Brucella in Tajikistan - Zoonotic Risks of Urbanized Livestock in a Low-Income Country

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Abstract

One of the most powerful megatrends of our time is urbanization. Urban and peri-urban (UPU) farming is a common practice in many low-income countries because it increases the incomes of families that are often restrained by limited economic resources. However, there is a concern that the growing number of people and livestock living close together in UPU areas will increase the transmission of different zoonotic pathogens such as *Brucella*. Brucellosis is one of the most common and economically important zoonoses globally and Central Asia represents an area with high incidence among humans and livestock. This thesis aims to assess the occurrence of *Brucella* among livestock in an UPU area and to elucidate how farmers understand and respond to this zoonosis. The results of this thesis might contribute to raising awareness of how livestock in urban areas can constitute a public health risk if they are infected with *Brucella* or other zoonoses. The four studies included in this thesis were conducted among small-scale livestock farmers in the UPU region of Dushanbe, the capital of Tajikistan. Blood samples were collected from 904 dairy cows and 667 sheep and goats and analysed with enzyme-linked immunosorbent assay (ELISA). The *Brucella* seroprevalence was 4.1% among the dairy cows at the herd level and ranged between 1.0% and 15.6% among sheep and goats at the individual level in the four included districts. Furthermore, 564 cow milk samples were analysed for *Brucella* DNA by *IS711*-based real-time PCR and 13.7% were found to be positive. All seropositive cows were positive by PCR, but 11.8% of the seronegative cows were also positive by PCR. Further characterization of the *Brucella* DNA suggests that there is a reservoir of *B. abortus* in the cattle population and a spillover of *B. melitensis* from small ruminants to cattle. A knowledge, attitudes and practice study targeting 441 households revealed poor knowledge of brucellosis and several high-risk behaviours, such as consumption of unpasteurized dairy products and not wearing protective clothing when handling potentially infectious materials like aborted foetuses and discharges.

Brucella is widespread among the livestock in the UPU area of Dushanbe and this might constitute a serious risk to public health and cause significant economic losses. The discrepancy between serology and PCR results suggests that implementing complementary diagnostic strategies to detect false serological negative individuals might be warranted in *Brucella* control programmes. Poor knowledge, several high-risk behaviours and a willingness to learn more provide the rationale for developing campaigns to raise awareness of brucellosis and its associated risks among farmers.

Keywords: Brucella, ELISA, *IS711*, KAP, PCR, *rpoB*, Tajikistan, urban/peri-urban

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Dedication

To my family

“The disease rarely kills anybody, but it often makes a patient wish he were dead”

TIME magazine 1943 reporting on brucellosis

Contents

List of Publications

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

I Lindahl E, Sattorov N, Boqvist S, Sattori I, Magnusson U (2014). Seropositivity and risk factors for *Brucella* in dairy cows in urban and peri-urban small-scale farming in Tajikistan. *Tropical Animal Health and Production* 46(3), 563-569.

II Lindahl Rajala E, Grahn C, Ljung I, Sattorov N, Boqvist S, Magnusson U (2016). Prevalence and risk factors for *Brucella* seropositivity among sheep and goats in a peri-urban region of Tajikistan. *Tropical Animal Health and Production* 48(3), 553-558.

III Lindahl Rajala E, Hoffman T, Fretin D, Godfroid J, Sattorov N, Boqvist S, Lundkvist Å, Magnusson U. Detection and characterization of *Brucella* spp. in bovine milk in small-scale urban and peri-urban farming in Tajikistan. *Submitted manuscript*.

IV Lindahl E, Sattorov N, Boqvist S, Magnusson U (2015). A study of knowledge, attitudes and practices relating to brucellosis among small-scale dairy farmers in an urban and peri-urban area of Tajikistan. *PLoS One* 10(2), doi: 10.1371/journal.pone.0117318.

Abbreviations

C-ELISA	Competitive ELISA
Ct	Cycle threshold
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organization of the United Nations
I-ELISA	Indirect ELISA
ILRI	International Livestock Research Institute
KAP	Knowledge, Attitudes and Practices
OIE	World Organisation for Animal Health
OR	Odds ratio
PCR	Polymerase chain reaction
qPCR	quantitative or real-time PCR
SNP	Single nucleotide polymorphism
UPU	Urban/peri-urban
WHO	World Health Organization

1 Introduction

Brucellosis is one of the most common and economically important zoonoses globally (McDermott *et al.*, 2013). Central Asia and the Middle East are among the regions with the highest incidence of brucellosis in humans and livestock worldwide and the incidence is rising (Pappas, 2010; Seleem *et al.*, 2010; Pappas *et al.*, 2006). Humans can become chronically infected if not treated adequately and osteoarticular manifestation is a common complication (Dean *et al.*, 2012). Inadequate treatment might result in loss of work and income at the individual level (Seleem *et al.*, 2010). Furthermore, loss of human productivity and increased costs within the public health sector cause economic losses at the national level. Brucellosis in livestock mainly affects the reproductive organs and causes abortion, reduced fertility and a reduced milk production (Corbel, 2006). A decrease in productivity, loss of export and costs caused by movement restrictions and vaccinations of livestock also cause significant economic losses within the livestock sector (McDermott *et al.*, 2013). Brucellosis is a zoonosis, meaning that the disease is transmissible between animals and humans. The rapid urbanization currently prevailing in many countries is drastically changing how societies are organised and is laying the foundation for great economic advancements (United Nations, 2014; WHO, 2010). However, urbanization of humans also implies urbanization of their livestock. This development raises concerns that the growing number of people and livestock living close together in urban/peri-urban (UPU) areas will increase the transmission of different zoonotic pathogens such as *Brucella* (Steinfeld, 2004). Like in many other low-income countries, the people of Tajikistan are dependent on small-scale farming (Jackson *et al.*, 2007) and at the same time are quickly urbanizing (United Nations, 2014). *Brucella* is endemic among the livestock in the region, there is no control programme in place and resources are scarce. The need for dealing with zoonotic infections in

the face of accelerating urbanization and economic constraints is shared by many low-income countries.

1.1 *Brucella* – a neglected disease of the poor

The World Health Organization (WHO) has classified brucellosis as one of seven neglected zoonotic diseases (WHO, 2005). These diseases are rarely in the spotlight for research and mainly affect poor, marginalized people. Furthermore, the International Livestock Research Institute (ILRI) has listed brucellosis as one of the 13 most important zoonotic infections in terms of impact on human and livestock health, amenability to agricultural-based control and emergence or severity of disease in people (Grace *et al.*, 2012). According to the WHO, there are three underlying mechanisms for why the consequences of this group of neglected diseases are so serious, especially for poor people (WHO, 2005). First, poor people are more likely to acquire a zoonotic infection like brucellosis because they often live in close proximity with animals. Poor people are also more likely to consume low-quality food products from informal markets such as unpasteurized dairy products or meat from sick animals and thus are at greater risk of becoming infected. Second, poor people are less likely to get a proper diagnosis and to receive adequate treatment. For *Brucella*, this implies that they are more likely to become chronically infected and be at greater risk of permanent disability. Third, poor households have lower economic margins and often only keep a small number of livestock. Loss of income due to a family member´s inability to work, additional costs for medical treatment and production losses among the few livestock they own can be devastating for a poor household´s economy.

1.2 Brucellosis in Tajikistan

Tajikistan is located in Central Asia and borders Afghanistan to the south, China to the east, Kyrgyzstan to the north and Uzbekistan to the west and is populated by approximately 8 million people (CIA, 2016; Worldbank, 2016) (Figure 1). The pace of poverty reduction has been among the top 10% in the world over the past 15 years (Worldbank, 2016) and the poverty rate, measured as the percentage of the population that lives at or below USD 1.90 a day, has decreased from over 80% in 1999 to around 30% today. According to the 2015 Global Hunger Index report, 33% of the Tajik population suffer from malnutrition and Tajikistan is ranked as the country with the highest malnutrition rate among the former countries of the Soviet Union (von Grebmer *et al.*, 2015). Due to economic slowdown in Russia, tighter migration

restrictions in Russia resulting in a decline in remittances and weak global demand for its key export commodities, Tajikistan´s GDP growth has decreased from 6.7% in 2014 to 4.2% in 2015 – although this is still a considerable growth rate.

During the Soviet era, brucellosis among livestock was fairly well controlled in Tajikistan through a strategy of vaccination and test-and-slaughter (Ward *et al.*, 2012). After the collapse of the Soviet Union in 1991, the animal health sector was seriously affected and control for zoonotic infections like brucellosis became markedly impaired in the region. Over the last two decades, Tajikistan - as well as other Central Asian countries - have seen increased numbers of small farm units (Beauvais *et al.*, 2015; Ward *et al.*, 2012; Pappas, 2010), uncontrolled movement of livestock, poor infrastructure, deregulations of trade and decreased border controls (Ward *et al.*, 2012; Pappas, 2010). This could be one set of explanations for why Central Asia is currently a hotspot for *Brucella* infection among humans and livestock.

The Food and Agricultural Organization of the United Nations (FAO) started a *Brucella* control programme in 2004 in eight districts in Tajikistan with high *Brucella* burden in sheep and goats (Jackson *et al.*, 2007). That programme did not include the region around Dushanbe, which is the focus of this thesis. The program comprised mass-vaccination of sheep and goats in the first two years followed by biannual vaccination of young animals and non-vaccinated adults. The vaccine used was *Brucella melitensis* Rev 1 applied as eye drops. After six years, the seroprevalence had dropped from 8.9% to 1.8% in the eight best-vaccinated districts, making it one of the most successful programmes in the region (FAO, 2014; Ward *et al.*, 2012) .

1.3 Urbanization and UPU livestock production in low-income countries

One of the most powerful megatrends of our time is urbanization (United Nations, 2014). Today, 54% of the world's human population lives in urban areas and by 2050 the number is anticipated to rise to 66%. An often forgotten consequence of human urbanization is the urbanization of their livestock. Today, Asia and Africa are among the most rural regions of the world, but also the regions with the highest urbanization rates. As the farmers of these highly populated regions move themselves and their livestock closer to cities at an unprecedented scale, there are increased concerns for the spread of zoonotic diseases (Steinfeld, 2004). Due to population growth, increasing urbanization and higher disposable income, the demand for animal food products is expected to double by 2030 in low-income countries. This will likely result in

increasing large-scale livestock production close to urban centres. In Tajikistan, 27% of the population lives in urban areas (United Nations, 2014) and by 2050 this number is expected to rise to 41%. Living in a city often implies many advantages compared to living in rural areas, including better access to health services and higher levels of literacy and education. UPU livestock production offers an opportunity for people to improve their livelihood but it also poses a public health threat if the risk of zoonotic infections is not addressed wisely (Flynn, 1999).

