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Brucellosis in small ruminants – a seroprevalence study in peri-urban farming around the region of Dushanbe, Tajikistan

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Brucellos hos små idisslare – en serprevalensstudie i periurbana områden omkring Dushanbe, Tadzjikistan

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SUMMARY

The mountainous Central Asian and former Soviet country Tajikistan is the least advantaged country economically among the former Soviet Union states. Approximately 6.5 % of the land is arable in a country where roughly 80 % of the households typically own small numbers of sheep and goats. Management practices and animal husbandry in the villages such as uncontrolled breeding, mixing of animals on pastures favor transmission of infectious diseases.

Brucellosis is a zoonotic bacterial disease caused by *Brucella* spp. The disease is endemic in Tajikistan. B. *melitensis* causes disease primary among sheep and goats. An important clinical sign amongst animal is abortion during the last third of pregnancy.

Aims of the current study were to, 1) describe the farm structure of peri-urban located villages around the capital Dushanbe. 2) To investigate the seroprevalence of brucellosis in sheep and goats from small households in peri-urban villages in four districts around Dushanbe.

Sera were collected from 908 animals (329 sheep and 579 goats respectively). Samples were tested for antibodies to *Brucella* spp. by i-ELISA. All positive or suspicious positive samples were tested with Brucella-Ab c-ELISA.

Homogenous animal husbandry and management practises were observed with most farmers owning a small number of sheep and goats (<20) and the commingling of animals occurred frequently.

Inadequate use of vaccination, mixing of animals, common summer pastures, the high number of small households, and poor knowledge among owners about brucellosis were all risk factors for brucellosis present in several of the villages

The study showed evidence of infection among sheep and goats with B*rucella* spp. An overall seroprevalence of 9.5 % in the study area was observed. A large variation in seroprevalence between the districts and also between villages was seen, indicating a difference in spatial distribution of seropositive animals. A higher seroprevalence was observed in sheep than in goats and the majority of seropositive sheep and goats were between four and five years old.

SAMMANFATTNING

Det bergiga centralasiatiska landet Tadzjikistan är det minst gynnade landet ekonomiskt av länderna i det forna Sovjetunionen. Cirka 6.5 % av marken är odlingsbar, i ett land där cirka 80 % av hushållen främst äger ett mindre antal får och getter. Djurhållning bedrivs vanligen genom bland annat okontrollerad avel och sammanblandning av djur på betesmarker vilket således gör det lättare för spridning av smittsamma sjukdomar.

Brucellos är en zoonotisk bakteriell sjukdom som orsakas av *Brucella* spp. Sjukdomen är endemisk i Tadzjikistan. B. *melitensis* orsakar sjukdom primärt hos får och getter. Ett viktigt klinisk symtom är abort under den sista tredjedelen av dräktigheten.

Syftet med denna studie var att; 1) beskriva djurhållningen i periurbana byar runt huvudstaden Dushanbe. 2) Undersöka seroprevalensen av brucellos hos får och getter från små hushåll i fyra distrikt omkring Dushanbe.

Sera från 908 djur (329 får och 579 getter) insamlades och alla prover testades med avseende på antikroppar mot *Brucella* spp. med i-ELISA. Alla positiva eller misstänkt positiva prover konfirmerades med Brucella-AB c-ELISA.

En homogen djurhållning och skötselpraxis observerades. De flesta hushållen hade ett mindre antal får och getter(<20) och dessa blandades frekvent på byns gemensamma beten.

Bristfällig vaccinering, gemensamma sommarbeten, blandning av djur, hög antal hushåll med få djur samt dålig kunskap bland hushållen om brucellos var alla riskfaktorer för sjukdomen.

Seroprevalensstudien visade på att infektion hos små idisslare med *Brucella* spp var vanlig med en total seroprevalens på 9.5 %. En stor variation i seroprevalens mellan distrikten och bland byarna observerades, vilket indikerar en skillnad i spatial fördelning av seropositiva djur. En högre seroprevalens observerades hos får än hos getter. Majoriteten av seropositiva får och getter var mellan fyra och fem år gamla.

ABBREVIATIONS

c-ELISA	Competitive ELISA
ELISA	Enzyme Linked Immuno-sorbent Assay
FAO	Food and Agriculture Organization of the United Nations
i-ELISA	Indirect ELISA
KAP	Knowledge, Attitude and Practice
OIE	World Organization for Animal Health
SLU	Swedish University of Agriculture Sciences
TAU	Tajik Agriculture University
WHO	World Health Organization

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INTRODUCTION

Tajikistan

The Republic of Tajikistan is a Central Asian and former Soviet country, situated west of China, south of Kyrgyzstan and also borders Afghanistan in the south and Uzbekistan in the west. It is a mountainous country where the Pamir and Alay mountains dominate the landscape. Tajikistan experiences a midlatitude continental climate with hot summers and mild winters and in the Pamir Mountains semiarid to polar. Due to its mountainous landscape approximately 6.5 % of the land is arable and cotton is the most important crop. The country's major natural resource is hydropower and to less extent petroleum, uranium and other minerals.

