 0-3 months ago 2. 3-6 months ago 3. 6-12 months ago 4. More than a year ago	
4. Where was the cow bought?		
5. Who supported the purchase of the cow?		
6. The goal for the dairy herd size.	1. For subsistence 2. Expand the herd 3. Reduce the herd 4. As saving scheme 5. Trading commodity	
7. Breed	1. Local, mixed 2. Local, mixed improved 3. Pure 4. Both local breeds 5. Both pure and mixed local 6. Both pure and improved local 7. All three kinds	

8. Cow identification	1. No specific id/Signalement 2. Name 3. Tag/Id-number 4. Name and Id-number	
9. Age at first calf	1. < 25 months 2. 25-35 months (2-3 years) 3. >36 months (3 years or more) 4. Unknown	
10. Milk yield/day at the moment **Average/cow in L**	1. <5 2. 5,1-10 3. >10 4. Unknown	
11. Average weaning age of calf	1. 0-3 months 2. >3 months 3. Natural weaning 4. Unknown	
12. How long is the dry period before calving?	1. < 1 month 2. 1-2 months 3. > 2 months 4. No dry period	
13. System of registration/ journal applied	1. Yes 2. No	
14. If yes, specify which type of	1. Animal health 2. Reproduction 3. Production/Economy	
15. Milking equipment	1. By hand 2. Automatic 3. By hand and automatic	
16. How often are the cows milked (per day)?	1. Once 2. Twice 3. Three times	

17. Are the cows divided into specific groups during milking?	1. Yes 2. No	
18. If yes, which groups do you have?	1. Cows that recently had a calf 2. Cows with mastitis 3. Cows with a high milk yield 4. Cows in the end of lactation 5. Other, specify in comment	
19. Are the cows fed after milking?	1. Yes 2. No	

Questions related to individual cows with mastitis

20. Time after calving	1. 0-3 months 2. 3-6 months 3. 6-12 months	
21. How many calves has she had?	Write number of calves	
22. How much milk/day?	1. <5 2. 5-10 3. >10 4. Unknown	
23. Known illness? If yes, which one?	1. Yes 2. No	
24. Is she being treated with antibiotics? What	1. Yes 2. No	

kind? (Show the bottle or brand name)		

Observation form

25. Cow cleanliness	1 2 3 4	
26. Handwash before milking	Yes No	
27. Use of milking gloves	Yes No	
28. Washing of the udder	Yes No	
29. Wiping of the teats	1. With wet cloth 2. With dry cloth 3. No wiping 4. Same cloth to all/many cows 5. One cloth/cow 6. Teat dipping	
30. Wiping dry	Yes No	
31. Is the milk controlled before machine is put on?	Yes No	
32. Is the udder stimulated?	Yes No	
33. Time from first contact to machine is put on	1. 0-30 s 2. 30-60 s 3. > 60 s	
34. Teat dipping postmilking?	Yes No	