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Epidemiology, cost and surveillance of brucellosis in people and livestock of Kyrgyzstan

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List of abbreviations

AT	Tube Agglutination Test
CAR	Central Asian Region
CFT	Complement Fixation Test
CIS	Commonwealth of Independent States
DALY	Disability-Adjusted Life Year
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked Immunosorbent Assay
EPH	Department of Epidemiology and Public Health
FAO	Food and Agriculture Organisation
FMD	Food and Mouth Disease
FPA	Fluorescence Polarization Assay
FSU	Former Soviet Union
GIS	Geographic Information System
HAHU	Human and Animal Health Unit
KGS	Kyrgyz local currency (Som)
LDPS	Livestock Development Planning System
MALDI–TOF	Matrix-Assisted Laser Desorption Ionization–Time of Flight
MLVA	Multiple Loci VNTR Analysis
MoA	Ministry of Agriculture
MoH	Ministry of Health
MS	Mass spectrometry
OIE	World Animal Health Organisation
PCR	polymerase chain reaction
PPR	peste des petits ruminants
RBT	Rose Bengal test
SVD	State Veterinary Department
Swiss TPH	Swiss Tropical and Public Health Institute
TB	Tuberculosis
UPGMA	Unweighted pair-group method with arithmetic averages,
USD	United States Dollar
VNTR	Variable Number of Tandem Repeats
WHO	World Health Organization

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2. Summary

Brucellosis is a livestock disease which is also transmissible to humans and thus it is of major public health concern. Brucellosis is considered as a major zoonotic disease of public health importance worldwide. However, its prevention and control poses a number of problems to national authorities, particularly to the Veterinary Services and the Public Health sector. The prevalence of brucellosis in Kyrgyzstan is one of the highest worldwide and has been increasing for animals and humans in recent years.

Currently, there is very limited understanding of brucellosis transmission both between livestock species and to humans at the national level. It is important to understand the main transmission routes in order to establish a control strategy of this zoonosis. Brucellosis can ultimately be eliminated only if the disease is controlled in the animal reservoir since animal and human health is inextricably intertwined. It is therefore necessary to consider human and animal health strategies as two aspects of the same aim.

The goal of the current study was to describe the distribution and the transmission dynamics of brucellosis in Kyrgyzstan and to determine its impact on livestock production and public health. The results of the study should contribute to the development of an efficient brucellosis control strategy in Kyrgyzstan.

The specific objectives are: 1) a historical review and analysis of brucellosis control measures used in Kyrgyzstan; 2) a representative sero-survey of brucellosis prevalence for humans and animals; 3) assessment of molecular epidemiology of animal and human brucellosis in Kyrgyzstan; 4) brucellosis cost estimations for livestock owners, brucellosis patients and society; 5) assessment of the potential of abattoirs for brucellosis surveillance; 6) evaluation of the current mass livestock vaccination campaign and promotion of effective brucellosis control in Kyrgyzstan.

This research has been carried out within an interdisciplinary study with the participation of different projects and operational teams involving veterinarians, health workers, epidemiologists, molecular biologists, and laboratory and field veterinary professionals. The study included: serological studies for humans and animals (2006, 2007 and 2012) and the molecular characterisation of *Brucella* cultures isolated from aborted foetuses of cattle and sheep, (2007-2011) as well

as surveillance of abattoirs (2012) and the survey of patients through questionnaires (2013). Based on the collected data, a cross-sector estimation of the societal cost of brucellosis was done. Livestock demographic models were used to estimate the losses in the livestock production. Health provider and patient information was used to estimate the public health costs. Abattoir surveillance was tested for its usefulness to estimate vaccination coverage of brucellosis and the prevalence of PPR.

A national representative cross-sectional study using cluster sampling proportional to size tested a total of 4,936 livestock sera and 1,774 human sera. The overall apparent seroprevalences of brucellosis were 8.8% in humans (95% CI 4.5–16.5), 2.8% (95% CI 1.6–4.9%) in cattle, 3.3% (95% CI 1.5–6.9%) in sheep, and 2.5% (95% CI 1.4–4.5%) in goats (Bonfoh et al., 2012). To confirm the circulating strains of *Brucella* in Kyrgyzstan, aborted foetuses were collected in Naryn oblast for the strain isolations. Overall, 17 B. *melitensis* strains were isolated from aborted foetuses of sheep and cattle. Multilocus variable number tandem repeat analysis showed low genetic diversity. Kyrgyz strains seem to be genetically associated with the Eastern Mediterranean group of *Brucella* global phylogeny. We identified and confirmed transmission of B. *melitensis* to cattle and a close genetic relationship between B. *melitensis* strains isolated from sheep sharing the same pasture (Kasymbekov et al., 2013).

We developed a demographic model for livestock and estimated the livestock productivity taking into consideration the real cost of disease and accurate calculations of final losses in the livestock productivity. The losses for Kyrgyzstan were estimated for the period from 2006 to 2011 considering the seroprevalence of brucellosis: 2.8% in cattle, 3.3% - in sheep and 2.5% - in goat.

The societal cost of estimate of brucellosis to Kyrgyzstan includes the cost of public and private health and the livestock production system costs. We developed a demographic model for livestock to estimate cost of disease with and without brucellosis.

Net present cost of brucellosis to the public health sector (2006 – 2011) was estimated at 1.38 million USD (95% CI 1.22–1.55) and the private net present health cost was 6.02 million USD (5.5- 6.5). The overall net present health cost was 23.0% of the societal net present cost of 32.5 million USD (25.7– 39.6). For 2006-2011, losses of the net present value were 13.7 million USD (7.1 – 20.7) for cattle, 0.78 million (0.49 – 2.0705) for sheep and 0.75 million (0.08 – 1.43) for goat products. The incremental asset value was estimated at 2.66, 1.63 and 0.11 million USD for cattle, sheep and goats, respectively. We carried out an abattoir and field study on brucellosis and PPR sero-surveillance. Our finding of field prevalence for brucellosis was in a similar range to the abattoir prevalence. Abattoir prevalence in the area under the study made up 9.8% (95% CI 8.0 - 11.5%) and brucellosis seroprevalence in the field studies made up 10.7% (95% CI 8.9 -12.6%). When the abattoir prevalence was adjusted to the national population structure, the brucellosis seroprevalence made up 10.4% (95% CI 8.6 – 12.2%).

However the PPR prevalence was lower in the field when compared to abattoir surveillance. Field surveillance is two times more expensive than abattoir surveillance. For certain cases, abattoir surveillance is feasible and sufficiently accurate when compared with field surveillance.

The abattoir surveillance was predictive for brucellosis field prevalence when adjusted to the national demographic composition but cannot be used to estimate vaccination coverage without good traceability systems at the slaughterhouses.

3. Кириш сөз

Бруцеллез малдан адамга жугуучу ылаң жана дүйнөдө адам азтына салымдуу зыян алып келе турган дарт деп белгиленген. Ошондой эле бул дарт менен күрөшүү жана алдын алуу улуттук мекемелерге, негизинен мал-чарбачылына жана саламттык сактоого чоң кыйынчылыктарды алып келет. Кыргызстанада адамдардын бруцеллезу салыштырмалуу, дүйнөдө эң жогорукулардын арасында деп табылган жана акыркы жылдардын ичинде көбөйгөндүн үстүндө болгон.

Азыркы маалда бруцеллездун таралышынын жолун жана малдан-малга жугуу жолун түшүнүп билүү жолу чектелген. Ылаңдын алдын алууда же дарт менен күрөшүү стратегиясын кабыл алууда жогорудагы себертерди билүү маанилүү.