Figure 1. World map and close up of Central Asia and Tajikistan (ArcGIS® software by Esri, www.esri.com).

1.4 *Brucella* spp.

Brucella is a genus of gram negative bacteria named after David Bruce (1855-1931) who was the first person to isolate *B. melitensis*. This strain was isolated from the spleen of a British soldier, suffering from a febrile illness called Malta fever in 1887 (Nicoletti, 2002).

1.4.1 *Brucella*: species and biovars

To date, twelve different *Brucella* species have been described (Scholz *et al.*, 2016; Whatmore *et al.*, 2014) (Figure 2). The six classical species are *B. melitensis* and *B. abortus* (Meyer & Shaw, 1920), *B. suis* (Huddleson, 1929), *B. ovis* (Buddle, 1956), *B. neotomae* (Stoenner & Lackman, 1957) and *B. canis* (Carmichael & Bruner, 1968). *Brucella melitensis*, *B. abortus* and *B. suis* are further classified into biovars. After *B. canis* was isolated in the late 1960s, no novel *Brucella* species were identified for many decades (Pappas, 2010). In the 1990s, two new *Brucella* species were found in marine mammals (Ewalt *et al.*, 1994; Ross *et al.*, 1994) and these were subsequently categorized as *B. ceti* and *B. pinnipedialis* (Foster *et al.*, 2007), both with zoonotic potential (Whatmore *et al.*, 2008). Another newly described species, *B. microti* was isolated from common voles and red foxes (Scholz *et al.*, 2008b). Two additional novel strains have recently been isolated from humans and the first one was isolated from an infected human breast implant (Scholz *et al.*, 2010). This strain was named *B. inopinata* and the second strain showed similarity to *B. inopinata* and was isolated from a patient with chronic lung disease (Tiller *et al.*, 2010). The two most recently described species are *B. papionis*, which was isolated from two baboons with retained placenta (Whatmore *et al.*, 2014) and *B. vulpis* which was isolated in Austria from the mandibular lymph nodes of two red foxes (Scholz *et al.*, 2016) (Figure 2).

Species	Biovars	Preferred natural host	Main geographical area	Pathogenicity for man
B. melitensis	1, 2, 3	Sheep, Goats Wild ungulates	Mediterranean countries Middle & Near East	High
B. abortus	1, 2, 3, 4, 5, 6, (7), 9	Bovines Wild ungulates	Europe, Americas, Africa, Asia	Moderate
B. suis	1	Suids	Americas, Asia, Oceania	High
	2	Suids, Hares	Central & Western Europe	Very low
	3	Suids	USA, China	High
	4	Reindeer	USA, Canada, Russia	Moderate
	5	Wild rodents	Russia	High
B. neotomae		Desert wood rat *Neotoma lepida*	USA	Unknown
B. ovis		Sheep (males)	Mediterranean countries	No
B. canis		Dogs	USA, South America Central/Eastern Europe	Low
B. ceti		Cetaceans	-	High / Unknown
B. pinnipedialis		Pinnipeds	-	High / Unknown
B. microti		Common vole	Central Europe	Unknown
B. inopinata		Unknown	USA / Oceania	Unknown
B. papionis		Unknown	Unknown	Unknown
B. vulpis		Unknown	Unknown	Unknown

*Figure 2. Brucella s*pecies and biovars, from B. Garin-Bastuji, 2014.

1.4.2 Diagnostic methods

If there is suspicion of *Brucella* infection among livestock, such as the occurrence of abortions or stillbirths during the last trimester of pregnancy, appropriate samples should be collected (OIE, 2016). Because *Brucella* is one of the most easily acquired laboratory infections, strict safety precautions must be implemented when handling infected materials or cultures.

The golden standard in *Brucella* diagnostics is to isolate, identify and molecularly characterize the bacteria from an infected host as described below. This is essential in order to find the infection source and to plan suitable control measures (Godfroid *et al.*, 2013).

Collection of samples

Depending on the symptoms of the animal, different clinical samples should be collected (OIE, 2016). These include aborted foetuses, foetal membranes, vaginal secretions (swab), milk, semen, and arthritis or hygroma fluids. The number of excreted *Brucella* bacteria can be low in milk and colostrum and to increase the probability of successfully isolating *Brucella* from milk, it is recommended to centrifuge the milk and use the cream and deposit for culture. From animal carcasses, samples from the head, lymph nodes (mammary and genital), spleen, uterus and udder can be collected.

Staining methods

Brucella bacteria can be demonstrated in smears of organs or biological fluids by different staining methods like Gram or Stamp staining (OIE, 2016; Godfroid *et al.*, 2010). *Brucella* are coccobacilli or short rods approximately 0.5-1.5 µm long and 0.5-0.7 µm wide. They are usually arranged singly or occasionally in pairs. *Brucella* are non-motile and do not form spores.

Culture

Due to the very low infectious dose, *Brucella* is an easily acquired laboratory infection and bio-safety level 3 laboratories are recommended for culturing of all zoonotic *Brucella* spp. that infect livestock (Schwarz *et al.*, 2015). Phenotypic analysis of *Brucella* includes growth on different media, colony morphology and organism morphology after different staining methods like Gram or Stamp staining (OIE, 2016; Godfroid *et al.*, 2010). Further identification of *Brucella* spp. includes the requirement of CO_2-enriched atmosphere for growth, metabolic profile (oxidase production and urease activity), growth in the presence of dyes, production of H_2S, lysis by *Brucella*-specific bacteriophage and agglutination with *Brucella* antiserum.

Culture of *Brucella* can be performed on both basal and selective media (OIE, 2016). Direct isolation is often performed on basal solid media whereas liquid media can be used for enrichment purpose.

Selective media can be prepared from basal media by adding antibiotics to suppress the growth of other microorganisms (OIE, 2016). One of the most commonly used selective media is Farrell´s medium, which contains six different antibiotics (OIE, 2016; Farrell, 1974).

The *Brucella* colonies can be visible on solid media after two to three days of incubation. After four days they can be seen as round colonies 1-2 mm in diameter with smooth margins, except for *B. ovis* and *B. canis*, which present with rough margins (OIE, 2016; Godfroid *et al.*, 2010).

Molecular DNA technology

Species within the genus *Brucella* show a high level of nucleotide similarity (> 90%) and most genetic differences between the species consist of single nucleotide polymorphisms (SNPs) (Foster *et al.*, 2009; Whatmore, 2009; Verger *et al.*, 1985). Culture of *Brucella* and further characterization into species and biovars is time consuming, requires experienced personnel and involves exposure to living *Brucella* organisms (Whatmore, 2009; Marianelli *et al.*, 2006). Therefore, methods of genetic characterization using molecular DNA technology have been developed. Today, there are several molecular methods described for the detection, identification and to some extent

differentiation of *Brucella* spp. and biovars. Specific sequences of *Brucella* spp. such as the 16S rRNA (Romero *et al.*, 1995; Herman & De Ridder, 1992), the *bcsp31* gene (Bricker, 2002; Baily *et al.*, 1992) and the *IS711* insertion sequence (Halling *et al.*, 1993) have been used for the detection of *Brucella* DNA with conventional polymerase chain reaction (PCR) assays. There are also real-time or quantitative PCR (qPCR) assays that have been developed for rapid and safe detection of *Brucella*, including assays targeting the *bcsp31* gene or the *IS711* insertion sequence (Bounaadja *et al.*, 2009). To enable epidemiological tracking, further typing is necessary. A number of different PCR-based assays have been used to find DNA markers to enable further molecular typing of *Brucella*, including assays based on the *rpoB* gene (Marianelli *et al.*, 2006). Furthermore, different multiplex PCR assays such as the Bruce ladder (García-Yoldi *et al.*, 2006) and the AMOS-PCR (Bricker & Halling, 1994), are widely used. Recently developed techniques, such as multiple variable number of tandem repeat (VNTR) loci and multi-locus VNTR analysis (MLVA), have made it possible to classify *Brucella* into biovars as well as to discriminate *Brucella* isolates within a given biovar (Allen *et al.*, 2015; Godfroid *et al.*, 2010; Le Flèche *et al.*, 2006).

Serology

Serological methods are valuable tools for screening purposes in *Brucella* surveillance, control and eradication programmes but all serological tests have limitations, especially when it comes to testing of individual animals. When performing serology, it is important to use standardized serological methods and reference sera according to the World Organisation for Animal Health (OIE) standards (OIE, 2016). There are different serological tests for detecting *Brucella*-specific antibodies and the OIE recommends that positive samples should be confirmed as positive with a suitable confirmation test. The buffered plate agglutination test (BPAT), the rose bengal test (RBT), the complement fixation test (CFT), the fluorescence polarisation assay (FPA) and the indirect enzyme-linked immunosorbent assay (I-ELISA) are suitable screening tests among cattle, buffaloes, sheep, goats and camelids. The competitive ELISA (C-ELISA) may be used in some situations but is considered costly (OIE, 2016).

An efficient way to screen a dairy herd for *Brucella* infection is to analyse bulk milk with I-ELISA (OIE, 2016). A shortcoming with bulk milk is that pregnant individuals in the last trimester are not producing milk and hence will not be discovered as seropositive if only analysing bulk milk. Therefore, the testing of these individuals should be repeated after parturition. Bulk milk sampling is more convenient than performing individual blood sampling and

also more cost effective. If a positive bulk milk result is obtained, it is recommended to investigate the herd further by collecting individual blood samples and analysing them with appropriate methods. Another test commonly used for analysing bulk milk is the milk ring test (OIE, 2016; Godfroid *et al.*, 2010). None of the serological tests have the ability to differentiate with 100% certainty antibodies induced by recent vaccination for brucellosis and antibodies induced by natural infection (OIE, 2016; Godfroid *et al.*, 2010). Therefore, it is important to have knowledge of the animals' vaccination status in order to draw the right conclusions from the serology results. Another shortcoming with serology is the difficulty in discriminating between serological reactions due to *Brucella* infection and those due to cross-reacting bacteria like *Yersinia enterocolitica* O:9 (OIE, 2016; Godfroid *et al.*, 2010). According to the OIE Terrestrial Manual, the C-ELISA can eliminate some but not all false positive reactions due to cross-reacting bacteria (OIE, 2016; Munoz *et al.*, 2005).