The official language is Tajik which is closely mutual to the Persian language. With a population of nearly 8 million, approximate 80 % of the population is Tajiks, 15 % Uzbeks and the remaining 5 % consist of numerous nationalities. Russian is widely spoken particularly in the cities and is the preferred language used within the government and business sector. Approximately 26 % of the total population lives in urban settings and the rate of urbanization was in 2010 2.2 % annually. Islam is the dominating religion with 85 % Sunni Muslims and 5 % Shia Muslims. The major city is the capital Dushanbe, populated by approximately 700.000 inhabitants.

Tajikistan became independent in 1991 after the resolution of the Soviet Union. From 1992 to 1997 Tajikistan suffered a devastating civil war and consequently the already fragile economic infrastructure was damaged severely. Today, Tajikistan is the least advantaged country economically among the former Soviet Union states (CIA, World Factbook, 2012).

Figure 1. Map over Tajikistan (www.nationsonline.org/oneworld/map/tajikistan-political-map.htm (1997) political map of Tajikistan, 1:1000 000, info@nationsonline.org | Copyright © 1998-2013.

Importance of livestock in many low income countries

According to a report published 2006 by World Health Organization (WHO) it was estimated by various sources that there are approximately 500-900 million poor livestock holders worldwide. Livestock play a major part of their survival strategy thus healthy and well-managed animals can in many cases be a way out of poverty. It is usually women and children who are main responsible for taking care of small-stock herds thus providing an income that goes directly to women when meat, egg and milk are being sold. Economic marginalized people normally have few animals and will consequently be more exposed in case of illness or death among their animals. Poor households are also unlikely to afford treatment in case of illness and/or to have good access to veterinary services and healthcare information. The lack of veterinary means will therefore be, in many cases, at the expense of the ill animal and subsequently create an even more unstable economic situation for the households (WHO, 2006).

Livestock in Tajikistan

During the Soviet era most farms were state-owned and managed through a collective farming system. After Tajikistan gained independence in the early 1990s, subsistence farming is commonly practiced thus many state-owned farms transcended to a private ownership of animals. Approximately 80 % of the households own livestock, generally sheep and goats and a fewer numbers of cattle (Jackson *et al*., 2007). Over 90 % of all small ruminants in year 2009 were owned privately by smallholders (Ward *et al*., 2012). In each village, household flocks grazed together as a close unit for the most part of the year. Nevertheless it is still is not uncommon for many villages to yearly relocate livestock to high altitude summer pastures where flocks and herds graze jointly. Uncontrolled breeding, low reproduction rates, poor utilization of pastures and high mortality rates due to infectious diseases are all factors which reduces the productivity of the livestock (Jackson *et al*., 2007).

Figure 2. Pasture belonging to a village in Rudaki district (private photo).

Brucellosis

Brucellosis is a zoonotic bacterial disease caused by *Brucella* spp. and is primarily a disease of animals whereas humans are accidental hosts (Corbel *et al*, 2006). The disease is one of the most widespread zoonoses and is endemic in many countries including Tajikistan. It is also considered a neglected zoonosis by the WHO (WHO, 2006). There are six identified species and numerous biotypes. B. *melitensis* causes disease primary among sheep and goats and is also the most pathogenic for humans. The bacteria show a strong host preference although cross-species infections happen, particularly with B. *melitensis* (Corbel *et al*, 2006).

Clinical manifestation among humans is acute febrile illness which may persist and develop into a chronic disease with serious complications, such as joint illness, organ failure and symptoms of mental illness (Corbel *et al*, 2006; Quinn *et al*, 2002). The mortality rate is relatively low, especially when the patient is treated with adequate antibiotics; however this is not the case for everyone in low income countries (Corbel *et al*, 2006). In endemic countries like Tajikistan humans get infected mainly by drinking unpasteurized milk and/or exposure to aborted foetuses, placentas or infected animals (FAO, 2010).

Brucellosis affects many animal species but can cause several problems for livestock holders when the disease occurs among food-producing animals. Important clinical signs are; abortions usually during the last third of pregnancy, premature births, retained placenta, reduced fertility and lowered milk production. Epididymitis and orchitis in males are two important clinical signs. Correct diagnosis is reliant on isolation of the bacteria or detection of; genetic material, antigen, antibodies or cell-mediated immune responses since the clinical signs are not pathognomonic (Corbel *et al*, 2006).

The agent erythritol (polyhydric alcohol) is found in animal placental tissue but worth mentioning not in human placental tissue. Erythritol acts as a growth factor for *Brucella* spp. and promotes infection in placenta and foetus and often followed by abortion. The same agent can also be found in mammary glands and epididymis (Quinn *et al*, 2002).