Мал менен адам тыгыз байланышта болгондуктан алардын ден-соолугун бирдей кароо керек, , ошондуктан дарттын очогун жана дартты контролдоо менен бруцеллезду азайтуу мүмкүнчүлүгү пайда болот. Мунун негизинде мал менен адамдын ден-соолугунун стратегиясын бир максаттагы эки аспект караты кароо керек.

Бул изилдөөнүн долбоорунун максаты катары бруцеллездун Кыргызстандагы таркоо динамикасын жана малчарбачылыгы менен саламаттык сактоого келитрген таасирин мазмуундоо болуп эсептелет. Изилдөөнүн натыйжасында бруцеллезго каршы эффективдүү стратегия иштеп чыгууда салым кошот деп ишенебиз.

Изилдөөнүн негизги максаттары: 1) Бруцеллез тууралуу тарыхый маалымат жана бруцеллез менен күрөшүүдө пайдаланылган ыкмаларды талдоо; 2) адамдын жана малдын бруцеллезунун таркашын серологиялык изилдөө; 3) Кыргызстандагы малдын жана адамдардын бруцеллезун молекулярдык эпидемиологиясы; 4) бруцеллезду контролдоого, бруцеллездун мал ээлерине жана коомго келтирген чыгымдарын эспетөө; 5) бруцеллезго көзөмөл кылуу үчүн мал сою жайларынын абалын мүнөздөө; 6) учурда болуп жаткан жалпы эмдөө компаниясын жана бруцеллезду контролдоодогу жылыштарын тастыктоо.

Бул изидөө ар-тармактагы ар кандай долбоорлордун жана ошондой эле саламаттык сактоо кызматкерелинин, эпидемиологдордун, молекулярдык биологдордун, лаборатория кызматкерелинин жана мал доктурлардын катышуусу менен аткарылды.

Изилдөөдө серологиялык тастыктоонун (2006, 2007, 2012), молекулярдык изилдөөнүн – козу жана музоо салгандан чогултулган матриалдардын негизигдеги культараларды тибин талдоо (2007-2011), мал сою жайларынын изилдөө (2012), ыландаган оорулардын жоопторунун (2013) негиздери алынган.

Изилдөөнүн негизинде иштеп чыккандар: малдын демографиялык түзүлүшү; эмдөөнүн камтылышы; бруцеллез менен ыландаган жана ылыңдабаган мал чарбачылыгынын экономикалык чыгаша-кирешелери. Булардан сырткары изилдөө учурунда майда жандыктардын “кыргыныны” жана эчкилердин жугуштуу плевропневмониясы тастыкталган..

Изилдөөнүн жыйынтыктары. Буга чейинки серологиялык изилдөө (2006-2011) көргөзгөндөй Кыргызстан эндемикалык болуп эсептелип, бодо малда - 2.8%, койдо - 3.3% жана эчкиде 2.5% көргөзгөндүгү (Bonfoh et al., 2012), кийинки молекулярдык изилдөө менен тастыкталды, ошондой эле бодого койдон *B. melitensis* жуккандыгы аныкталды (Kasymbekov et al., 2013).

Бруцеллездун мал чарбачылыгына келтирген чыгымын эсептөө үчүн эки бөлөк моделдеги эки башка ыкмада баалосу сунушталган.

Эсептөөдө төмөнкү бааны алыш үчүн биз 2006 жылдын бааларын алдык жана ошол тапта акча алмушуу курсу 1 АКШ долларына 41.3 сомду түзгөн.

Жалпы коомго келтирилген чыгым 1.3 миллиард (95% МА 1.06 – 1.63 миллирад) сом, саламаттыкты сактоого 306 млн (95% МА 279.5 – 332.3 млн), анын арасынан 248.7 млн (95% МА 228.9- 268.1 млн) жеке жана 57.3 млн (95% МА 50.5 – 64.0 млн) сом коомдук саламаттыкты сактоого тиешелүү болгон. Жеке менчиктин кирешеси 385.2 млн (95% МА 381.1 – 390.0 млн) сомго зыян тартып, изилдөө мөөнөтүндө (2006-2011) мал чарбачылыгында 635.7 млн сом, анын ичинен бодо малга 572.3 млн (95% МА 291.1 – 857.6 млрд), кой чарбасына 32.1 млн (95% МА 20.2 –84.8 млн) жана эчки чарьасына 31.2 миллион сом (95% МА 3.5 -59.0 млн) чыгым келтирилген деп эсептелген.

Биз мал чарбачылыгына карта демографиялык модель иштеп чыктык жана мал чарбачылыгына келтирилген накталай зыянды эсептептедик. Мал чарбасынын продукциясы боюнча бодо малдын наркы 32.5 млрд (95% МА 23.8 – 37.8 млрд), кой чарбасы 10.0 млрд (95% МА -7.77 – 12.25 млрд) жана эчки чарбасынын наркы 1.01 млрд (95% МА 801.5 млн – 1.2 млрд) сомду түзгөн.

Салыштырмалуу үй чарбачылыгын активдуу көзөмөлгө алуу мал союлуучу жайдын көзөмөлүнө кеткен каражаттан эки эседен жогору болот. Ылаңдын жайылышын изилдөө катарында үй чарбасын мал союлуучу жайларга салуштыруу ирээтинде жүргүзүлгөн изилдөөдө биздин жыйынтык болуп мал союлуучу жайда бруцеллез 9.8% (95% МА 8.0 – 11.5 %) жана үй чарбасында 10,7% (95% МА 8,9 -12,6 %) болду. Мал союлуучу жайдын структурасын өлкөдөгү жалпы малдын структурасына тууралаганда ылаңдын жайылышы 10,4% (95% МА 8,6 - 12,2%) түздү. Ал эми майда жандыктын “кыргынын” изилдегенде мынчалык окшош болгон жок, буга себепкер элдин жашоо турмушу же мамлекеттин өнүгүшү болсо керек, анткени биздин изилдөөдө ыландаган мал союшка же сатыкка кетсе, ал эми өнүккөн мамлекеттерде ден соолугу таза гана мал союлат экен деген тыянакка келдик.

Негизинен мал союлуучу жайларды улуттук жалпы малдын структурасына тууралаca мамлекеттеги ылаңдын жайылгындыгын алдын ала айтууга болот, бирок ушул эле учурда мындай көзөмөлдөө малдын идентификациясы болбосо малдын эмделгендигин билүү үчүн пайдалануу кыйынчылыкка турат деген чечимге келдик.

Эгерде бул кыска малымат түшүнүксүз болсо, англис тилинде жазылган маалыматты караңыз, анткени диссертацианын негизи жана так маалыматы англис тилинде берилген.

4. Резюме

Бруцеллез заболевание животных, передающееся человеку, следовательно, является одним из основных проблем здравоохранения и ветеринарной службы. Бруцеллез был определен в качестве основного зооноза для общественного здравоохранения во всем мире. Тем не менее, его профилактика и контроль создает ряд проблем для национальных властей, в частности, для ветеринарного сектора и также общественному здравоохранению. За последние годы, заболеваемость животных и людей бруцеллезом в Кыргызстане резко увеличилась и являлся третьим в мире после Сирии и Монголии. (Pappas et al., 2006).

В настоящее время понятие о путях передачи бруцеллеза, межвидовой передачи, пути передачи для человека на национальном уровне ограничено. Важно понять основные пути передачи в целях создания стратегии управления зоонозов. В конечном счете, бруцеллез может быть устранен, только если болезнь в резервуаре и находится под контролем, так как здоровье животных и человека непременно переплетаются. Поэтому необходимо рассмотреть стратегии здоровья человека и животных как два аспекта с одной целью.