1.5 Control of brucellosis

In order to reduce the incidence of many zoonotic infections among humans, the pathogens must be controlled in the animal population (WHO, 2005). Unfortunately, few low-income countries have the capacity and resources necessary to monitor and control emerging zoonotic infections. The *Brucella* species mainly concerning livestock and their principal farm animal hosts are *B. abortus* (cattle), *B. melitensis* (sheep and goats) and *B. suis* (swine) and all have zoonotic potential (Godfroid *et al.*, 2011; Seleem *et al.*, 2010). The most common cause of human brucellosis worldwide is *B. melitensis* (Blasco & Molina-Flores, 2011).

The economic cost of bovine brucellosis can be high. For example in Argentina, with a prevalence around 5%, the cost has been estimated to be about USD 60 million per year or USD 1.20 per bovine (McDermott *et al.*, 2013). The corresponding figure in Nigeria, with a prevalence of 7% to 12%, has been estimated to be USD 3.16 annually per bovine. A study from India reports that the economic loss is USD 6.8 per bovine, USD 0.7 per sheep and USD 0.5 per goat and emphasize that the economic costs and social consequences of human infection are not included in these figures (Singh *et al.*, 2015). As long as sufficient funding is provided, the knowledge and tools on how to control and eventually eradicate *Brucella* and many other neglected diseases often already exist (WHO, 2005). *Brucella* in livestock has been eradicated from many high-income countries. This has been achieved by an initial compulsory whole-flock vaccination strategy until the prevalence drops to 1–2% followed by a test-and-

slaughter strategy. A prerequisite for success has been financial compensation to farmers for loss of livestock as well as financial incentives to farmers to gain and preserve the status of a *Brucella*-free herd (Godfroid *et al.*, 2013; WHO, 2005). Sweden was one of the first countries to eradicate the disease among livestock in the 1950s (SVA, 2015; Cerenius, 2010).

Countries suffering from high rates of *Brucella* infection among livestock are often poor countries with limited financial resources, hence endemic *Brucella* infection among livestock is often ignored (WHO, 2005). In these countries, test-and-slaughter programs are not feasible for many reasons, especially lack of funding and weak institutions (Blasco & Molina-Flores, 2011). In such cases, a mass vaccination program targeting livestock can be a way forward to reduce the incidence among humans and livestock (FAO, 2014; Smits, 2012).

1.5.1 Brucellosis vaccine

There are effective brucellosis vaccines both for cattle and for sheep and goats (OIE, 2016). Among sheep and goats, the best and most commonly used vaccine is the live *B. melitensis* strain Rev. 1 vaccine (OIE, 2016; Blasco & Molina-Flores, 2011; Blasco, 1997). Rev. 1 is often given to young animals (aged 3–6 months) as a single subcutaneous or conjunctival inoculation. However, subcutaneous vaccination can induce long-term persistence of vaccinal antibodies that interfere with serological tests for *Brucella* infection and the use of conjunctival vaccines has been reported to minimize this problem (OIE, 2016). Despite this, in an eradication programme where mass vaccination has been conducted, it is recommended to avoid serological testing within two years after vaccination to avoid culling of healthy but seropositive adult vaccinated animals (Blasco, 2010). Also, vaccinated animals kept in an infected environment can be exposed to *Brucella* field strains and produce antibodies that can be detected with serological tests, which makes the interpretation of the results difficult. Conjunctival vaccines are quick and easy to administer and these are the most preferred vaccines in control programmes (OIE, 2016). Reported side effects for Rev. 1 vaccine include induced abortions in pregnant animals. As Rev. 1 is a live vaccine, bacteria can be excreted in the milk of the vaccinated animal and might therefore constitute a public health risk. In control programmes, the recommendation is to use conjunctival vaccine only on non-pregnant sheep and goats or during the last month of pregnancy. Another reported side effect is accidental human infection when handling the vaccine. Therefore, the vaccine should be handled with protective glasses and gloves (Blasco & Molina-Flores, 2011).

In cattle, the most widely used vaccine is the live *B. abortus* S 19 vaccine (OIE, 2016). It is often given to calves (aged 3–6 months) as a single subcutaneous dose. The vaccine can also be given conjunctival as eye drops. For adult cattle, conjunctival inoculation is often preferred to minimize the risk of persistent antibody response which can interfere with serological tests. Furthermore, S 19 vaccine given via the conjunctival route decreases the risk for abortions and milk shedding in adult cattle to less than 1% (Godfroid *et al.*, 2011). Another commonly used vaccine for cattle is the *B. abortus* strain RB51 vaccine. The side effects reported for S19 and RB51 are similar to those for Rev. 1 in sheep and goats. Vaccination of pregnant cattle should therefore be avoided and care should be taken to avoid accidental human infection when handling the vaccines (OIE, 2016).

1.6 *Brucella* in mammals

Despite the knowledge and tools for controlling *Brucella* among cattle, sheep and goats, the disease is re-emerging among humans and livestock in many regions of the world (Pappas, 2010). The regions with the highest incidence rates among humans and livestock are Central Asia and the Middle East but increasing numbers of human and animal brucellosis cases have recently been reported from the Balkan Peninsula and sub-Saharan Africa. The genus *Brucella* can infect a wide range of hosts, including humans, livestock and wild animals. Over the last two decades, six novel species have been discovered and the complexity of the *Brucella* genus has become evident (Scholz *et al.*, 2016; Whatmore *et al.*, 2014; Pappas, 2010). Although each *Brucella* species has a preferred host, cross-infection between animal species can occur (Corbel, 2006).

Brucella bacteria can survive and replicate within a variety of host cells and reproductive failure in the host is due to the replication of *Brucella* within placental trophoblasts (Roop II *et al.*, 2009). *Brucella* can persist within macrophages for prolonged periods and can therefore produce chronic and sometimes lifelong infections.

1.6.1 *Brucella* in humans

The incidence of human brucellosis is reported to be 500.000 new cases every year and it is considered to be one of the world's most widespread zoonotic infections (Pappas *et al.*, 2006). The true number of human cases is believed to be much higher because many cases are never diagnosed or reported (Pappas *et al.*, 2006; WHO, 2005). Recently published data suggests that the incidence of human brucellosis exceeds 800.000 cases per year with a 95% uncertainty level

of 0.34 – 19.6 million cases (Kirk *et al.*, 2015). Close to 50% of these cases are estimated to be caused by contaminated food (Havelaar *et al.*, 2015). Furthermore, 40% of the *Brucella* cases are estimated to result in chronic infection and 10% of cases to result in orchitis in men (Kirk *et al.*, 2015).

Brucella melitensis is the most frequently reported *Brucella* spp. causing human infection (Blasco & Molina-Flores, 2011) and other *Brucella* species with high zoonotic potential are *B. abortus* and *B. suis* (biovars 1, 3, 4 and 5) (Whatmore, 2009). Despite the low zoonotic potential of *B. canis,* small outbreaks have been reported in humans (Lucero *et al.*, 2010). The recently described species *B. ceti* and *B. pinnipedialis* (Foster *et al.*, 2007), both isolated from marine mammals, are also considered to be zoonotic (Whatmore *et al.*, 2008) (Figure 2, p. 14).

The most prevalent routes of human infection are through consumption of unpasteurized milk products and close contact with infected animals (Corbel, 2006). Furthermore, laboratory staff working with *Brucella*-infected material and cultures are exposed to the risk of contracting infection (OIE, 2016; Corbel, 2006). Indirect transmission through a contaminated environment, such as water sources contaminated by aborted animals, might also play a significant role in transmission to humans (Corbel, 2006). Infected humans often present with weakness, undulant fever, anorexia, headache and joint and muscle pain (Dean *et al.*, 2012; Solera *et al.*, 1999; Young, 1995). If left untreated, the infection can become chronic with osteoarticular manifestation which in turn can have a disabling outcome.

1.6.2 *Brucella* in livestock

The most common cause of cattle brucellosis is *B. abortus* (Godfroid *et al.*, 2010; Whatmore, 2009; Corbel, 2006), but *B. suis* and *B. melitensis* also have the potential to infect cattle (Corbel, 2006). The predominant cause of brucellosis in sheep and goats is *B. melitensis* (Godfroid *et al.*, 2010; Whatmore, 2009; Corbel, 2006), and although *B. ovis* also infects sheep, it is reported to lack zoonotic potential. Brucellosis in pigs is mainly caused by *B. suis* (Figure 2, p. 14).

The most prevalent route of transmission to livestock is through direct contact between an infected animal and a susceptible animal (Whatmore, 2009; Corbel, 2006). Large numbers of bacteria are shed with aborted foetuses and discharges, and contaminated pastures or animal barns can constitute important transmission sites (Corbel, 2006). Venereal transmission can also occur and is mainly a problem in dogs, sheep and pigs (Whatmore, 2009). Therefore, male individuals used for natural breeding should be proven *Brucella*-free before being introduced into a herd (Corbel, 2006). Furthermore, semen collected for

artificial insemination must come from animals tested free from *Brucella* infection.

Brucella has a predilection for the reproductive organs of both males and females (Whatmore, 2009; Corbel, 2006). Sexually mature female animals are most susceptible to infection and symptoms of *Brucella* infection include abortions in susceptible replacement animals, stillbirth, weak offspring, retained placenta, metritis, reduced fertility and decreased milk production (Corbel, 2006). Infected males can present with orchitis, epididymitis and decreased fertility. Young animals are often resistant to infection but if infected in utero or in the early post-natal period the infection can become latent. These individuals can become important disease transmitters after their first abortion or parturition. Approximately 3.5% of infected cows are estimated to deliver latent-infected offspring (Saegerman *et al.*, 2010).

1.6.3 *Brucella* in other animal species

In addition to causing infection in humans and livestock, *Brucella* spp. have been isolated from a wide range of animal species, including *B. neotomae* isolated from rodents (Stoenner & Lackman, 1957), *B. microti* isolated from common voles and red foxes (Scholz *et al.*, 2008b), *B. vulpis* isolated from red foxes (Scholz *et al.*, 2016) and *B. papionis* isolated from baboons (Whatmore *et al.*, 2014) (Figure 2, p. 14). Furthermore, *B. abortus* has been isolated from buffalo, elk, yak and camels, and wild boar, reindeer, caribou and rodents have all been shown to be infected with *B. suis* (Pappas, 2010). Two novel species were described in 2007, *B. ceti* isolated from porpoises, dolphins and whales and *B. pinnipedialis* isolated from seals (Foster *et al.*, 2007). Furthermore, *B. melitensis* have been isolated from fish (El-Tras *et al.*, 2010), and *B. microti* has been recognized as a soil contaminant (Scholz *et al.*, 2008a).