Brucellosis in small ruminants

B. *melitensis* primarily affects small ruminants and while the bacteria shows strong host preference B. *melitensis* can also be found in other species, due to the bacteria's ability to cross-infect to other animal species. Transmission occurs mainly after abortion when the bacteria can be found in fluids and tissues connected with pregnancy like the placenta, dead foetuses and the udder (Corbel *et al*, 2006; OIE, 2009).

B. *melitensis* is highly pathogenic and infection in sheep and goats create a great risk for people living in close vicinity to their animals to contract the disease. Animal owners generally mix small ruminant from different herds to higher extent than cattle and the density of the flocks and

herds causes a high number of bacteria shed in the environment and consequently generate a main route for animal-to-animal transmissions (Corbel *et al.*, 2006; FAO, 2010).

The disease in goats is usually more severe and prolonged than in sheep due to the fact that the susceptibility to B. *melitensis* is generally higher in goats compared to in sheep (Quinn *et al*, 2002).

Small ruminants; prevention and control

The B. *melitensis* REV 1 vaccine is an attenuated strain of B. *melitensis* and an effective method to reduce the prevalence of brucellosis among whole flocks or herds in low income countries and/or endemic countries (Corbel *et al*, 2006; OIE, 2009).

In unvaccinated animals abortions are more prevalent and the amount of bacteria shed from birth fluids and tissues are much greater than in vaccinated animals. Previous stated enhances the importance to vaccinate small ruminants especially in low income countries (Corbel *et al*, 2006) where Brucellosis causes substantial economic losses due to abortions, reduced fertility and lowered milk production in livestock (WHO, 2006).

Conjunctival administration with REV 1 has shown to be a well-applied and an effective method to acquire fast herd immunity. With conjunctival vaccination a strong antibody response is induced but does not induce persistent antibody response. Subcutaneous vaccination with REV 1 however induces a long lasting serological response which interferes with serological testing (Corbel *et al*, 2006; OIE, 2009).

When using serological tests it is important to take vaccination status into consideration given that there are currently no serological tests that could differentiate vaccinated sheep and goats from natural infected animals (Blasco & Molina-Flores, 2011; OIE, 2009). A study conducted by Stournara et al. (2007) indicated that it is not recommended to test conjunctively vaccinated adult ewes due to low specificity with Indirect Enzyme Linked Immunosorbent Assay (i-ELISA) for at least 330 days post vaccination. The study also illustrated that additional research is needed to find correct cut-offs for competitive Enzyme Linked Immunosorbent Assay (c-ELISA) or i-ELISA especially in areas where vaccination of small ruminants with Rev 1 is practiced (Stournara *et al*, 2007).

Brucellosis in Tajikistan

Brucellosis in small ruminants was rather well controlled in the Soviet times mainly by a government-owned test and slaughter programme and vaccination (Jackson *et al*, 2007). Since Tajikistan became independent from the Soviet Union in 1991, the veterinary service has diminished which has had a deleterious effect on animal health and production. Uncontrolled movement of livestock, numerous small farm units and lack of funding within the veterinary

sector which makes it more difficult to maintain a sufficient disease control program are believed to be the main reasons for this development (Magnusson *et al*, 2005).

Since brucellosis is such an important zoonotic disease worldwide affecting human welfare, livestock health and food security it is of great importance to map out its expansion (FAO 2010).

Introduction to the study

In order to get a clearer view over the brucellosis situation in endemic countries, it is important to have recently updated serological data. Knowing the extent and spread of the disease among the sheep and goats will obviously be helpful when control and eradication programmes will be implemented.

Cross-sectional serological studies were conducted in year 2003 and in 2009 in approximately 12.500 small ruminants from different districts in Tajikistan. The aim of these studies was to assess the efficiency of control based biannual conjuctival vaccination with Rev 1[1]. In districts where vaccination was well implemented the seroprevalence was reduced by 80 % in five years. The prevalence on household level with evidence of infection in their small ruminants also decreased from 25.1 % to 7.5 %. In districts where no vaccination was carried out no changes in seroprevalence were to be seen. The positive results from mass-vaccination of sheep and goats illustrate the importance of well managed vaccination programmes together with serological testing (Ward *et al*, 2012).

According to oral information given by local authorities, the latest vaccination campaign among small ruminants in districts neighboring the capital Dushanbe were conducted in year 2010.

The current study was conducted in cooperation with the Tajik Agrarian University (TAU) in Dushanbe. The seroprevalence study took place concurrently with a KAP-study (Grahn, 2013) whose study included the same animal owners that participated in the current study.

Objectives of the study

1. To describe the farm structure of peri-urban located villages around Dushanbe, Tajikistan.

2. To investigate the seroprevalence of brucellosis in sheep and goats from small households in peri-urban villages in four districts; Varzob, Gissar, Rudaki and Vahdat around the capital Dushanbe.