Целью данного исследования является описание распределения и динамики передачи бруцеллеза в Кыргызстане и определение его влияния на здравоохранение, производства животноводства и ее продукции. Результаты данного исследования могут способствовать разработке эффективной стратегии по борьбе с бруцеллезом в Кыргызстане.

Конкретные цели: 1) исторический обзор и анализ мер борьбы бруцеллеза, использовавшихся в Кыргызстане; 2) серологическое исследование распространенности бруцеллезом людей и животных; 3) Молекулярная эпидемиология бруцеллеза животных и людей в Кыргызстане; 4) оценка затрат на контроль бруцеллеза, выгоды владельцев животных и общества; 5) оценка потенциала убойных пунктов для надзора за бруцеллезом; 6) оценка текущей кампаний массовой вакцинации скота и продвижения эффективного контроля бруцеллеза в Кыргызстане.

Данное исследование было проведено в рамках междисциплинарного исследования различных проектов и групп с участием ветеринаров,

работников здравоохранения, эпидемиологов, молекулярных биологов, лабораторных и практических ветеринарных специалистов. В диссертацию основном включены - серологическое исследование (2006, 2007, 2012), молекулярное исследование - типирование культур из абортированных плодов крупного рогатого скота и овец (2007-2011), а также надзор за убойными пунктами (2012), опрос пациентов инфицированных бруцеллезом (2013). В результате исследований были разработаны: демографический состав сельскохозяйствкнных животных; распространенность заболеваний; экономические затраты и выгоды производства животноводства с участием и без участия бруцеллеза. А также проведены дополнительные серологические исследования для выявления чумы в мелких жвачных животных и контагиозной плевропневмонии коз.

Результаты исследования. Предыдущие серологические исследования (2006-2011) показали, что бруцеллез в Кыргызстане является эндемичным и распространенность бруцеллеза составляет у овец – 3.3%, крупного рогатого скота – 2.8% и коз - 2.5% (Bonfoh et al., 2012). Эти данные были подтверждены молекулярным исследованием, типированием абортированных плодов овец и коров. Также исследованием доказано межвидовая передача инфекции (Kasymbekov et al., 2013).

Оценка стоимости бруцеллеза на производственную систему животноводства в Кыргызстане представлены в двух моделях, которые основаны на разных методах оценки.

Для вычисления минимальных затрат, мы взяли за основу 2006 год, по обменному курсу того времени 41.3 сом за 1 доллар США.

Мы разработали демографическую модель для животноводства и произвели расчет продуктивности животноводства по реальной стоимости болезни и точные оценки конечных потерь продуктивности скота. Потери для Кыргызстана оценивались за период с 2006 по 2011 год с учетом распространенности бруцеллеза: 2.8% для крупного рогатого скота, 3.3% - у овец и 2.5% - у коз.

В период исследования общий социальный ущерб от бруцеллеза составил 32.1 млн. долларов (95 % ДИ $25.6 – 39.5 млн.долл.) стоимость здравоохранения составляет 7.4 млн. долл. (95% ДИ 6.7 – 8.0 млн.долл.), из которых 6.0 млн.долл. (95% ДИ 5.5 – 6.5 млн.долл.) для частного здравоохранения и 1.4 млн. (95% ДИ 1.2 – 1.5 млн.долл.) для общественного здравоохранения. Частные потери доходов от бруцеллеза в этот период составили 9.3 млн. долл. (95% ДИ 9.2 – 9.4 млн долл.). Стоимость бруцеллеза для животноводческого сектора составили 15.4 млн долларов, из которых скотоводство теряет 13.8 млн.долл. (95% ДИ 7.0 - 20.8 млн.долл), овцеводство теряет 0.78млн.долл (95% ДИ 0.48 – 2.05 млн. долл) и козоводство теряет 0.75 млн. долл (95% ДИ 0.08 – 1.4 млн.долл).

Средняя стоимость активов животноводческой продукции оценена на сумму 1.7 млрд. долл (95% ДИ 1.48 – 2.05 млрд долл), Если пересчитать расходы с учетом текущих цен, то сумма будет значительно увеличена.

Активный надзор в домохозяйствах два раза дороже в сравнении с надзором на убойных пунктах. Мы провели исследование на убойных пунктах и в домохозяйствах и разработали демографическую модель.

Наши выводы о распространенности домохозяйств был в близком диапазоне с надзором на убойных пунктах. Распространенность бруцеллеза при серологических исследованиях в убойных пунктах составил 9.8% (95% ДИ 8.0 -11.5 %) и 10.7% (95% ДИ 8.9 -12.6 %) при исследовании домохозяйств. Мы скорректировали структуру состава стада убойного пункта к национальному уровню, и серологическая распространенность была 10.4% (95% ДИ 8.6 – 12.2%). Однако распространенность чумы МРС не было одинаковым при надзоре домохозяйств по сравнению с убойным пунктом, составила 11% и 20.4% соответственно. При надзоре убойных пунктов с корректировкой состава стада на национальном демографическом составе можно предсказать распространенность инфекции, но такой вид надзора не может быть использован для оценки охвата вакцинацией без хорошей прослеживаемой системы, то есть идентификации животных.

Основным источником информации является английская версия данной диссертации, если что-либо не понятно, пожалуйста, смотрите английскую версию как основную.

5. Introduction

5.1 *Infection and disease*

Brucellae are small, non-motile, non-sporulating, non-toxigenic, non-fermenting, aerobic, Gram-negative coccobacilli that may, based on DNA homology, represent a single bacterial species (Moreno et al., 2002, Rodriguez et al., 1992). Conventionally, *Brucella* spp. are classified into seven species each comprising multiple biovars (Rodriguez et al., 1992). Through discovery of new *Brucella* species, especially in wild animals, this number is increasing (Rodriguez et al., 1992, Boschiroli et al., 2001). *B. melitensis*, *B. suis* and *B. abortus* can infect humans (Zinsstag et al., 2005, Diaz Aparicio, 2013). Human infection with *B. canis* has also been reported. Infection of humans with *B. ovis*, *B. neotomae* (Wallach et al., 2004) and the newly identified *B. maris* has not been described (Godfroid et al., 2005). *B. melitensis* mainly infects sheep and goats (Garin-Bastuji et al., 1998), and *B. abortus* is the major cause of abortion in cattle (Ocholi et al., 2005, Taleski et al., 2002). There are some reports that even in the countries where *B. abortus* has been eliminated in cattle, in some areas *B. melitensis* has re-emerged in sheep, goats and cattle (Taleski et al., 2002). Still, *B. melitensis,* with the main reservoir in sheep and goats, remains the principal cause of human brucellosis (Cloeckaert et al., 2002). *B. suis* is also re-emerging as an agent of infection in cattle, and as the organism is shed in milk, thus is a risk factor for human infection (Salehi et al., 2006).

Two novel species *B. ceti* and *B. pinnipedialis*, isolated from marine mammals, have evolved rapidly in recent years with the potential to cause human disease. Another novel species *B. microti* has been isolated from wildlife, whilst *B. inopinata* has been isolated from a human case (Taleski et al., 2002, Pappas, 2010, Maquart et al., 2009d, Zygmunt et al., 2010, Maquart et al., 2008, Seco-Mediavilla et al., 2003, Scholz et al., 2008). Brucellosis is considered to be globally one of the most wide-spread zoonoses – a disease transmissible from animals to humans and vice versa.

Brucellosis remains a major preventable zoonosis, which continues to cause significant medical, veterinary and socioeconomic problems, mainly because the overall burden remains underestimated and neglected (Aleixo et al., 1999, Pappas, 2010). Compared to the highly contagious transboundary animal diseases like foot and mouth disease (FMD), brucellosis has sometimes been regarded as a second priority for control. As a consequence, insufficient resources have been allocated for the implementation of brucellosis control programmes (Nikolaos, 1998).