There are still many unanswered questions regarding the epidemiology of *Brucella* in wildlife, but the findings of wildlife infected with *Brucella* have raised concerns that wildlife might act as an important reservoir for disease transmission (Godfroid *et al.*, 2010). This is the case with transmission of *Brucella* between wild boars and domestic pigs in some parts of the world (Pappas, 2010). Furthermore, in the USA, there have been concerns that elks might act as *Brucella* disease transmitters to cattle during the grazing season in the Yellowstone National Park area (Beja-Pereira *et al.*, 2009). This has led to elk being trapped, tested for *Brucella,* and, if positive, being killed (Pappas, 2010). When implementing control strategies, it is important to demonstrate whether *Brucella* infection in wildlife is a spillover from domestic animals or if it is a persistent infection in the wild-life population (Godfroid *et al.*, 2010).

The major cause of brucellosis among dogs is *B. canis* (Whatmore, 2009). However, dogs can also become infected with *B. abortus*, *B. melitensis* and *B. suis,* most commonly due to consumption of placental or foetal material (Corbel, 2006). Thus dogs can constitute a zoonotic risk as well as serve as a disease transmitter to livestock. Disease transmission between dogs is similar to that described for livestock, and the primary route is through direct contact between a susceptible animal and an infected female either aborting or giving birth. Venereal transmission can also be an important factor because infected male dogs can excrete large numbers of bacteria in their semen (Corbel, 2006).

2 Aims of this thesis

The overall aim of this thesis was to assess the occurrence of *Brucella* among livestock in Tajikistan and to elucidate how the farmers understand and respond to the threat posed by this neglected zoonotic disease. The results presented in this thesis might contribute to raising awareness of how livestock in UPU areas can constitute a public health risk if infected with *Brucella* or other zoonoses.

The more specific objectives were to:

- Describe small-scale UPU livestock farming in Dushanbe, Tajikistan.
- Assess the *Brucella* seroprevalence in dairy cows, sheep and goats in small-scale UPU farming in Dushanbe, Tajikistan.
- Identify factors associated with *Brucella* seropositivity among cattle, sheep and goats.
- Investigate the presence of *Brucella* DNA in bovine milk, compare the results to serology and perform sequence analysis of *Brucella* DNA extracted from bovine milk.
- Identify and evaluate knowledge, attitudes and risk practices with regards to brucellosis among dairy farmers involved in small-scale UPU farming in Dushanbe, Tajikistan.

3 Methodological considerations

This section presents an overview of the methodological considerations that led to the choice of methods in papers I – IV. The overview focuses on why the methods were chosen. How the materials and methods were applied is described in detail in each paper.

3.1 Study area (papers I–IV)

In papers I, III and IV the study area was set to a radius of 20 km from the central part of Dushanbe. The goal was to collect data as close as possible to centres of dense human and livestock populations in Dushanbe. Figure 6 (p. 33) shows the location of herds included in paper I and Figure 7 (p. 34) shows the locations of the study subjects included in paper III. Paper II targeted the peri-urban area of Dushanbe (areas more distant from the city centre than in papers I, III and IV) and the radius was therefore extended to 30 km (Figure 8, p. 34).

As the distance from an urban centre increases, the characteristics of the environment gradually changes from urban to peri-urban. A clear distinction between urban and peri-urban farming is difficult to make, but the following guidelines from a report published by the Special Programme for Food Security within the FAO (FAO, 2001) were used when categorizing different parts of the study area.

- Urban areas have higher population densities than peri-urban areas.
- Urban farming most commonly consists of small-scale subsistence, whereas peri-urban areas most commonly have access to more land with a market-oriented production.
- Urban farming is often a part-time job, whereas peri-urban farming is more commonly a full time job.

- Urban areas have more infrastructure/construction compared to peri-urban areas.
- Urban areas have more services (banks, schools, medical centres etc.) compared to peri-urban areas.
- There is a lower availability of natural resources in urban areas compared to peri-urban areas.
- Urban areas have easy access to markets, whereas peri-urban areas have more difficult access to markets.
- Labour and land costs are higher in urban areas compared to peri-urban areas.

3.2 Study population (papers I and II)

In papers I and II, sexually mature female animals were targeted because they play an important role in transmission of *Brucella* (Corbel, 2006). In none of the studies did we include male individuals. In paper I, this was mainly due to difficulties in performing safe sample collection of bulls because it was not possible to immobilize the animals. There were a few farms raising bulls in each village, and these bulls comingled with the cows during grazing season in villages that practiced communal grazing. In the villages where the cows were kept within limited pastures or were tethered, the dairy farmers could pay the farmers raising bulls a mating-fee each season. Because each village used the same few bulls for mating, it would have been interesting to also include the bulls in paper I because *Brucella* infection can be transmitted during natural breeding (Corbel, 2006).

Mating among the small ruminants included in paper II took place during collective grazing in August. The remaining part of the year the rams and bucks were kept separated from the ewes and dams. Sexually mature females are considered to play the major role in disease transmission because *Brucella* invades the reproductive organs and causes placentitis followed by abortion, with large numbers of organisms shed in the birth fluids of pregnant females. Sexual transmission is probably more important among sheep and goats compared to cattle (Corbel, 2006). In retrospect, extending the selection in paper II to also include male individuals could potentially have contributed to a deeper understanding of a less studied disease transmitter.

3.3 Epidemiological unit (papers I and II)

The main route of transmitting *Brucella* infection is through direct contact between an infected and a susceptible animal (Corbel, 2006). Hence, when

defining the epidemiological units, animals raised together were assumed to have similar probabilities of being exposed to the pathogen.

In paper II, we considered the village to be the epidemiological unit because all sheep and goats within every village grazed collectively during the summer and winter. In paper I, the herd was considered to be the epidemiological unit because a majority of the cows were kept in limited pastures or tethered in a pasture for most of the year. Furthermore, most farmers in the area kept their cows within the farms instead of on the open pastures/natural rangelands for the month prior to and during calving (Sattorov, personal communication 2016). This practice might decrease the risk of contaminating the pastures and natural rangelands with *Brucella* spp. excreted with aborted foetuses and the discharges of infected animals and might reduce the importance of disease exposure in such pastures.

3.4 Data collected anonymously

In none of the four studies did we collect any data regarding the identity of the animals or farmers. The owners of animals that tested positive by serology or PCR could therefore not be informed that their animals might be infectious to themselves or other animals. Additionally, we would not have had the capacity to take action such as compensating farmers financially for test-positive animals that were subsequently culled. A previous study of brucellosis performed in Tajikistan had a similar set-up as the current studies with no collection of personal data (Jackson *et al.*, 2007). The authors of that study concluded that informing the owners of test-positive animals would not have had any real impact on the overall distribution of *Brucella*-infected animals or on the risk that *Brucella*-positive animals might be sold to other farmers rather than being slaughtered.

3.5 Serology (papers I and II)

To assess the *Brucella* seroprevalence among cattle, sheep and goats in the study area, blood samples were collected and analysed with ELISA. Animals were regarded as seropositive for *Brucella* spp. if they tested positive in both I-ELISA and C-ELISA. Because none of the animals in the two studies had been vaccinated against brucellosis, seropositivity was considered to be caused by natural exposure to *Brucella*. Also, because papers I and II were based on serology, it was not possible to conclude which *Brucella* spp. had induced antibodies in the hosts.

There are different serology tests that can be used to assess serological responses to *Brucella* antigens. In papers I and II, all samples were analysed with I-ELISA (SVANOVA Biotech AB, Uppsala, Sweden) because this test is, according to the OIE, suitable for testing of individual animals (OIE, 2016). Positive samples were confirmed with C-ELISA (SVANOVA Biotech AB, Uppsala, Sweden) in an attempt to avoid false positive reactors. Because the two ELISAs detect different classes of antibodies, an option would have been to analyse all samples with I-ELISA and C-ELISA and present the results separately. This set-up would have enabled us to detect true infected individuals that were positive only in I-ELISA or C-ELISA. Due to economic and time constraints, this was not possible.

3.6 Detection and analysis of *Brucella* DNA (paper III)

Because cultivation of *Brucella* requires strict safety measures, it was not a feasible option to culture *Brucella* in the milk samples taken in paper III because there was no bio-safety level 3 laboratory available in Dushanbe. A possibility would have been to perform bacteriological cultivations in Sweden, but because milk samples have to be heat inactivated in order to be transported to Sweden, and cultivation of milk should preferably be performed directly from fresh samples, it was decided to exclude cultivation of the milk samples. According to the OIE, milk samples collected for cultivation should contain milk from all quarters of the udder and a minimum of 10–20 ml should be collected from each teat (OIE, 2016). Collecting more milk from each cow than was done in paper III would probably have increased the amount of DNA extracted from each sample and thus increased the number of samples that were successfully characterized at the species level. Unfortunately, it was not possible to transport such large amounts of milk to Sweden.

To investigate the presence of *Brucella* DNA in the milk samples, we chose to analyse all samples with primers targeting the *IS711* insertion sequence because *IS711* is a specific and highly sensitive method for the safe detection of the genus *Brucella* (Bounaadja *et al.*, 2009). The first choice for further typing of *Brucella* would have been to perform a MLVA analysis. Unfortunately, the amount of DNA extracted from the milk samples was too low to enable such analysis. Therefore, further typing of *Brucella* was performed by using primers targeting the *rpoB* gene, which allows for rapid differentiation of all *Brucella* species (Marianelli *et al.*, 2006).

3.7 Interviews (paper IV)

Paper IV is based on face-to-face interviews performed during household visits. A questionnaire with approximately 50 questions was developed by the authors and pre-tested with three students at the Tajik Agrarian University to allow for improvements. The same questionnaire was used for all interviews, and we chose to target the family member responsible for the daily management of the cows, such as milking the cows and handling the milk. In 78% (342/441) of the households, this person was a female. The option to engage all adults within the family so as to obtain a comprehensive understanding of the knowledge, attitudes and risk practices relating to brucellosis was considered. The reason for targeting the main person responsible for the daily management of the cows was to avoid all members of the family participating during the interviews because this could have caused information bias.

4 Main results and discussion

4.1 Small-scale UPU livestock farming

Papers I, III and IV were focused in the UPU area of Dushanbe, while paper II specifically targeted the peri-urban area. Figure 3 shows a local breed of dairy cows in an urban area of Dushanbe and Figure 4 shows tethered cattle in a peri-urban village. The reason for excluding the urban area in paper II was because it was more common to keep small ruminants in peri-urban areas compared to urban areas, while cattle could be found in both areas. A reason for not rearing small ruminants in urban areas could be due to the lack of land and peri-urban areas often have easier access to natural rangelands (Figure 5). A previous study from Tajikistan reported that urban households have higher proportions of cattle in relation to sheep and goats compared to rural areas (Jackson *et al.*, 2007). The findings of the current studies suggest that a similar pattern can be seen between urban and peri-urban located households.