[1] Full-strenght (1 x 10^8) quality-assured Rev 1 *Brucella melitensis* live attentuated vaccine (BRUCEVAC, Jordan Bio-Industries Center (JOVAC); CZV REV 1 CZ Veterinaria (Ward *et al*, 2012)

MATERIAL & METHODS

Study area

The study was conducted in 2012 during October till November in peri-urban located villages surrounding the capital Dushanbe. The villages were, in advance, partly randomly selected while others were chosen by convenience. The aim was to get an even distribution through the city and also the location of the villages were preferably not to be located more than approximately 30 km away from Dushanbe. All villages were located in the four districts; Varzob, Gissar, Rudaki and Vahdat neighboring the capital Dushanbe. Coordinates were collected with a hand-held global position system (GPS) receiver at every visit. Single households were selected randomly in each village upon arrival. All animals tested were currently on pastures close to their villages and due to collective grazing, trading and mixing of animals, every village were considered to be one epidemiological unit.

Study population

All sheep included in the study were fat-tailed sheep of Gizar breed and all goats included in the study were local Tajik breed (N. Sattorov, personal communication). All sheep and goats were privately owned. The unit of interest was female sheep or goat older than six months. A motive for selecting sexually mature animals is due to the higher susceptibility to brucellosis in this category of livestock. Young animals are usually resistant, however it is possible for young animals to have a latent infection and thus become an eventual source of infection when mature (Corbel *et al*, 2006).

Figure 3. Gizar sheep on their way to pasture, (private photo).

Figure 4. Tajik local breed on pasture (private photo).

Study design and sample collection

The sampling strategy in each village was to sample sheep and goats from an even distribution of households in the four districts rather than to collect numerous samples within one small geographical area. The aim was to include a minimum of 400 animals per species to estimate the seroprevalence of *Brucella* spp. on individual level with an expected prevalence of 5%, a confidence interval (CI) of 95 % and a desired absolute precision of 5 %.

An even spreading of villages around the city was also prioritized instead of collecting numerous samples within one village. Official numbers of sheep and goats within the four chosen districts were approximately 300.000 (N. Sattorov, personal communication).

If one or several households opposed having their animals tested the sampling team proceeded to the next village within the same district in order to get an even distribution within the district.

Descriptive data collected at the same time were; the total numbers of sheep and goats owned by each household, species, age, pasture type and vaccination status of the tested animals.

Blood-samples were collected from the jugular vein in sterile tubes. Each sample was individually identified by district, village, species, and age and vaccination status.

Collected blood samples were kept in a cool-box and transported to the laboratory at the Tajik Agrarian University in Dushanbe where the serum were removed and kept in freezer until analyzed.

Serology

All samples were tested for antibodies to *Brucella* spp. by i-ELISA. All positive or suspicious positive samples were tested with Brucella-Ab c-ELISA according to recommendations from the World Organization for Animal Health (OIE, 2009). All analyses were made according to instructions from the manufacturers (Svanova Biotech AB, Uppsala, Sweden). Both negative and positive controls were included in every assay. Each control and samples were run in duplicates for both the i-ELISA and c-ELISA. Each ELISA-plate had to pass the validation criteria set up by the manufacture for a valid result.

Statistical analysis

The data was entered in Excel (Microsoft) and analysed with descriptive statistics on district and village level in Excel (Microsoft). Relevant individual factors such as age, species and vaccination status are described in order to see any associations between serological results and the animal factors mentioned above. To describe the farm structure in the villages concerned,

analyses concerning total number of sheep and goats owned by each household were being included.

RESULTS

Farm structure and sampling

The study involved 97 households from 22 villages. Approximately 4 households (range two to eight) were selected in each village.

Management practices and animal husbandry in the villages studied were highly homogenous. The majority of the owners had a small number of sheep and goats kept for meat production and all animals were privately owned. The average herd size was 18.5 sheep and goats in total (range three to 130) and the number of sheep and goats owned by the 97 households was approximately 1795 in total. Among these 1795 sheep and goats, 908 females were tested (table 1).

Sheep and goats from different households grazed jointly together during the day and housed at night, usually in cramped conditions. Most households owned both sheep and goats. Approximately 93 % of the households owned at least one cattle together with their sheep and/or goats (Grahn, 2013).

Sera were collected from 908 animals (329 sheep and 579 goats respectively).

On average, 227 (range 212 to 248) blood-samples from each district were collected in five to six villages within each district.

The average number of sheep tested per district was approximately 80 (range 51 to 125). The average number of goats tested per district was 145 (range 95 to 185).

The average age of tested sheep and goats were for both species 3.5 years (range 0.5 ≥ to ≥7 years).

Table 1. Total number of sheep and goats (n=1795 from 97 households, total number of tested sheep and goats and total proportion (50.6 %) of above-mentioned

Species	Total no. of sheep and goats in the 97 households	No. of tested sheep and goats from selected households	Percentage of sheep and goats tested from selected households
Sheep	809	348	43.0
Goats	986	560	57.0

Seropositivity to *Brucella* spp.

The overall seroprevalence for brucellosis among sheep and goats were 19.4 % (n = 176) on i-ELISA.