Since the end of the socialist period, human and animal brucellosis has become a growing problem and one of the most important zoonoses in Kyrgyzstan and other countries of the Central Asian region (CAR) (Nikolaos, 1998). Although continuous progress is claimed in brucellosis control, it still remains a major public health hazard which explains the ever-increasing concern in many other countries.

In economic terms, brucellosis is one of the most important diseases of livestock. The economic cost in annual gross volume of livestock products, loss of livestock from brucellosis due to abortions, meat-shortfall, decrease in milk production and infertility, death of young infants and loss of breeding stock caused by *Brucella* is never, or very rarely, estimated (Ivanov et al., 2010, Roth et al., 2003, Zinsstag et al., 2005).

Brucellosis, particularly *Brucella melitensis*, has been identified as the major zoonotic disease of public health importance in Kyrgyzstan. However, prevention and control pose considerable problems to national authorities, particularly for Veterinary Services and Public Health, in Kyrgyzstan which has the highest brucellosis morbidity rate of all the independent countries of the Former Soviet Union (FSU) (Wolfram et al., 2010) and incidence worldwide (Zinsstag et al., 2009). Since the country gained independence, all the livestock has been distributed to private owners and as a result the incidence of brucellosis in humans has been increasing annually.

The complexity of the epidemiology of brucellosis and the serious difficulties for effective control measures arise because of the involvement of both livestock (cattle, sheep, goats) and humans (Ayman and Nermeen, 2010) in the disease process. Seven republics of the former Soviet Union are included in 25 countries with the highest incidence of the disease worldwide (Pappas, 2010, Zinsstag et al., 2007, Pappas et al., 2006) In Kyrgyzstan, brucellosis control has become a national priority, as a result of the high prevalence observed in livestock and humans (Kozukeev et al., 2006, Pappas et al., 2006) (Figure 5-1).

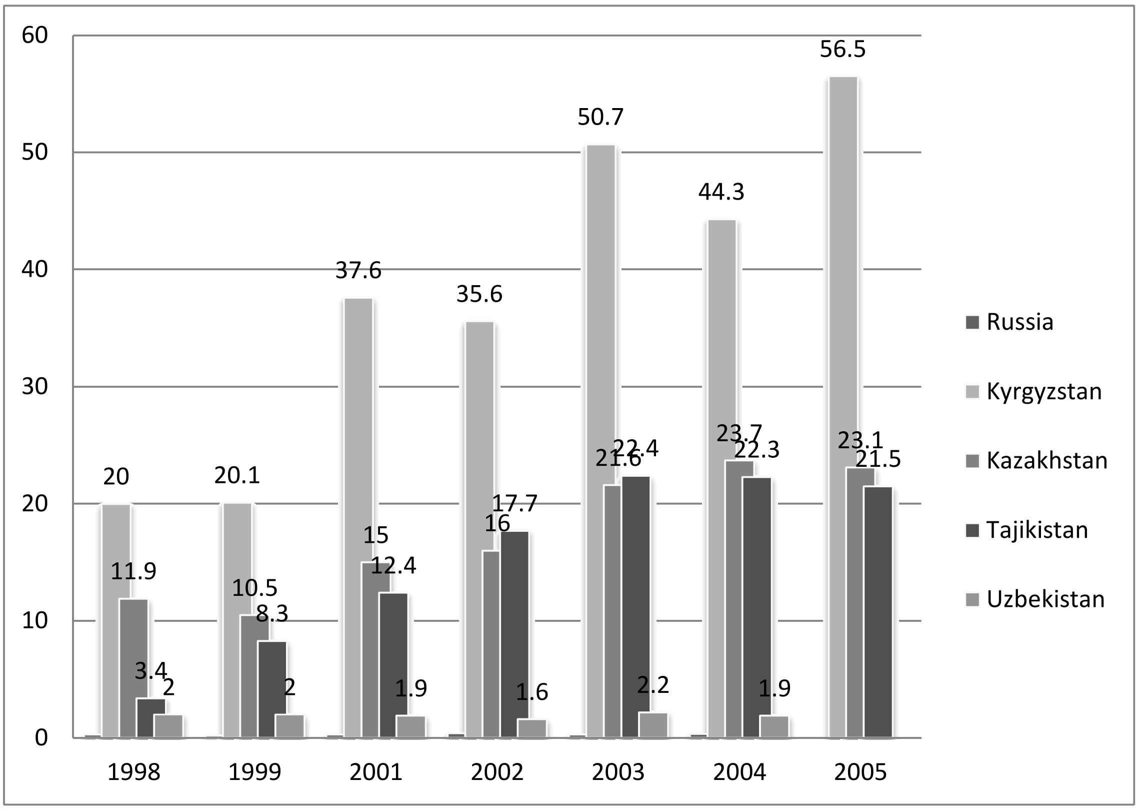

Figure 5-1. Human brucellosis incidence rate per 100,000 inhabitants in CIS countries.
Note the sharp increase of human brucellosis incidences reported in Kyrgyzstan.

5.2 *Burden and epidemiology of brucellosis in Kyrgyzstan*

Although *B. melitensis* is still endemic in some areas of Southern Europe (Health Protection, 2010), *B. abortus* has been eliminated or is on the verge of elimination in these countries (Nikolaos, 1998, Health Protection, 2010). In Kyrgyzstan, where the programs have been implemented, several technical problems challenge the veterinary services, such as animal movement control and identification, vaccination coverage and the emergence of *B. melitensis* in cattle. Brucellosis caused by *B. melitensis* in small ruminants is major problem in almost all of the Asian region (Benkirane, 2006, Donev, 2010). Poor infrastructure is among the major obstacles to effective prevention and control of the disease, which, remains endemic in some countries of the Asian region.

Given the massive infection of sheep and cattle, it is not feasible to slaughter all seropositive livestock to eliminate brucellosis foci. Other important factors that have led to disease propagation in the country were: i) no systematic 30 day quarantine for newly arriving animals, ii) neglecting veterinary examination (and provision of veterinary services), iii) lack of effective meat inspection at processing plants, iv) brucellosis-infected cattle keeping, especially of breeding stock, v) failure of hygienic precautions during veterinary interventions, and vi) general lack of zoo-hygienic and veterinary requirements for transportation and feeding and construction and fencing of livestock facilities.

Among the methods for brucellosis control, epidemiological surveillance of human and animal brucellosis is considered a high priority and of essential strategic importance for endemic and disease-free countries (Nikolaos, 1998, Roth et al., 2003). Experience has shown and proven that control in livestock is one of the pillars upon which any monitoring control programs, irrespective of the country, should be based. Moreover, essential tools for organized control of zoonotic disease in general and brucellosis in particular are an efficient surveillance system at the national level, effective co-operation and information exchange between public health and veterinary sectors, as well as regular co-operation between developed countries where *B. abortus* has been eliminated and the neighbouring countries (Nikolaos, 1998, Kozukeev et al., 2006, Roth et al., 2003, Kim, 2004).

The natural conditions and economic drivers in Kyrgyzstan have led to uneven development of animal husbandries. After independence in 1991, all livestock was distributed to private owners, and veterinary services were no longer available at all the farms. Lack of knowledge on animal keeping at newly formed private farms further favoured the propagation of brucellosis in all parts of Kyrgyzstan, especially in the lowland areas. Since that time, human incidence has increased annually, and Kyrgyzstan has now one of the highest brucellosis incidences worldwide (annual incidence: 78 per 100 000 in 2007) (NatStatCom, 2013) (Fig. 5-2). In addition, in terms of incidence of human brucellosis, Kyrgyzstan (362.2 cases) is in the lead compared to neighbouring CIS countries such as Russia, Kazakhstan and is ranked third in the world after Syria (1603.4 cases) and Mongolia (605.9 cases) according to the Pappas global status in 2002 (Pappas, 2010, Pappas et al., 2006).