Figure 3. Urban farming in Dushanbe (author's photo).

Figure 4. Tethered cattle in the peri-urban region of Dushanbe (author's photo).

Figure 5. Natural rangeland (author's photo).

In paper I, 904 serum samples were collected from dairy cows of breeding age belonging to 443 herds in 32 villages (Figure 6). The median herd size was four cattle (range 1–24 cattle), which is in line with previously published data on small-scale farming in Tajikistan (Jackson *et al.*, 2007). Twenty per cent of the herds included small ruminants and 10% of the farmers reported having bought new cattle during the previous year. Rearing small ruminants together with cattle increases the risk of transmitting *B. melitensis* infection from sheep and goats to cattle and trading of livestock is a common cause of transmitting *Brucella* infection between herds (Godfroid *et al.*, 2013; Blasco, 2010). Four per cent of the herds were reported as having cows that aborted during the previous year which corresponds to the seroprevalence at herd level. In some areas abortion is reported to be a relatively uncommon sign of brucellosis and most infected animals only abort once (Corbel, 2006).

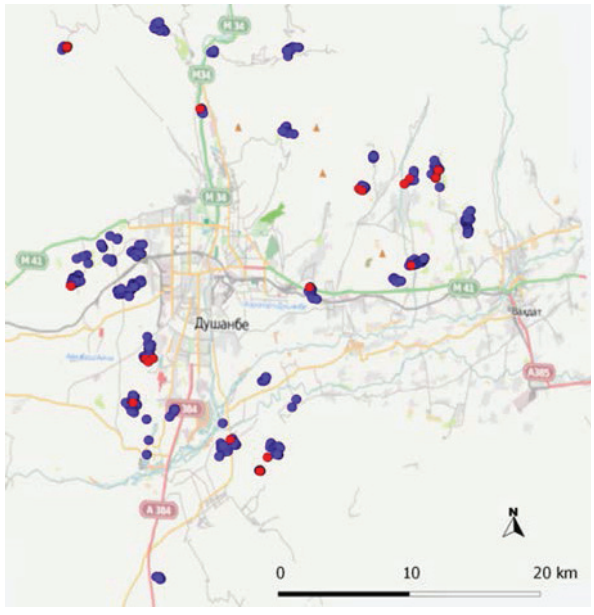

Figure 6. Map of the study area and *Brucella* serology results at herd level (n = 441). *Brucella* seropositive herds (n = 18) are represented by red dots and seronegative herds (n = 423) are represented by blue dots. © OpenStreetMap contributors (www.openstreetmap.org) (paper I).

In paper III, the study population was the same as in paper I with two exceptions – non-lactating cows were excluded and milk samples were only collected during October 2011. In total, 564 cow milk extracts from 326 herds in 21 villages were analysed for *Brucella* DNA (Figure 7).

In paper II, 667 individual blood samples were included from 260 sheep and 407 goats in 21 villages located in the peri-urban area of Dushanbe in four different districts (Figure 8). Only one goat was reported to have a history of abortion/stillbirth. Because abortion is a common consequence of *Brucella* infection (Corbel, 2006), this result might not represent the true number of abortions in the study area considering that there was a high seroprevalence in some districts. A reason behind this low reported number could be that the farmers fail to observe the abortions/stillbirths during the grazing season. Also, it has been reported that abortions/stillbirths may be uncommon in some areas where *Brucella* infection is prevalent (Corbel, 2006). All villages in paper II used natural rangelands for communal grazing, which corresponds to the characteristics of peri-urban areas that commonly have access to more open land compared to urban areas (FAO, 2001).

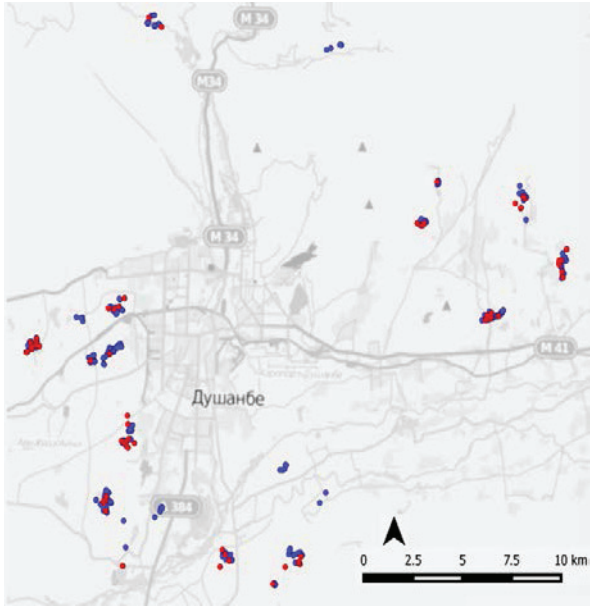

Figure 7. Map of the study area and results from the *IS711*-based qPCR at herd level (n = 324). Positive herds (n = 66) are represented by red dots and negative herds (n = 258) are represented by blue dots. © OpenStreetMap contributors (www.openstreetmap.org) (paper III).

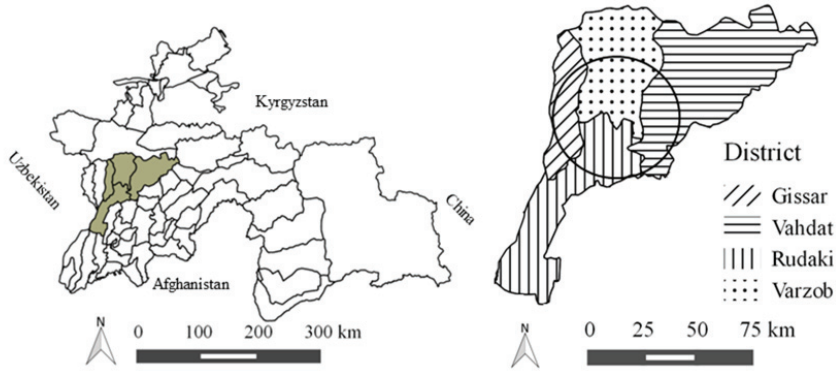

Figure 8. Left: General map of Tajikistan showing the four districts included in the study. Right: Detailed map of the four districts, with the approximate outer border of the study area represented by a black circle (Quantum GIS 2.4.0, Chugiak) (paper II).

In paper IV, women were responsible for the daily management of the cows in a majority (78%) of the 441 included households. The literacy rate among the farmers was almost 100% and the majority of the respondents had completed

secondary school which is in accordance with statistics from UNICEF (Unicef, 2013).

4.2 *Brucella* seropositivity (papers I and II)

In paper I, the herd seroprevalence among the dairy cows was 4.1% (95% CI, 2.6–6.3%) and the individual seroprevalence was 2.0% (95% CI, 1.3–3.1%). The distribution of *Brucella*-seropositive herds is shown in Figure 6 (p. 33). The seroprevalence at the individual level is in line with the findings in a previous study from 2007 comprising both small-scale and large-scale herds in Tajikistan (Jackson *et al.*, 2007). A nationwide study conducted in the neighbouring country of Kyrgyzstan showed similar results to those presented in paper I, with an individual seroprevalence in cattle of 2.8% (Bonfoh *et al.*, 2012). Higher figures have been reported from Kazakhstan, another neighbouring country to Tajikistan, where it was estimated that 14% of the lactating cows were *Brucella* seropositive (Beauvais *et al.*, 2016).

In countries where brucellosis has been eradicated, the typical eradication strategy has been compulsory whole-flock vaccination until the prevalence drops to 1–2%, followed by a test-and-slaughter strategy (Godfroid *et al.*, 2013). This eradication strategy has often taken more than 10 years in high-income countries. In Tajikistan and other low-income countries with scarce resources, an eradication programme is not a realistic approach because it requires elements such as financial compensation to farmers for production losses and to replace culled animals (Blasco & Molina-Flores, 2011), a legal framework for enforcing eradication measures, well-developed transportation systems, control of animal movements and an identification system for individual animals (FAO, 2014; Smits, 2012; Blasco & Molina-Flores, 2011). Instead, a mass vaccination strategy to reduce the incidence of *Brucella* among dairy cows together with campaigns promoting pasteurization of milk and dairy products might be a way forward to reduce the risk of human infection (Godfroid *et al.*, 2013).

In paper II, the true individual seroprevalence among sheep and goats ranged from 1.0% to 15.6% in the four districts. Table 1 shows the apparent individual seroprevalences and the total number of samples collected from sheep and goats. Fourteen villages had at least one seropositive sheep or goat, resulting in an apparent prevalence at the village level of 67%. In a serosurvey performed in 2009, the overall individual seroprevalence was 4.2% in rural districts near the Dushanbe area in the western part of Tajikistan (Ward *et al.*, 2012). From a public health perspective, it is unfortunate that the higher seroprevalence in sheep and goats as shown here geographically coincides with

higher human population density in the peri-urban areas. A recent study conducted in Mexico modelled whether a control programme targeting goats would be economically profitable or not (Montiel *et al.*, 2015). The results showed that a control programme including mass vaccination with Rev 1 would be economically profitable for the farmers, whereas a test-and-slaughter strategy would not. This implies that a test-and-slaughter strategy in Mexico would need to include financial compensation to the farmers for the culling of infected animals. To accomplish effective control of the high disease burden among sheep and goats in some districts in Tajikistan, long-term mass vaccination with Rev 1 would probably be the best alternative.

Table 1. *Descriptive results of Brucella seropositivity at the individual level (n = 667) (paper II).*

Variable	Category	% (Number)	Seropositive % (Number)
Species	Sheep	39 (260)	11 (28)
	Goat	61 (407)	5 (20)
District [a]	Varzob	26 (174)	3 (5)
	Rudaki	23 (156)	2 (3)
	Gissar	23 (156)	8 (12)
	Vahdat	27 (181)	15 (28)

[a] Including both sheep and goats

4.3 Factors associated with *Brucella* seropositivity (papers I and II)

In paper I, the multivariable logistic regression analysis at herd level showed that abortions were significantly associated with seropositivity ($p = 0.02$) (Table 2). This finding is in line with the pathobiology of *Brucella* (Corbel, 2006), and similar results have been described in other field studies (Matope *et al.*, 2011; Al-Majali *et al.*, 2009; Silva *et al.*, 2000). It is generally acknowledged that abortions and decreasing milk yield can be of major economic importance in an infected herd (McDermott *et al.*, 2013).