After confirmation with c-ELISA, 86 sheep and goats tested positive resulting in a seroprevalence of 9.5 % (n=86) (figure 5).

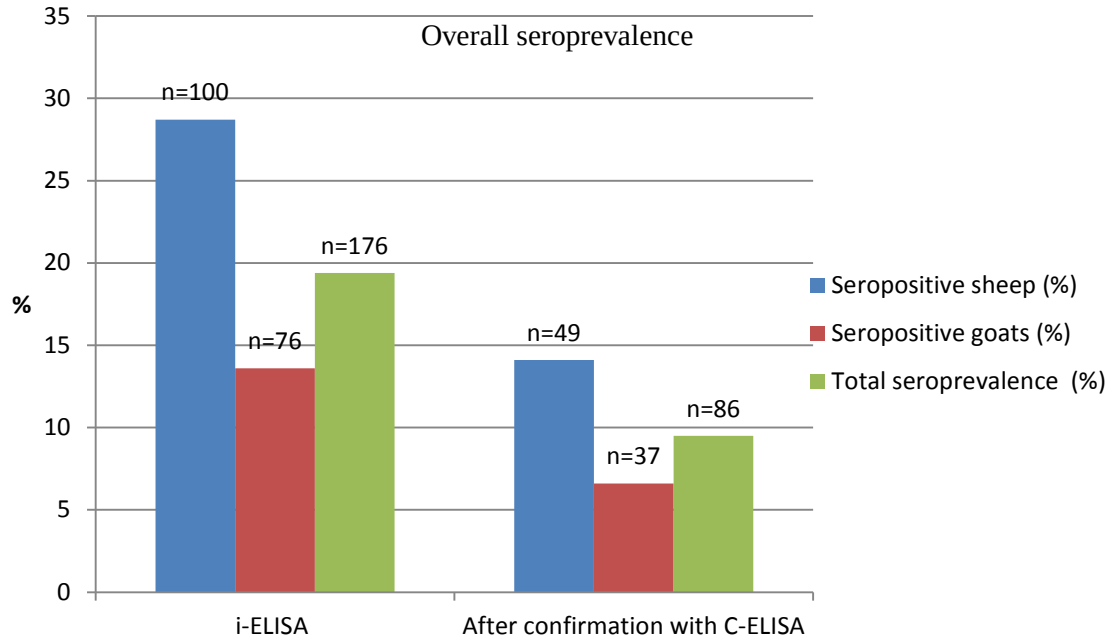

Figure 5. The overall seroprevalence in sheep and goats and total seroprevalence. A total no. of 908 samples (sheep n=348 and goats n=560) were tested with i-ELISA (Svanova, Biotech, Uppsala, Sweden) and seropositive samples (n=176) were tested with c-ELISA for confirmation.

All serological results presented as from now on are based on c-ELISA confirmed seropositive animals.

The average age of seropositive sheep (n=49) was 4.8 years and the average age of seropositive goats (n=37) was 4.7 years (range 0.5≥ to ≥7 years) (figure 6).

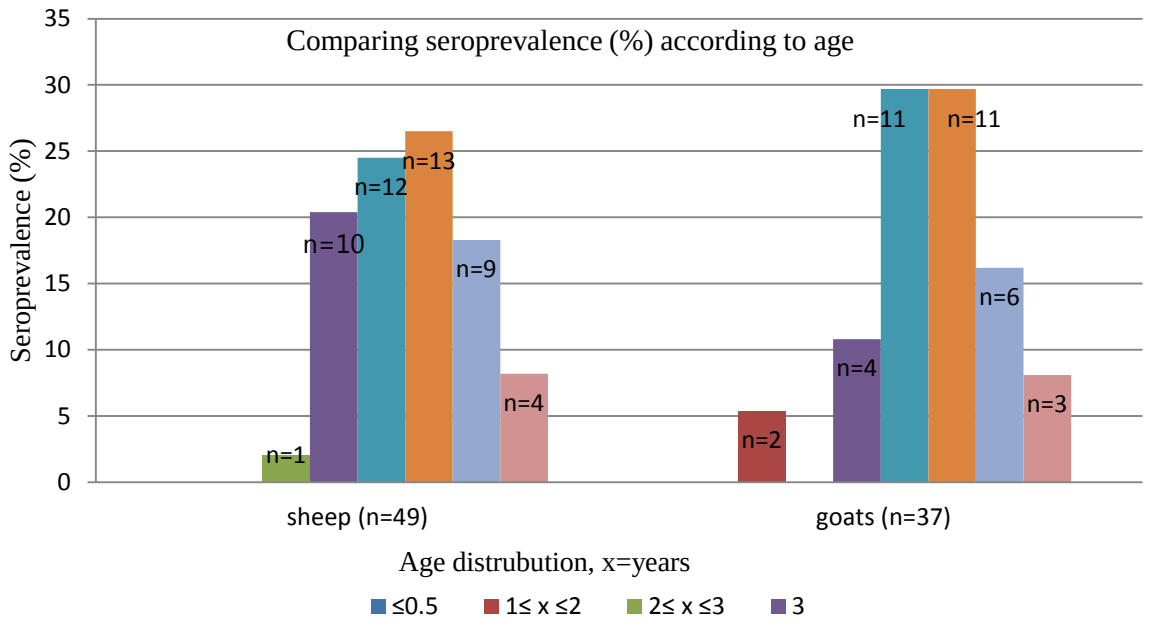

Figure 6. Comparing seroprevalence (%) of c-ELISA (Svanova, Biotech, Uppsala, Sweden) positive sheep (n=49) and goats (n=37) according to age.