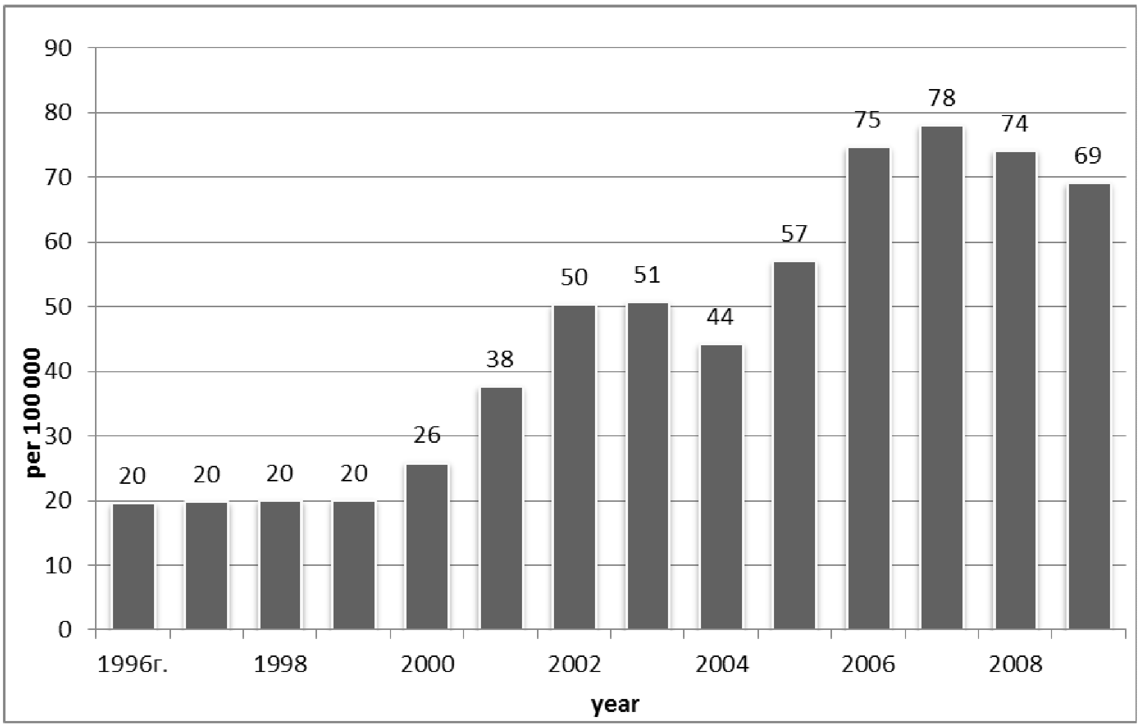

Figure 5-2. Human brucellosis incidence rate per 100,000 inhabitants from 1996 – 2009.

Currently, the communities are concerned with strategic planning and effective reduction of the occurrence and burden of this disease in humans and animals. However, current knowledge on the transmission within and between livestock species and to humans does not facilitate epidemiological description of brucellosis in Kyrgyzstan. It is important to understand the main transmission routes in order to establish a control strategy. Brucellosis can ultimately only be eliminated when the disease is controlled in the animal reservoir, since animal and human health is inextricably intertwined. It is therefore necessary to consider human and animal health strategies as two aspects of the same aim.

5.3 *Previous work on brucellosis in Kyrgyzstan*

Since 1992 as part of a set of measures to combat brucellosis in small ruminants, mass immunization of animals with *B. abortus* S19 was implemented. Ewes and lambs were tested for brucellosis by serological methods (AT, CFT, and RBT). Positively reacting animals were isolated and those with negative results were immunized. In the following year for the 1-2 months before mating, sheep were re-vaccinated with the same vaccine without preliminary serological testing. Particular attention was paid to the timely isolation and removal of aborting sheep from the flocks.

Despite annual vaccination of sheep during this long time period, there was not a noticeable decline of brucellosis incidence, also due to poor vaccination coverage. The specialists advised use of the internationally recommended *B. melitensis* Rev-1 instead of S19, which was successfuly administered in 2006-2007. Since 2008, the conjuncitval application of Rev-1 vaccine was adopted.

5.4 *Institutional collaboration*

A representative study on brucellosis sero-prevalence in livestock and humans, including the first attempt for comprehensive research in brucellosis prevention, was undertaken in 2006-2007 by the Swiss Tropical Institute and the Institute of Livestock, Veterinary and Pastures as a case study of the Transversal Partnership Project "Extensive production Systems", within the Swiss National Centre of Competence in Research North-South. The project collaborated closely with the local Veterinary and Public health institutions of the country and the Kyrgyz-Swiss-Swedish Health Project in Bishkek. This study took place in the context of the Kyrgyz-Swiss Health Reform Support Project, funded by the Swiss Development Cooperation through the Swiss Red Cross, Bishkek.

Within the Swiss TPH project, in close collaboration with NCCR North-South and JACS CAS (Bishkek), and in partnership with the Republican State Centre for Veterinary Diagnostic, Labor Spiez, Spiez, and the Cantonal Microbiological Laboratory (Istituto di Microbiologia Cantonale, IMC), Bellinzona, Switzerland, aborted material was collected from April – May 2009 in Naryn district at the Naryn Oblast veterinary diagnostic centre. Routine abortion diagnostic testing was established by upgrading culture capacity at the Naryn Oblast laboratory and the Central Veterinary Laboratory in Bishkek. The isolated primary cultures were shipped to Switzerland for molecular diagnostic-characterisation. The cultures were sub-cultured at Labor Spiez, and *B. melitensis* was confirmed through biochemical and PCR tests. The isolated cultures were investigated for biochemical and antibiotic resistance. Variable Number of Tandem Repeats (VNTR) testing of the first strains was done by Marie Ballif at Swiss TPH and Matrix-Assisted Laser Desorption Ionization–Time of Flight (MALDI–TOF) characterization of strains was done at IMC in Bellinzona. Through this process, the Human and Animal Health unit in collaboration with Swiss and Kyrgyz partners successfully established the laboratory and logistical capacity for isolation and characterization of *Brucella* spp.

6. Research rationale

6.1 *Goal*

The 'One Medicine' concept by Calvin Schwabe has seen an unprecedented revival in the last decade and has evolved towards 'One Health' conceptual thinking, emphasising epidemiology and public health. A 'tool box' translating the 'One Health' concept into practical methods in the fields of integrated disease surveillance, joint animal-human epidemiological studies and health services development has been proposed (Pappas et al., 2006, Zinsstag et al., 2009, Frank, 2008). In this study, we seek to apply and validate available tools to foster cooperation between animal and human health sectors based on evidence of the best control options adapted to the context of Kyrgyzstan. Cross-sector approaches in the epidemiology of brucellosis are new to Kyrgyzstan and may lead to novel effective control strategies.

The current test and slaughter programme is inefficient to control the disease, which is reflected by the high brucellosis incidence in humans. However, prior to proposing a modern Rev-1 vaccination programme for all livestock, cost-effectiveness of the control programme was estimated in a systematic way, including all involved sectors. The results showed that strategy would lead to considerable benefits for both the public and the veterinary sector.