In paper I, herds with more than eight cattle were significantly associated with seropositivity ($p = 0.02$) compared with herds with only one or two cattle (Table 2). This is consistent with other studies from Uganda, Jordan, sub-Saharan Africa and Kenya (Mugizi *et al.*, 2015; Al-Majali *et al.*, 2009; McDermott & Arimi, 2002; Kadohira *et al.*, 1997). Because *Brucella* is most commonly transmitted through direct contact between cattle following abortion, it is likely that the large herds provide more opportunities for transmission of *Brucella* infection between cattle.

Table 2. *Relationship between different factors and Brucella seropositivity at the herd level (n = 443) using multivariable logistic regression analyses (paper I).*

Variable	Category	β	SE	p	OR (95 % CI)
Abortion	Yes	1.7	0.7	0.02 [a]	5.3 (1.3–21.3)
	No				
Herd size (number of cattle)				0.07 [a]	
	1 or 2				reference
	3 or 4	1.6	1.1	0.14	5.0 (0.6–41.5)
	5-7	1.6	1.1	0.17	4.8 (0.5–43.9)
	≥ 8	2.6	1.1	0.02	13.9 (1.6–119)

[a] Likelihood ratio test

Pasture type was not found to be associated with *Brucella* seropositivity in paper I, although communal grazing is known to be an important risk factor for disease transmission (Corbel, 2006). The reason why pasture type was not found to be associated with *Brucella* seropositivity could be, as mentioned previously, that most farmers keep their cows within the farms instead of on the pasture one month prior to and during calving. This might decrease the risk of contaminating the grazing area with *Brucella* bacteria shed with aborted foetuses and discharges.

There was also no evidence of an association between the introduction of new cattle into a herd and seropositivity for *Brucella* in paper I. A likely explanation for this is that the number of herds reporting having acquired new cattle in the previous year was too small to see a significant association with seropositivity. Trade is otherwise known to be an important risk factor for disease transmission between herds (Corbel, 2006).

Raising sheep and goats together with dairy cows was not found to be associated with *Brucella* seropositivity among the dairy cows. However, mixed farming has been reported to promote the spread of *B. melitensis* from sheep and goats to cattle (Godfroid *et al.*, 2013; Blasco, 2010).

In paper I, the number of calves produced per cow was used as an indicator of age. The categories with one or two calves produced per cow ($p = 0.051$) and three or four calves produced per cow ($p = 0.07$) were borderline significant. These categories were less likely to be seropositive compared to the category with more than six calves produced per cow. A similar finding was reported in paper II where an increase in age among sheep and goats was associated with a significant increase in seropositivity ($p < 0.001$) and none of the animals younger than 1 year ($n = 59$) in paper II were seropositive for *Brucella*. It is generally acknowledged that sexually mature individuals are more susceptible to *Brucella* infection compared to younger animals (Quinn *et al.*, 2011) and this has also been verified by other studies (Akbarian *et al.*,

2015; Boukary *et al.*, 2013; Megersa *et al.*, 2011; Al-Majali *et al.*, 2009; Silva *et al.*, 2000). However, in papers I and II where we only sampled sexually mature individuals, the older animals had lived a longer time at risk of being exposed to infection compared to the younger animals.

In paper II, there was a significant difference in seroprevalence between the four districts (Table 3). Sheep and goats in Rudaki (p = 0.003) and Varzob (p = 0.024) were less likely to be seropositive than sheep and goats in Vahdat. One reason for the high seroprevalence observed in Vahdat district could be that many villages from other districts use part of Vahdat district as the main route for the movement of sheep and goats between summer and winter pastures. This could increase the density of animals grazing together and hence the risk for transmission of *Brucella* through direct contact between animals from different villages. Furthermore, there are three large animal markets in Vahdat district that could play an important role in transmitting *Brucella* infection (Figure 9). To reduce the risk of transmission between villages and districts, trade in animals should be restricted (Blasco & Molina-Flores, 2011).

Table 3. *Relationship between different factors and Brucella seropositivity at the individual level (n=667) using multivariable logistic regression analyses with village as the random effect, Tajikistan, 2012 (paper II).*

Variable	Category	β	p	OR (95% CI)
Species	Sheep	1.0	0.009	2.7 (1.3-5.5)
	Goat			
District			0.008	
	Varzob	− 1.3	0.024	0.3 (0.09−0.8)
	Gissar	− 0.5	0.191	0.6 (0.3−1.3)
	Rudaki	− 2.3	0.003	0.1 (0.03−0.4)
	Vahdat			Reference
Age (in years)	Continuous	0.3	<0.001	1.4 (1.2−1.6)

Figure 9. Animal market (author's photo).

In paper II, sheep were more likely to be seropositive than goats (p = 0.009) (Table 3). However, other literature suggests that goats are more susceptible to *B. melitensis* infection than sheep (Quinn *et al.*, 2011). There was no difference in seroprevalence between non-vaccinated sheep and goats in a previous study conducted in Tajikistan (Ward *et al.*, 2012), but differences in susceptibility have been observed among sheep, where the milking breeds seem to be most susceptible to *B. melitensis* (Corbel, 2006). More research is required to allow firm conclusions to be drawn on whether the fat-tailed Gissar breed of sheep that are common in the region are more susceptible to *Brucella* infection than goats.

4.4 Detection of *Brucella* DNA by PCR and comparison with serology (paper III)

In the current study, 564 cow milk extracts from 326 herds in 21 villages were analysed for *Brucella* DNA. In total, *Brucella* DNA was detected in 13.7% (n = 77) of the milk samples with *IS711* qPCR. A sample was considered to be positive if the Cycle threshold (Ct) was ≤ 40 (Al Dahouk *et al.*, 2007) in two runs. The apparent individual seroprevalence measured previously with I-ELISA and C-ELISA was 2.1%. All seropositive cows (n = 12) were positive by qPCR with Ct-values ranging between 26.9 and 31.9. Out of the 552 seronegative cows, 11.8% (n = 65) were qPCR positive for *Brucella* DNA with Ct-values ranging between 26.5 and 39.8. At herd level, 20% (n = 66) had at least one positive cow by qPCR (Figure 7, p. 34). Similar discrepancy between the serology and qPCR results was demonstrated in a study comparing *IS711*-based qPCR, serology and culture among wild boars. In that study, *Brucella* DNA was detected in tissue samples of 11.1% of the seronegative individuals

(Hinić *et al.*, 2009). The discrepancy between the serology and PCR results observed in the current study might indicate that the true number of *Brucella*-infected cattle within the study area could be underestimated by serology screening. False serological negative results have been reported previously (Mailles *et al.*, 2012; Al Dahouk *et al.*, 2003; Godfroid *et al.*, 2002) and one explanation could be that antibody titers reduce over time (Godfroid *et al.*, 2010). Hence, seronegative animals in the current study, which tested positive by qPCR, could have been exposed to *Brucella* and turned seronegative after a certain time period. Alternatively, if sampling at an early stage of the infection, i.e. within the first 14 days, the humoral immune response has not yet induced detectable levels of antibodies in the host (Gardner *et al.*, 2000). Furthermore, individuals infected in utero or in the early post-natal period can become latently infected and hence never become seropositive (Corbel, 2006). Approximately 3.5% of infected cows are estimated to deliver latent-infected offspring (Saegerman *et al.*, 2010). Furthermore, it has previously been reported that *B. suis* infection in cattle generates a shorter duration of antibody response in the host compared to □□□□□□□□□□(Godfroid *et al.*, 2002). Whether this is also true for *B. melitensis* infection in cattle is not known and needs to be investigated further. If this is the case, it might partially explain the discrepancy between the serology and qPCR results observed in the current study. Hypothetically, the discrepancy between the serology and qPCR results could be caused by previous vaccination against brucellosis as reported from a study in Egypt where cattle vaccinated with RB51 tested negative by serology tests but positive by qPCR (Gwida *et al.*, 2016). However, in the current study, the information given from the local official veterinarians that none of the cattle had been vaccinated against brucellosis is considered reliable because there is no national control programme for brucellosis among livestock in Tajikistan. The potentially significant number of serological false negative individuals observed in paper III highlights the importance of determining if there is a need for implementing complementary diagnostic strategies to detect false serological negative individuals in *Brucella* surveillance, control, and eradication programmes.

To draw firm conclusions regarding the zoonotic risk of consuming the milk from the qPCR-positive individuals is difficult because the qPCR can detect DNA from live, damaged or dead bacteria. However, because consumption of and trading with unpasteurized dairy products is commonly occurring among small-scale farmers in the study area, the significant numbers of cows with detectable levels of *Brucella* DNA in their milk could constitute a serious health risk.

4.5 Sequence analysis of *Brucella* DNA (paper III)

In total, only two samples had sufficient amounts of DNA to perform sequence analysis. The first sample was collected from a seropositive cow and the SNP allelic profiles corresponded to the SNP profiles described for *B. melitensis* and *B. suis* at codon positions 716 (CCG) and 737 (GTT) (Marianelli *et al.*, 2006). Because there is almost no pig production in Tajikistan, it is highly likely that this cow was infected with *B. melitensis*. This individual was not being kept together with small ruminants and the infection source in this particular case remains unknown. The prevailing epidemiological situation in the study area, with endemic *Brucella* infection among sheep and goats and where cattle are often kept in close proximity with small ruminants, could lead to a spillover of *B. melitensis* from small ruminants to cattle (Godfroid *et al.*, 2013; Blasco, 2010). A similar finding has been reported in a study from the neighbouring country of Kyrgyzstan where *B. melitensis* has been isolated from cattle (Kasymbekov *et al.*, 2013).

The other sample with a sufficient amount of DNA to perform molecular characterization came from a seronegative cow, and the SNP allelic profiles corresponded to *B. abortus* at codon positions 716 (CCA), 969 (CGT) and 985 (GCC). At one position – the codon at 737 (GTT) – the SNP was not described for *B. abortus*, although it has previously been reported to be GTC for *B. abortus*. Whether this SNP is a new marker for *B. abortus* in the region remains unclear, and more research is required to draw firm conclusions from this observation. A report by the FAO stresses that *B. melitensis* infection is much more common than *B. abortus* in Central Asia and the Middle East (FAO, 2010), but the findings of the current study suggests that *B. abortus* infection might also constitute a problem in the region. Thus vaccination of cattle with S19 in addition to vaccination of small ruminants with Rev 1 might be needed in order to control *Brucella* infections in the livestock population. To obtain a comprehensive understanding of the *Brucella* spp. circulation within the livestock population in this region, further research, including isolation of *Brucella* from cattle, sheep and goats, is required.