District level

The total seroprevalence amongst seropositive sheep and goats in studied districts varied from 7% to 45 % (figure 7).

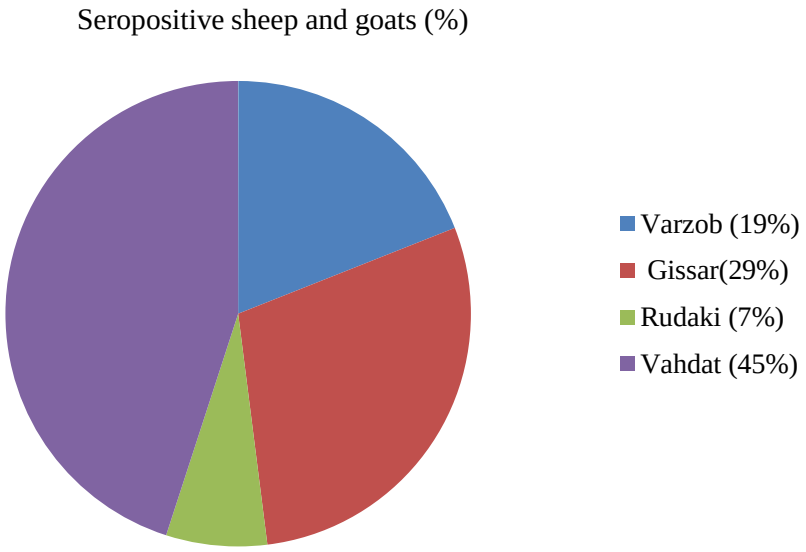

Figure 7. Distribution of seropositive sheep and goats on c-ELISA (Svanova, Biotech, Uppsala, Sweden) from seropositive samples (n=86) per district.

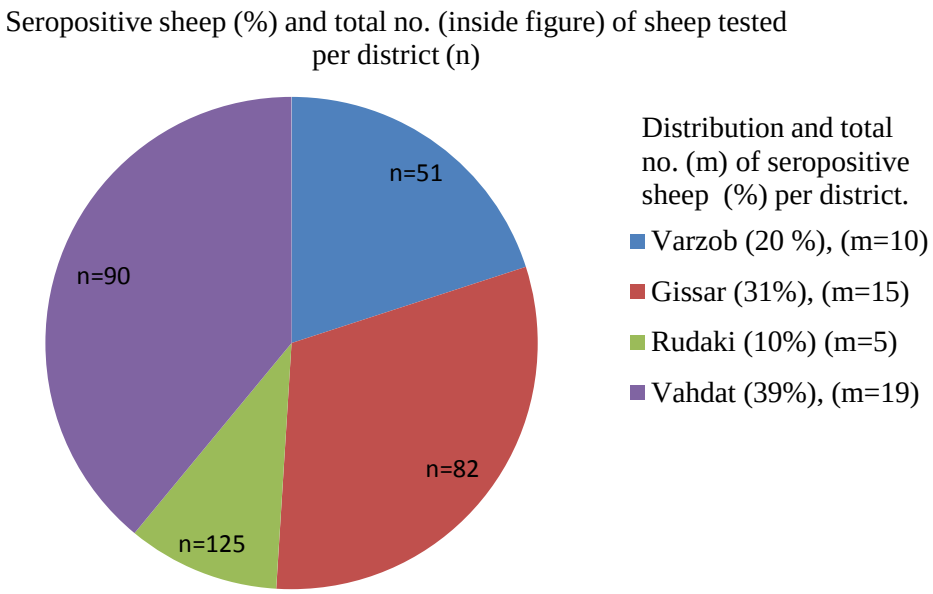

Figure 8. Distribution of seropositive sheep (m=49) on C-ELISA (Svanova, Biotech, Uppsala, Sweden) shown in percentage and numbers and total no. of sheep tested per district.