The most cost-effective method for disease surveillance needs be assessed to meet requirements for disease control in resource poor settings, but this is also of interest in industrialised countries (Ridley, 2004). Surveillance of infectious disease in livestock is expensive due to the cost of logistics, personnel and diagnostic laboratory testing. It could be reduced by sampling from abattoirs in place of costly farm surveys (Thornton, 1957). Despite the abattoir population not necessarily reflecting the total herd, the combination of information on animal origin through transport certificates and adequate meat inspection of the large number of animals processed could be sufficient to replace field surveillance thereby reducing the cost of surveillance (Caldow et al., 2001). In general, the use of abattoir information depends very much on the type of disease and surveillance system in question, but it would be applicable for the case of cattle and small ruminant brucellosis. Sensitivity and cost-effectiveness of different sampling scenarios are estimated. The present project investigates the possibilities and the feasibility of the use of abattoir as a data source for monitoring and control programmes in animals as a model of disease surveillance and estimate sampling cost in abattoirs to find the most effective sampling method and to assure that future implementation is accepted by the veterinary service.

If abattoir surveillance proves to be a cheaper and equally sensitive way for infection surveillance compared to field surveillance, the Kyrgyz government could save substantial resources. Finally, the analysis done under this study is an important one as an evidence base for policy dialogue with Kyrgyz authorities.

The goal of the proposed research project is to describe the distribution and the transmission dynamics of brucellosis in Kyrgyzstan and to determine its impact on livestock production and public health. The results will contribute to development of an efficient brucellosis control strategy in Kyrgyzstan.

6.2 *Specific objectives*

The specific objectives are:

- A short historical review and analysis of brucellosis control measures in Kyrgyzstan.
- Molecular epidemiology of animal brucellosis in Kyrgyzstan.
- Assessment of brucellosis costs to livestock owners, brucellosis patients and society.
- Assessment of the potential of abattoirs for brucellosis surveillance.
- Evaluation of current mass livestock vaccination campaigns and promotion of effective brucellosis control in Kyrgyzstan.

7. Methods

7.1 *Literature review*

A systematic literature review of the international literature and its comparison with the materials of FSU scientists was done. The most important parts of literature were translated into English. The efforts were focussed on the analysis and review of epidemiological and control policy documents in Russian and Kyrgyz languages to document epidemiological and policy transition from the Socialist to the market economy. In addition, the available reports were reviewed and synthesised. The systematic review method was 1) document the literature search from electronic databases and non-electronically available sources. A part of the literature was collected from sources in the library of the National Academy of Science, Research Institute of Veterinary, Ministry of Agriculture, Ministry of Health and National Library of Kyrgyzstan. Key words for literature searching included: Brucellosis, *Brucella melitensis*, *Brucella abortus*, incidence, prevalence, serology, policy, control. 2) Publications were restricted to the period of 1960-2011 and checked for duplicate entries. 3) Papers and reports were classified according to epidemiological or policy relevance. Category 1: Human brucellosis: relating to brucellosis infection in populations (i.e. disease frequency) or cases of human brucellosis (i.e. disease sequelae). Category 2: Animal brucellosis frequency, diagnostic methods or control. 4) Frequency studies were classified as prevalence studies if they stated a specified study population and area and an outcome expressed as the proportion of the study population identified as a brucellosis case (%) or as incidence studies if they described a time period of observation, a statement regarding the study population size and area and an outcome expressed as the number of new brucellosis cases per population at risk per time period. The study area was categorised in decreasing order of quality as being at the national, provincial, district or village level. Diagnostic methods were categorised by the prevailing test methods: Wright-Huddleston, complement fixation or Rose Bengal. Control policy documents were categorised separately depending on their legal or operational content aimed at documenting temporal trends. 4) Documents were given an overall quality grade depending on method descriptions and indication of diagnostic tests. Quality criteria required the following detail: indication of year of study, size and sex/age distribution of study population, indications of disease frequency and size of reference population. All references with notes were stored in the bibliographic referencing programs Zotero version 4.0.17.1 and EndNote X7.0.2.

7.2 *Analyses of existing quantitative data*

Available historical quantitative data on reported human cases and prevalence data in cattle, sheep and goats together with human and livestock demographic data were entered in an MS Access® database and analysed in STATA 12® for time trends from 1960 to 1990 (end of socialist period) and 1990 to 2011 and data collected from field and abattoir surveillance. Our hypothesis is that brucellosis control in livestock changed with the end of the Socialist period, resulting in increased transmission among livestock, which determines the growing epidemic in humans (Figure 1). Some data analysis was preformed under supervision of Jan Hattendorf. For Monte Carlo simulation,s we used Ersatz® software (www.epigear.com) in addition to Excel® with a range of probability distribution functions, the ability to draw randomly from these distributions and an automated sensitivity analysis for all parameters expressed as probability distributions. Monte Carlo simulations were used for the analysis of brucellosis cost in the Kyrgyz context.

7.3 *Interviews with key livestock experts in Kyrgyzstan*

Data on animal productivity were discussed with key livestock experts and veterinarians in Kyrgyzstan. Through this study we obtained their experiences and data on animal fertility and productivity needed for the development of the livestock demographic model.

Previous studies done in the past decades were discussed with brucellosis experts in Kyrgyzstan using key informant interviews. We documented their views on successes and failures of past zoonoses control programmes. All interviews were registered with notes, transcribed in a text editor and then analysed. Additional data were obtained from itinerary reports, documents, scientific journals and books of the Soviet time period and former Soviet Union (FSU) countries.

7.4 *Structure of thesis*

The thesis presents the results of several studies in five chapters as follows:

1. Effect of political, cultural and economic issues on brucellosis epidemiology and control in Kyrgyzstan

In this working paper the transition of the brucellosis control programme from the Soviet system to independence is reviewed and analysed. The effect of historical political system changes on the disease control programme is described in the paper.

2. Representative Seroprevalences of Brucellosis in Humans and Livestock in Kyrgyzstan

A cross-sectional study of human and animal brucellosis prevalence was conducted in three provinces in Kyrgyzstan, and it confirmed high seroprevalence of brucellosis.

3. Molecular epidemiology and antibiotic susceptibility of livestock Brucella melitensis isolates from Naryn oblast, Kyrgyzstan

This study was conducted to type and characterise brucellosis cultures isolated in Naryn province and to confirm the circulation of *Brucella melitensis* in the area and its transmission to cattle. The strains are compared with the global phylogeny.

4. Societal cost of brucellosis to Kyrgyzstan

In this study a cost analysis of brucellosis to the Kyrgyz society was conducted. For this purpose interviews with health care providers and 95 patients at the hospital were held. A livestock demographic model was developed to estimate livestock productivity with and without disease.

5. Slaughterhouse surveillance of infectious disease in Kyrgyzstan

This study was conducted to investigate the prevalence of brucellosis and vaccination coverage at the abattoir and household level. Abattoir surveillance was compared with the field surveillance. For this purpose abattoir population structures were corrected according to the demographic composition at the national level. Moreover the occurrence of Peste des petits ruminants (PPR) was investigated.

8. Effect of political, cultural and economic issues on brucellosis epidemiology and control in Kyrgyzstan*

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8.1 *Introduction*

Brucellosis to date is one of the major concerns in Kyrgyzstan as well as in many other countries, causing enormous economic damage to the government and much harm to human health.

Seven republics of the former Soviet Union are listed in 25 countries with the highest incidence of the disease worldwide. In addition, in terms of incidence of human brucellosis Kyrgyzstan is leading compared to other neighbouring CIS countries such as Russia, Kazakhstan, etc. and is ranked the third in the world after Syria and Mongolia according to the Pappas's Global status in 2002 (Pappas, 2010, Pappas et al., 2006).