4.6 Knowledge of brucellosis (paper IV)

In paper IV, a majority (85%) of the 441 respondents had never heard of brucellosis. Of those who had heard of the disease (n = 65), about half (55%) had received information from relatives or friends, and the majority (82%) knew that cattle, sheep or goats could become infected (Table 4). All interviewees who had heard of brucellosis (n = 65) knew that humans could become infected and 52 of these persons knew that arthritis was a common

symptom in humans. A majority (78%) did not know that cattle could be vaccinated against the disease and 91% of those who had heard of brucellosis knew at least one correct route of transmission from animals to humans, most commonly through the consumption of unpasteurized milk from infected cows. Fewer (22%) knew one or more correct route of transmission between animals. In five of the households that had heard of brucellosis, a family member had been diagnosed with the disease by a physician and in two of the households a veterinarian had diagnosed brucellosis among cattle, sheep or goats. A high awareness of brucellosis among farmers has been shown in Egypt where the majority of the farmers were aware of brucellosis, which the authors explained by endemic brucellosis in the study area (Holt *et al.*, 2011). The difference in awareness among farmers in Egypt and the current study might be explained by a lower herd seroprevalence among the dairy cows in UPU areas of Dushanbe compared to Egypt. However, it is noteworthy that the awareness of brucellosis was poor among the farmers in paper IV despite a control programme among small ruminants initiated in 2004 by the FAO. The programme did not include the region of Dushanbe, but included several areas in Tajikistan with high seroprevalences of *Brucella* spp. in sheep and goats (FAO, 2014; Ward *et al.*, 2012).

A study from Kyrgyzstan showed that a good knowledge of the transmission routes for brucellosis had a protective effect against human infection (Kozukeev *et al.*, 2006), and a study from Iran showed that knowledge of the mode of brucellosis transmission through fresh cheese was protective against disease transmission in humans (Sofian *et al.*, 2008). The human incidence of brucellosis is increasing in Tajikistan (Pappas, 2010) and the majority of the farmers in the current study with a low awareness of brucellosis could be exposed to a higher risk of contracting *Brucella* infection than the farmers with a high awareness of the disease.

The multivariable logistic regression model showed that participants with a lower level of education were less likely to have knowledge of brucellosis compared to those who had attended technical college or university ($p < 0.001$). The relationship between educational level and brucellosis has also been investigated in a study from Yemen, which showed that humans diagnosed with brucellosis were more likely to have a lower education level compared to controls (Al-Shamahy *et al.*, 2000). If this also is true for the current study area, farmers with a lower level of education could be at higher risk of contracting brucellosis than their peers with a higher level of education. Respondents who discussed animal health issues with family members or friends were less likely to have heard of brucellosis compared to those who often talked to veterinarians ($p = 0.03$). Discussing animal health issues with

veterinarians was common among the farmers (Table 5, p. 46), and information campaigns regarding the epidemiology of *Brucella* targeting veterinarians might thus be an effective way to transfer knowledge to farmers.

Table 4. *Knowledge about brucellosis among the respondents who had heard of the disease (n = 65) (paper IV).*

	Category	%
Information source	Relatives/friends	55
	Veterinarian	22
	Book	11
	Television	1.5
	Don´t know	11
Which animal species can become infected?	Cattle/Sheep/Goats	82
	All mammals	4.6
	Don´t know	14
Can humans become infected?	Yes	100
	No	0
Symptoms in humans	Arthritis	80
	Fever and arthritis	3.1
	Fatigue	1.5
	Skin lesions	3.1
	Don´t know	12
Does any vaccination for animals exist?	Yes	22
	No	78
Modes of transmission:		
Animal-to-animal	Correct [a]	22
	Incorrect [b]	78
Animal-to-human	Correct [a]	91
	Incorrect [b]	9.2
Previous *Brucella* infection within the household:		
Among humans	Yes	7.7
	No	92
Among cattle/sheep/goats	Yes	3.1
	No	97

[a] Stated at least one correct route of transmission
[b] Stated no correct route of transmission

4.7 Attitudes towards brucellosis (paper IV)

Sixty-three per cent (n = 279) of the households wanted more information about brucellosis, while the remaining 37% claimed that they did not need more information. Of the 279 respondents who wanted more information, the majority (58%) preferred to receive the information through an educational booklet while 23% preferred a course or information meeting in the village. The high literacy rate and educational standard, together with a positive attitude towards learning more, provides a good foundation for integrating information campaigns for brucellosis into future control programmes in Tajikistan.

Of the respondents who had heard of brucellosis (n = 65), the majority (n = 52) did not consider any family member to be at risk of contracting *Brucella* infection, and of all respondents (n = 441), only 2.5% perceived themselves as being at risk of contracting brucellosis. Among those who had heard of brucellosis (n = 65), 17% perceived a risk of contracting the disease and that the person in the household working most with the cows was exposed to the highest risk. Thus increasing the share of farmers who are knowledgeable of the existence of brucellosis might increase the number of farmers perceiving themselves as being at risk of contracting brucellosis. This could be a first step in building a platform for discussions regarding risk behaviours.

4.8 Self-reported practices (paper IV)

Consumption of unpasteurized dairy products is a well-known risk factor for human brucellosis (Earhart *et al.*, 2009; Sofian *et al.*, 2008; Kozukeev *et al.*, 2006), and close to 30% of the households reported consuming unpasteurized dairy products from the cows on a regular basis (Table 5). Seventeen per cent (n = 76) of the respondents sold unpasteurized milk or unpasteurized milk products directly to consumers on a regular basis. The majority (66%) of these 76 respondents sold their unpasteurized dairy products on an everyday basis. The results from paper I show that 4% of the households had at least one *Brucella*-seropositive cow. Furthermore, paper III shows that 13.7% of the dairy cows included in that study had detectable levels of *Brucella* DNA in their milk. Hence, the consumption of unpasteurized dairy products reported in paper IV could constitute a risk to public health. Changes in the political and economic situation in the region have led to increased privatization of collective farms in Tajikistan and other Central Asian countries (Jackson *et al.*, 2007; Kozukeev *et al.*, 2006), and Kozukeev et al. suggest that this development has led to more frequent trading with home-made animal source

foods in Kyrgyzstan and thus to decreased food safety (Kozukeev *et al.*, 2006). Because the pattern of privatization of collective farms has been similar in Tajikistan, there are reasons to believe that trading in Tajik home-made animal source foods has increased, putting food safety at risk.

Seventy-eight per cent reported hand washing as the only protective measure after having handled aborted foetuses or discharges, and only 21% used gloves (Table 5). If abortions in livestock occur due to brucellosis, the foetuses and aborted material will be heavily infected by *Brucella* (OIE, 2016), and using hand washing as the only protective measure to avoid transmission of infection to humans might not be sufficient. One explanation for why the majority of farmers were only using hand washing as a protective measure could be poor knowledge of the risk with this practice but also lack of access to protective clothing like gloves. Similar results have been reported in a study from Egypt (Holt *et al.*, 2011) and this practice is a known risk factor for humans to contract brucellosis from livestock (Earhart *et al.*, 2009; Kozukeev *et al.*, 2006).

Females were more likely to assist during calving (56% of the households) compared to males (31% of the households) (Table 5). This finding, together with the previously reported finding that females were responsible for the daily management of the cows in a majority of the households, could imply that females are exposed to a high risk of contracting *Brucella* infection through direct contact with *Brucella*-infected dairy cows in the study area. This is supported by a study from Mongolia that showed that women were more likely to be seropositive for *Brucella* compared to men (Zolzaya *et al.*, 2014). The authors of that study suggest that reasons for this could be that women more often take care of weak newborn animals and are more responsible for milking livestock.

It was almost as common to discuss animal health issues with veterinarians (48%) as it was with family members or friends (52%), and the majority of the farmers (81%) contacted a veterinarian if a cow showed symptoms of disease (Table 5). This is in line with findings from a study conducted in Egypt where most respondents would contact the local veterinarian if they suspected *Brucella* infection among their livestock (Holt *et al.*, 2011). The well-established relationship between many veterinarians and farmers could be useful for implementing information campaigns as part of a future brucellosis control programme.

Table 5. *Descriptive results of self-reported practices among dairy farmers (paper IV).*

	Category	n	%
Does the respondent sell unpasteurized milk or unpasteurized milk products directly to consumers? (n = 438)	Yes	76	17
	No	362	83
How frequently does the respondent sell unpasteurized milk or unpasteurized milk products? (n = 76)	Every day	50	66
	One to two times per week	14	18
	Once a month/sometimes	12	16
Does the respondent consume unpasteurized milk or unpasteurized milk products? (n = 441)	Yes	123	28
	No	318	72
Who in the household assist during calving? (n = 441)	Female	246	56
	Male	138	31
	Female & Male	56	13
	Always call veterinarian	1	0.2
Who does the respondent talk to about animal health issues? (n = 441)	Family member/friend	229	52
	Veterinarian	212	48
What does the respondent do with dead cattle foetuses? (n = 441)	Bury	413	94
	Call veterinarian	9	2
	Food for dogs	7	1.6
	Burn	2	0.5
	Don´t know	10	2.3
Does the respondent use protection when dealing with cows having an abortion or with aborted materials? (n = 441)	Use gloves	93	21
	Wash hands	344	78
	Always call veterinarian	1	0.2
	No / Don´t know	3	0.7
If the respondent buys a new cattle, does he/she take any action to assure it is healthy? (n = 441)	No	280	63
	Use more experienced people in the village	142	32
	Use veterinary inspection	19	4.3
What does the respondent do if a cattle is sick or shows signs of disease? (n = 441)	Seek veterinary assistance	359	81
	Treat	77	17
	Slaughter	4	0.9
	Don´t know	1	0.2

In paper IV, almost all households (94%) stated that they usually buried dead cattle foetuses (Table 5). This practice might reduce the risk of dogs consuming placental or foetal materials from aborting cows and thus reduce the risk of dogs acting as transmitters of *Brucella* to humans and livestock (Wareth *et al.*, 2016). In contrast to the finding in Tajikistan, a Mongolian study showed that almost half of the respondents usually fed their dogs with aborted foetuses and placentas (Zolzaya *et al.*, 2014). Burying dead animals (that were not slaughtered) is reported to be common practice in Tajikistan for religious reasons (Sattorov, personal communication, 2016).

When purchasing new cattle, the majority (63%) stated that they did not take any specific action to make sure the animal was healthy, whereas 32% used more experienced people in the village for help (Table 5). The introduction of new livestock into a herd from a source not declared free from *Brucella* infection is an important risk factor for transmitting infection between herds (Blasco & Molina-Flores, 2011). The practice of uncontrolled trading of cattle around Dushanbe might therefore increase the risk for transmission of *Brucella* infection between herds.