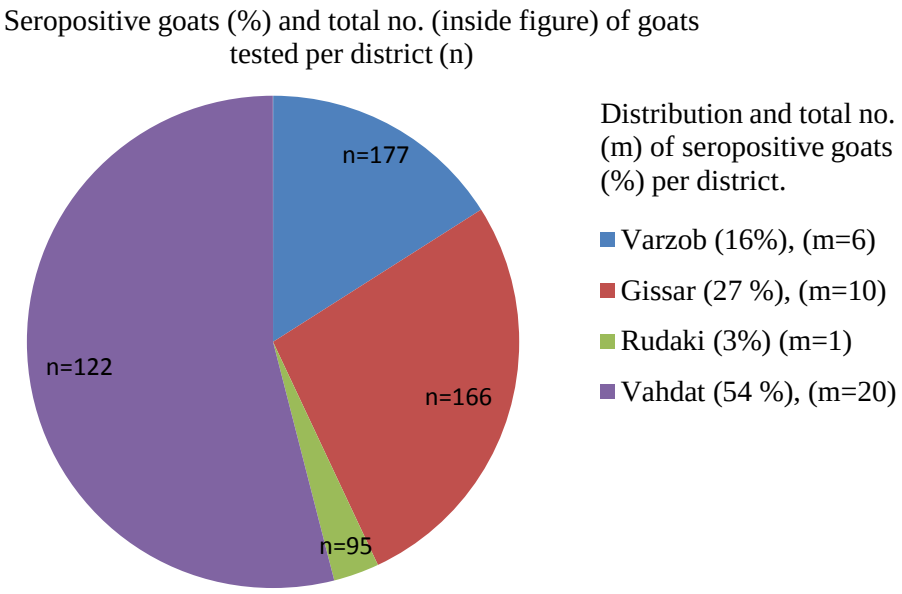

Figure 9. Distribution of seropositive goats (m=37) on c-ELISA (Svanova, Biotech, Uppsala, Sweden) shown in percentage and numbers and total no. of goats tested per district

Village level

Out of all the 22 villages, all except seven had seropositive sheep and goats thus giving a seroprevalence on village level of 68.2 %.

The individual prevalence of seropositive sheep and goats varied from 0 % to 31.7 % amongst different villages.

Distribution of seropositive villages and seronegative villages shown per district (table 2).

Table 2. Comparing seroprevalence between villages in the different districts

District	Number of villages tested per district	Number of villages with at least one seropositive sheep or goat.	Proportion (%) of villages with seropositive sheep or goats
Varzob	6	3	50
Rudaki	5	3	60
Gissar	6	4	66.7
Vahdat	5	5	100

DISCUSSION

The objectives of this study were primarily to describe the farm structure of peri-urban located villages in four districts around Dushanbe and to investigate the seroprevalence of *Brucella* spp among sheep and goats in the same area.

All animals in the study were kept under very similar conditions with joint pasture, where animals in each village were to a great extent mixed together during daytime. Most owners had a small number of sheep and goats and the majority (93 %) of the households owned at least one cattle together with their sheep and/or goats (Grahn, 2013). When the study was conducted, all animals were on pasture close to their villages.

The study showed evidence of infection among sheep and goats with B*rucella* spp. within the study area. Since *B. melitensis* practically always is the infecting species in sheep and goats (Corbel *et al.,* 2006) it is most likely that the seropositive animals had been infected with *B. melitensis*.

Brucellosis is a disease with several routes of transmission. There are numerous risk factors which are stated previously for spreading the disease within the animal population and animal-human transmission (FAO, 2010). In this study approximately 93 % of the owners owned cattle (Grahn, 2013). Drinking unpasteurized dairy products is a common way for animal-human transmission. Unfortunately, *B. melitensis* has the ability to establish itself in other species and the practice of keeping cattle together with small ruminants subsequently heightens the risk for humans (FAO, 2010).

As mention previously by Magnusson et al. (2005), Jackson et al. (2007), FAO (2009) and Ward et al. (2011), brucellosis in small ruminants can be attributed to lack of funding within the veterinary service, high prevalence of unvaccinated animals and a husbandry system (uncontrolled movement of animals, trading and mixing of animals and common pastures). The farm structure in concerned villages much illustrates the above-mentioned risk factors especially regarding the management practices of livestock.

The sampling strategy with a 95 % confidence that infection with *Brucella* spp. would be detected with an estimated prevalence of 5 % or more was fulfilled on district level. This aim was to a lesser degree achieved on village level when an even distribution of villages around the city was prioritized in front of collecting numerous samples within one village.

The study showed that evidence of infection was common, i.e. an overall seroprevalence of almost 10% after confirmatory testing. The study also demonstrates an unevenly distributed seroprevalence among different districts and villages. On district level the seroprevalence amongst seropositive sheep and goats varied from 7 % in Rudaki to 45 % in Vahdat. Causes for the differences in seroprevalence seen on district level are certainly due to various factors. It is possible that the risk factors stated previous were/are more widespread in Vahdat district than in

Rudaki district. However, this result highlights the need for more extended studies on risk factors and further interventions in areas with high seroprevalence.

All villages except seven had seropositive sheep and/or goats thus give a village-prevalence of 68.2 %. There is an uneven distribution of seropositive animals between villages with an animal-prevalence ranging from 0 to 31.7 %. The high overall prevalence and particularly in villages with a very high seroprevalence shows a heightened risk for transmission to humans and animals alike (Jackson et al., 2007).