Brucellosis control in Kyrgyzstan became a national priority as a result of high prevalence observed in livestock and humans (Pappas et al., 2006, Kozukeev et al., 2006, Bonfoh et al., 2012). Official data shows an increase of human incidence in the past two decades and makes up 78 per 100,000 in 2007(NatStatCom, 2013). Although there is rather good acknowledgement nowadays on the impact and importance of the disease (Wolfram et al., 2010) and despite continuous progress in brucellosis control, it still remains a major public health hazard (Zinsstag, 2012).

A representative serological study showing an apparent seroprevalence of brucellosis in Kyrgyzstan of 8.8% in humans (95%CI 4.5-16.5), 2.8% (95%CI 1.6-4.9%) in cattle, 3.3% (95% CI 1.5-6.9%) in sheep and 2.5% (95%CI 1.4-4.5%) in goats (Bonfoh et al., 2012). The Naryn oblast had the highest seroprevalences of sheep among other species and was related with human brucellosis (Bonfoh et al., 2012).

Recently *B. melitensis* was isolated in the Naryn oblast and has been characterized with molecular typing methods. This confirmed that in the Naryn oblast *B. melitensis* is endemic and sheep are apparently the main host of infection. *B. melitensis* is also transmitted to cattle (Kasymbekov et al., 2013).

The reasons for such situation are inadequate brucellosis interventions, inappropriate selection of vaccines and their use, non-compliance with the cold chain, minimum vaccination coverage, minimum use of diagnostic tools and low public awareness. The awareness of the type and prevalence of the circulating brucellosis is equally important. It is necessary to define brucellosis control strategy and approve brucellosis control programme at national or regional levels.

The natural conditions and economic drivers in Kyrgyzstan have led to uneven development of animal husbandry. Lack of knowledge on animal keeping of newly formed farms has further favoured the propagation of brucellosis in all parts of Kyrgyzstan, especially in the lowland areas.

Currently, the communities are concerned about strategic planning and effective reduction of the occurrence and burden of this disease in humans and animals. However, the current knowledge on the transmission within and between livestock species and to humans does not facilitate the epidemiological describing of of brucellosis in Kyrgyzstan. It is important to understand the main transmission routes in order to establish a control strategy. Brucellosis can ultimately only be eliminated if the disease is controlled in the animal reservoir since animal and human health is indispensably intertwined. It is therefore necessary to consider human and animal health strategies as two aspects of the same aim.

The outcomes of brucellosis control in animals in Kyrgyzstan during different periods have varied. In particular, the required interventions under the brucellosis control program were not implemented in full. It was not always feasible to ensure rapid and reliable recovery or replacement of infected animals to maintain the well-being as well as the on-going brucellosis control interventions often generated minimum effects, in other words brucellosis tends to re-emerge after a certain time span following the implementation of interventions. There is evidence that in some countries brucellosis control was backed up with a sound government program and financial support which generated lasting effects and as a result they are acknowledged as the "country free from brucellosis".

Currently, in many countries, especially in Asia and the Middle East (Pappas et al., 2006), there is a dramatic increase in the incidence of brucellosis in humans.

Rationale of this study is a synthesis of the systematic literature review, the historical analysis of brucellosis epidemiology faciliatates tracing back brucellosis control policy and its effect on brucellosis disease frequency across the decades, spanning the transition from Socialist system to the current market economy.

8.2 *Materials and methods*

8.2.1 Data collection

A literature review was conducted in Kyrgyzstan using mainly published literature in Kyrgyz and Russian languages but also international peer-reviewed articles. Local data collection was ensured from the national as well as the regional provincial databases of the National Statistical Committee (NatStatCom, 2013). Bibliographic databases such as PubMed were used to retrieve international articles. In addition, available reports were reviewed and synthesised. All references together with available abstracts were stored in the bibliographic reference program EndNote X7.0.1.

The incidences of human brucellosis cases were collected from annual reports of the Republican Centre for Quarantine and Especially Dangerous Diseases, Ministry of Health and National Statistic Committee of the Kyrgyz Republic.

The incidences of animal brucellosis were collected from annual reports of the State Veterinary Department, Republican State Center for Veterinary Diagnostic, Ministry of Agriculture, Veterinary Research Institute, Research Institute of Livestock and Pasture, Institute of Biotechnology of the National Academy of Science of the Kyrgyz Republic.

8.2.2 Analyses of existing data

Historical quantitative data on reported human cases and outbreaks in livestock were collected and analysed in STATA 12 and additionally visualised with graphics in MS Excel. Also the situation of past vaccination programmes was carefully evaluated. During the past years, brucellosis control programmes have used different livestock vaccines. Therefore a critical evaluation on advantages and disadvantages of different vaccines was performed.

8.2.3 Interviews with key brucellosis experts in Kyrgyzstan

Previous studies and developments over several decades were discussed with key brucellosis experts and veterinarians in Kyrgyzstan. We became aware of their views on successes and failures of past zoonoses control programmes. All interviews were noted, transcribed in a text editor and then analysed. Additional data were captured from itinerary reports, documents, scientific journals and books published in former Soviet Union (FSU) countries.

8.3 *Investigation outcomes*

8.3.1 Pre-Soviet time in Kyrgyzstan and role of animals in human livelihood

Kyrgyzstan is mainly a mountainous country harbouring two of the highest mountain ranges of Central Asia, namely the Tien-Shan and the Pamir-Alay ranges. They cover more than 80% of the Kyrgyz territory with numerous glaciers, lakes and a particularly difficult mountainous relief composed of ridges, spurs, high mountain valleys and canyons.

Eighty-three per cent of the usable agriculture land is located within mountains and is presented by high pastures and plateaus with dry steppe and short grass vegetation. The climate is considered as dry continental (FAO, 2010).

Due to the different microclimatic conditions in mountainous areas of the country, livestock herder’s transhumance follows the vegetation growth. This means they move to distant pastures, rich with vegetation in summer and lower pastures in autumn where winter season begins later. The typical extensive use of natural feed resources contributes to the development of sheep, cattle and horse breeds adapted to this situation.

Environmental conditions lead to the development of the country's livestock husbandry in a mobile pastoralist husbandry system which was the main component of the economy of Kyrgyzstan (Arnold and Jongma, 1978). Prior to the Soviet regime livestock farming has been developed as the main source of income and people used the skins and wool of animals for the manufacture of various household furniture like felt, decorative items or clothes. For Kyrgyz people, livestock was an integral part of the economy and animal products were used wasteless. In 1916 before the Soviet Revolution there were: 2’544’000 sheep, 519'000 cattle and 708’000 horses in Kyrgyzstan (book, 1973).

Today livestock production remains as one of the most important Kyrgyz economic activities. Two thirds (2/3) of the Kyrgyz population make their livelihoods from livestock production (FAO, 2010).

8.3.2 Political change and livestock production systems

The evolution of the political system in Kyrgyzstan influenced the development of traditional livestock industry, especially the sheep breeding (Schillhorn van Veen, 2004). With the rise of the Soviet regime large sheep breeding and collective farms (Kolkhoz) have been organised through collectivisation and deprivation of livestock from the population (McKee et al., 2006) tab.1.