Households with a history of reported *Brucella* infection among humans, cattle, sheep or goats were equally inclined to sell and consume unpasteurized dairy products as those who had not had the infection within the household or who had never heard of the disease. This might imply that there is a lack of information from physicians and veterinarians to affected farmers regarding the modes of transmission of brucellosis. It could also reflect the fact that awareness of risks might not translate into changed behaviors similar to what was shown in a study of avian influenza in Cambodia (Osbjer *et al.*, 2015).

5 Conclusions

This thesis reports on the occurrence of *Brucella* among livestock in an UPU area of Tajikistan, identifies the *Brucella* spp. extracted from bovine milk and elucidates farmers´ knowledge, attitudes and self-reported risk practices related to brucellosis. The following are the main conclusions of this thesis.

- *Brucella* infection is widespread among livestock in the UPU area of Dushanbe, with high seroprevalence among sheep and goats in Vahdat and Gissar districts. This can constitute a public health risk and cause significant production losses.
- Previously known risk factors for *Brucella* seropositivity were also demonstrated here, including herd size (among cattle) and age (among cattle, sheep and goats). Contrary to existing data, sheep are more likely to be seropositive compared to goats in the study area.
- *Brucella* DNA was commonly detected in milk from cows in the UPU area of Dushanbe, both among *Brucella* seropositive and seronegative individuals. The discrepancies between the serology and PCR results highlight the need to further investigate whether there is a need for implementing complementary diagnostic strategies to detect false serologically negative individuals in *Brucella* surveillance, control and eradication programmes.
- There is likely a reservoir of *B. abortus* in the cattle population and a spillover of *B. melitensis* from small ruminants to cattle. This suggests that vaccination of cattle with S19 in addition to vaccination of small ruminants with Rev 1 might be needed in order to control *Brucella* infections in the livestock population.

- The knowledge of *Brucella* is poor among UPU dairy farmers and a high literacy rate and willingness to learn more strengthens the logic for including campaigns aiming to raise awareness of brucellosis and its associated risks in control programmes.
- Several known high-risk practices, such as consumption of unpasteurized dairy products and not wearing protective clothing when dealing with aborted foetuses or discharges, were reported among the UPU dairy farmers. Health education aiming to change such risk behaviours should therefore be promoted in future control programmes.

6 Future perspectives

Tajikistan is a low-income country in a politically turbulent part of the world. With continued development and economic growth, gaining control of some important zoonotic infections as many high-income countries have done, is a likely progression. However, such a positive development cannot be guaranteed with ongoing conflicts in neighbouring countries, weak institutions and strong economic dependency on Russia. Many high-income countries have successfully gained control of *Brucella* through the implementation of control and eradication programmes in livestock, and the question of whether Tajikistan can follow suit needs to be addressed.

A cost-benefit analysis conducted in Mongolia, another low-income country in the region, suggests that implementation of a brucellosis control programme consisting of vaccinations of livestock can be cost effective (Roth *et al.*, 2003). To determine whether a control programme among livestock in Tajikistan would also be cost effective, the cost of implementing a control programme as well as the national economic gain from such a programme needs to be investigated (Smits, 2012). Also, it must be acknowledged that Tajikistan is a country with limited economic resources that is forced to make difficult prioritizations. Although a control programme might be profitable, it might very well be the case that it should not be implemented if there are other national interests of even higher urgency. Furthermore, even if a control programme makes economic sense after having considered other national needs, the feasibility of conducting a successful and long-lasting control programme for brucellosis in Tajikistan must be critically analysed. Not only is Tajikistan an economically constrained country, it is also a young nation. The functioning of infrastructure, border control, enforcement of policies and regulations, long-term political commitment, and funding of the veterinary sector cannot be taken for granted. Understanding if Tajikistan will have the

institutional functions in place to support a long-term control programme must therefore complement the economic analysis.

Long-term changes must be taken into consideration when evaluating the benefits of long-term interventions. One important driver of change in Tajikistan, and many other low-income countries, is urbanization. Today, every fourth Tajik citizen lives in an urban area and in 2050 almost every second Tajik is projected to live in an urban area (United Nations, 2014). Such a drastic development will surely affect the practice of livestock keeping and therefore needs to be better understood. One plausible development might be an increasing number of small-scale UPU farmers continuing to trade with home-made animal source foods and thus putting food safety at even greater risk. Another trajectory might be that urbanization leads to specialisation with large scale livestock production and well-controlled food value chains. These two scenarios would imply different set-ups of a proper *Brucella* control programme. Whether the described scenarios, or some other scenarios, are the most likely to materialize would be of great value to understand when projecting the future benefits of a control programme.

This thesis shows that the knowledge of brucellosis is poor among UPU dairy farmers in Dushanbe, Tajikistan. Furthermore, several known high-risk practices, such as consumption of unpasteurized dairy products and not wearing protective clothing when dealing with aborted foetuses or discharges, were commonly reported among the farmers. A future control programme should therefore promote health education aiming to increase public awareness of brucellosis and change such risk-behaviours among livestock farmers.

The fact that the majority of published brucellosis studies rely on serology is a matter that has recently received some critical attention because serology does not reveal which *Brucella* spp. is causing the infection in the host and therefore precludes the possibility of identifying the source of infection (Godfroid *et al.*, 2013). Some of the factors in favour of serology are that serology is rapid, easy to perform and does not require a well-equipped laboratory, all of which are beneficial in low-income countries. Today, there are different rapid typing tools for molecular characterization of *Brucella*, but the challenge is to transfer the technology and knowledge to low-income countries. The introduction of such tools in low-income countries would probably increase the number of publications investigating which *Brucella* spp. is causing infection in humans and livestock and thereby contribute to the understanding of how to better control *Brucella* in the future.

7 Populärvetenskaplig sammanfattning

Brucellos är en av de vanligaste zoonotiska sjukdomarna i världen och drabbar framför allt människor och djur i fattiga länder. En zoonos är en sjukdom som kan smitta mellan djur och människor. Brucellos orsakar stora ekonomiska förluster i de länder där sjukdomen är vanlig. Centralasien och Mellanöstern är hårt drabbade områden med många fall av brucellos hos människor och djur.

En av vår tids stora samhällsförändrande krafter är urbaniseringen, dvs. att människor flyttar från landsbygden till städer. När människor flyttar till städer tar de ofta med sig sina livsmedelsproducerande djur såsom kor, får, getter, grisar och fjäderfä. Att bedriva småskalig djurhållning i städer kan ge fattiga människor en extra inkomst och även utgöra en stor del av familjens mat-försörjning men urbaniseringen bidrar också till att stora koncentrationer av djur och människor bor tätt tillsammans. I dessa miljöer är handel med mjölk, kött och ägg vanlig. Dessa livsmedel kan nå många människor i staden, ofta via informella handelskanaler. Om livsmedelsproducerande djur, som hålls i en stadsmiljö, är infekterade med zoonotiska sjukdomar såsom brucellos, kan de därför utgöra en stor risk för folkhälsan.

Målen med denna avhandling är att inventera förekomsten av *Brucella* hos kor, får och getter och att klarlägga hur människor i ett låginkomstland, med en snabb förväntad urbaniseringstakt, förstår och agerar utifrån de risker denna sjukdom för med sig.

De fyra studier som ingår i denna avhandling är utförda i hushåll med småskalig djurhållning belägna i och i utkanten av Dushanbe, som är huvudstaden i Tajikistan. Totalt togs blodprov från 904 kor och 667 får och getter samt mjölkprover från 564 kor. Vidare intervjuades 441 hushåll om kunskaper, attityder och riskbeteenden med anknytning till brucellos. Andelen av kor, får och getter som bar på antikroppar mot *Brucella* undersöktes. Ett

djur som bär på antikroppar mot *Brucella*, och som tidigare inte vaccinerats för brucellos, har någon gång stött på smittämnet och räknas därmed som infekterad med *Brucella*. Fyra procent av de hushåll som ägde kor hade minst en *Brucella*-positiv ko. Positiva samband påvisades mellan antikroppar mot *Brucella* och antal kor i hushållet, ålder på korna samt tidigare aborter hos djuren. Bland får och getter var det en skillnad i förekomst av antikroppar mot *Brucella* i de fyra distrikten som undersöktes. I det distrikt med lägst förekomst bar 1.0% av de testade djuren på antikroppar mot *Brucella* medan det i distriktet med högst förekomst var 16% av fåren och getterna som hade antikroppar mot *Brucella.* Ett positivt samband mellan antikroppar mot *Brucella* och ålder på djuren påvisades och får visade högre sannolikhet att vara antikroppspositiva jämfört med getter.

Brucella DNA påvisades i 14% av mjölkproverna med polymeras-kedjereaktion (PCR). Alla kor som hade antikroppar mot brucellos hade även *Brucella* DNA i sin mjölk. *Brucella* DNA kunde också påvisas i mjölken hos 12% av de kor som inte hade antikroppar mot brucellos. Analyser av DNA:t från två mjölkprover tyder på att korna utgör en reservoar för *B. abortus* och att det även med hög sannolikhet skett en spridning av *B. melitensis* från får och getter till korna.

Kunskapen om brucellos bland de tillfrågade hushållen visade sig vara mycket bristfällig. Ett flertal riskbeteenden, såsom konsumtion av opastöriserad mjölk och att inte bära skyddskläder vid hantering av aborterade foster och fostervätskor, var vanligt förekommande.

Denna avhandling visar att *Brucella* finns bland livsmedelproducerande djur i och i utkanterna av Dushanbe. Dessutom pekar denna avhandling på hög förekomst bland får och getter i vissa av de undersökta distrikten samt på stor förekomst av *Brucella* DNA i komjölk. Detta kan utgöra en risk för folkhälsan och orsaka stora ekonomiska förluster i området. Skillnaden i resultaten mellan antikroppstesterna och PCR visar på ett behov av att undersöka om PCR bör användas som ett komplement till antikroppsanalyser i olika övervaknings-, och kontrollprogram för brucellos. För att bättre kunna förebygga brucellos i framtiden behövs fler studier som syftar till att skapa en helhetsbild över vilka *Brucella* arter som förekommer bland både djur och människor i Tajikistan.

Med tanke på den låga kunskapsnivån, den frekventa förekomsten av riskbeteenden och en vilja bland hushållen att lära sig mer, bör en informationskampanj riktad mot hushåll med livsmedelsproducerande djur vara en del av insatsen i ett framtida kontrollprogram mot brucellos i Tajikistan.

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