The different result between seropositive animals when using i-ELISA and confirming the i-ELISA positive samples with the c-ELISA were to some degree notable and various reasons for the difference can be debated but will not be discussed in detail in the current report. However the result raises several interesting questions. The study *"The diagnosis of brucellosis in sheep and goats, old and new tools"* by B. Garin-Bastuji et al. (*2006)* mentions that animals infected with cross-reacting bacteria can lower the specificity when using i-ELISA.

Oral information given to the field team by the owners and/or local veterinarian regarding vaccination status revealed that the majority of the owners reported that none of their sheep and goats ever been vaccinated against brucellosis. This contradicts the information given by local authorities that the latest vaccination campaign (conducted in 2010) included all small ruminants in districts neighbouring the capital Dushanbe. The co-current study conducted at the same time (Grahn, 2013) showed a clear lack of knowledge about brucellosis especially when it came to identify the clinical picture in animals. Furthermore answers from the KAP-study and the information collected during sampling regarding current vaccination status among sheep and goats were very confusing. Seven out of the 86 c-ELISA positive animals had been vaccinated against brucellosis in 2010 according to the owner or local veterinarian. Nevertheless vaccinated animals showed generally no heightened serologic response compared to other seropositive animals.

There are few studies concerning antibody response after vaccination with Rev 1 in small ruminants. The study "*Assessment of serological response of young and adult sheep to conjunctival vaccination with Rev 1 vaccine by fluorescence polarization assay (FPA) and other serological tests for B. melitensis*" conducted by Stournara et al. (2007) indicated that it is not recommended to test conjunctival vaccinated adult ewes due to low specificity with i-ELISA for at least 330 days post vaccination.

The current study took place approximately two years since the latest vaccination campaign and therefore seropositivity is considered to be caused by natural exposure to *Brucella* spp. Further research is needed on the subject as the present study included a relative small population of animals.

A higher seroprevalence was observed in sheep (14.1 %) than in goats (6.6 %). The current result is interesting when considering that the disease in goats usually is more severe and prolonged

than in sheep due to higher susceptibility to *B. melitensis* in goats (Quinn et al., 2002). Notably, more goats than sheep were tested in this study. It is interesting to speculate if the result is merely a coincidence or if there are other influencing factors. Corbel et al. (2006) mention that breed, particularly sheep of milking breed, could be a factor when it comes to susceptibility to *B. melitensis*. However it is also stated that management practices are much more important when to evaluate the risk of infection. All sheep tested for this study were of Gizar breed and were usually kept for meat consumption. Furthermore latent infections among sheep have been documented and such animals could be seronegative until first abortion or parturition (Corbel et al., 2006).

The study "*Brucellosis control in Tajikistan using Rev 1 vaccine: change in seroprevalence in small ruminants from 2004 to 2009"* by Ward et al. (2012) showed no difference between seroprevalence between non-vaccinated sheep and goats. Also, vaccinated sheep and goats were more likely to be seropositive compared to non-vaccinated animals. Animals included in the study could have been vaccinated from five years to the last vaccination campaign just over four months before testing. However, an effort was made to exclude sampling sheep and goats from the most recently vaccination campaign.

In the current study, the average age of tested sheep and goats were for both species 3.5 years (range 0. 5 ≥ to ≥7 years). The average age of seropositive sheep was 4.8 years and 4.7 years for goats. The present findings coincides with previous studies that sexually mature animals have higher susceptibility to brucellosis than younger animals who are usually resistant (Corbel et al., 2006). Reasons for adult animals to be more susceptible to infection could perhaps be explained by the fact that erythriol and sex hormones usually increase in concentration with age and sexual maturity. The same components also stimulate bacterial growth and multiplication (Radostits et al., 2000)

Finally, management practices and husbandry in the villages studied were highly homogenous. Vaccination is the most effective method for prevention. To reduce the prevalence of infection, wherever the disease is endemic and widespread mass-vaccination of whole herds can be recommended. However education of the population regarding brucellosis including risk-factors concerning transmission can be useful to reduce the impact of the disease (Corbel et al., 2006). The need for more information and education were clearly shown in co-current KAP-study (Grahn, 2013). The knowledge among owners and village veterinarians concerning brucellosis were very inadequate in general.

CONCLUSIONS

- Inadequate use of vaccination, mixing of animals, common summer pastures, the high number of small households, and poor knowledge among owners about brucellosis were all risk factors for brucellosis present in several of the villages.

- Infection with *Brucella* spp. is present and widespread among sheep and goats within the study population, especially in some of the investigated villages, indicates high risk for transmission to humans and animals alike.

- There was a large variation in seroprevalence between the districts. The seroprevalence for brucellosis was highest in Vahdat district and lowest in Rudaki district, indicating a difference in spatial distribution of seropositive animals.

- A higher seroprevalence was observed in sheep than in goats.

- The majority of seropositive sheep and goats were between four and five years old.

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