Table 1. Animal population in Kyrgyzstan (picked for each 10 years)

	Animal Population (thousand)		
Years	**Cattle**	**Sheep & goat**	**Horses**
1916	519	2544	708
1927	587.3	3736.6	618
1936	360.6	1241.2	299.7
1946	440.3	2272.3	290.2
1956	653.7	4530.6	364.4
1966	857.3	8303.2	230
1976	941.7	9850.5	265.3
1986	1110	10200	276.5
1991	1190.219	9524.935	320.468
1996	847.641	3716.081	314.066
2006	1116.733	4046.949	347.526
2011	1338.583	5288.115	388.971

The natural conditions and economic drivers in Kyrgyzstan have led to uneven development of animal husbandry. After its independence in 1991, all livestock has been distributed to private owners and veterinary services were no longer available at all the farms. Lack of knowledge on animal keeping of newly formed private farms has further favoured the propagation of brucellosis in all parts of Kyrgyzstan, especially in the lowland areas.

8.3.3 Brucellosis intervention in Kyrgyzstan

In the last century scientists of Kyrgyzstan have concluded that the infection was artificially imported to the country. For example, Smirnov quoted that in the opinion of Soviet scientists of the 20-th century brucellosis in cattle and sheep was introduced on multiple occasions to pre-revolutionary Russia from abroad with pedigree animals in the late 19th and early 20th century. G.T. Lindtrop (1928) and N.N. Stepanov (1950) (Smirnov, 1960) consider that brucellosis existed in goats in the south-east of the USSR since long time (Smirnov, 1958). L.A. Andreev (1946) determined that the type of brucellosis penetrated into Abkhazia from the Mediterranean coast and then spread across the territory of Armenia, Georgia and the Northern Caucasus. Sheep from the Northern Caucasus transmitted brucellosis across Kazakhstan and other Central Asian countries and, apparently, brucellosis was introduced to Kyrgyzstan through the same communication routes (Smirnov, 1960).

According to V.I. Kim brucellosis was imported from the Baltic countries (Lithuania, Latvia and Estonia) along with animals brought to improve the pedigree status of animals (Kim, 2004).

In Kyrgyzstan brucellosis has been known since the early 30s. Veterinary authorities started using vaccines only in the 50s, whereas the population of cattle and small ruminants has grown significantly in comparison with the 30s.(Vozhdaev et al., 1971)

In the 80 - 90s, many scientists in the country were making enormous efforts to eradicate brucellosis (Kim, 2004); however, it didn't yield any tangible effects (Smirnov, 1960). There were different reasons for that, for example, it was considered more important to reproduce and improve the animal productivity (milk yield, wool production) but little attention was paid to the health of animals and their resistance as the accomplishment of the production target by the Communist Party was of top priority.

Even in the 50-60-s appropriate measures were taken to eradicate brucellosis, however, according to the statements and opinion of Smirnov all undertaken interventions “come to naught" (Smirnov, 1960) (fig 8-1 and 8-2).

Table 2. Evaluation changing policies and interventions

Historical Period	Pre-socialist <1918	Early Socialist 1918-1945	Post WW2 1945-1990	Period 1990-2000	21st Cent >2000
Epidemiology in Livestock	0	3	4	1	2
Epidemiology in humans	0	1	4	2	3
Diagnostic procedures	0	1	4	2	3
Herd management	2	3	4	0	1
Vaccine intervention	0	1	4	2	3

The fact that veterinary services had no experience with brucellosis infections when the first outbreaks occurred largely contributed to the expansion of the disease. In addition, brucellosis control was mainly conducted in cattle by carrying out mainly organisational and veterinary-sanitarian measures that were inadequate (Vozhdaev et al., 1971).

During the Soviet regime, control programs and vaccination campaigns of brucellosis were approved by the Ministry of the Soviet Union in Moscow (Ivanov et al., 2011, Ivanov et al., 2010).

Therefore, almost all farms in Kyrgyzstan as well as in the whole Soviet Union used the same system for animal breeding, control of brucellosis and disease recovery measures. Vaccines, diagnostic tools, methods and provision of veterinary equipment's were all centralised (Salmakov et al., 2010).

8.3.4 The spread of infection

Furthermore, the situation and management of some remote pasture hampered the implementation of sanitary measures at dairy farms. For example, cattle originated from some district of the Chui valley were annually driven to winter pastures on Kazakhstan's territory or in the south of the country animals have been set aside for grazing on pastures neighbouring the republics of Uzbekistan and Tajikistan (Kim, 2004, Vozhdaev et al., 1971, Smirnov, 1958). Fundamental veterinary rules and principles have often been violated, thus leading to on-going brucellosis transmission.

During the grazing period on summer pastures animals were tested by allergic test. If the animals reacted positively they were examined through serological tests and positive animals were slaughtered. Winter enclosures were disinfected before the herds returned from summer pastures (Smirnov, 1960, Kim, 2004).

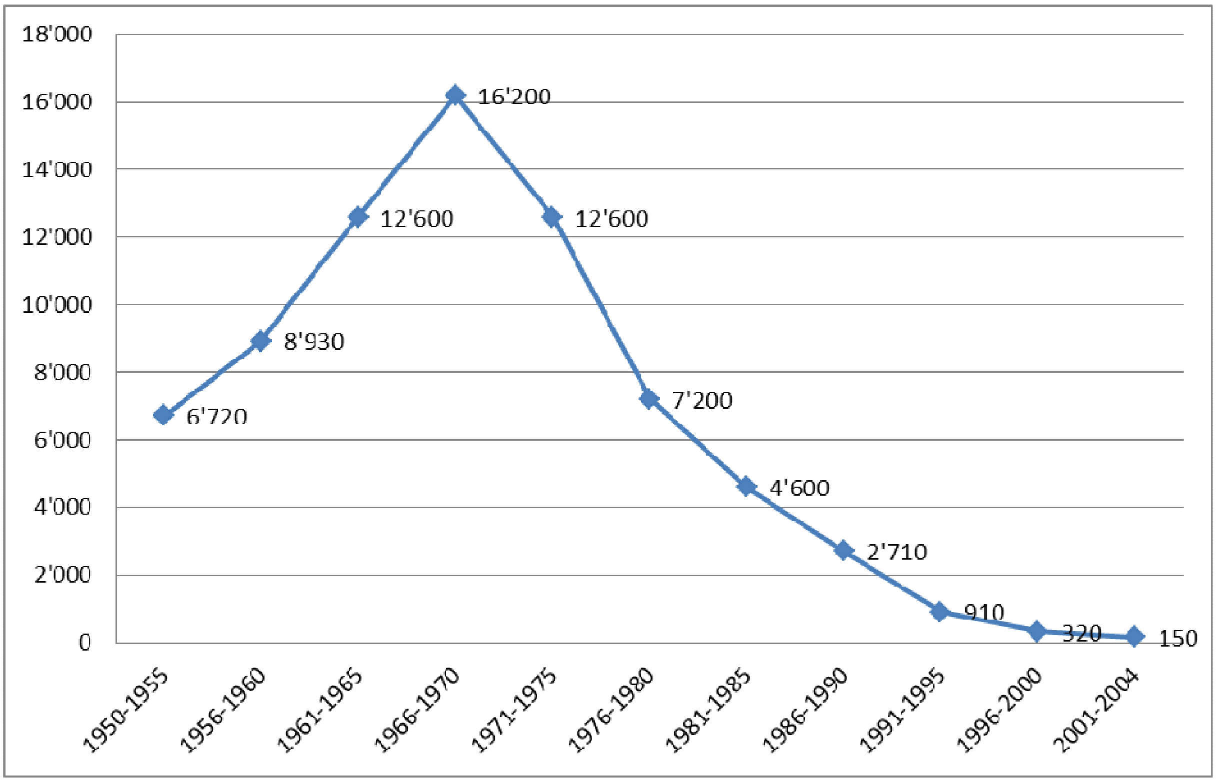

Figure 8-1. Dynamics of brucellosis – sheep breeding farms

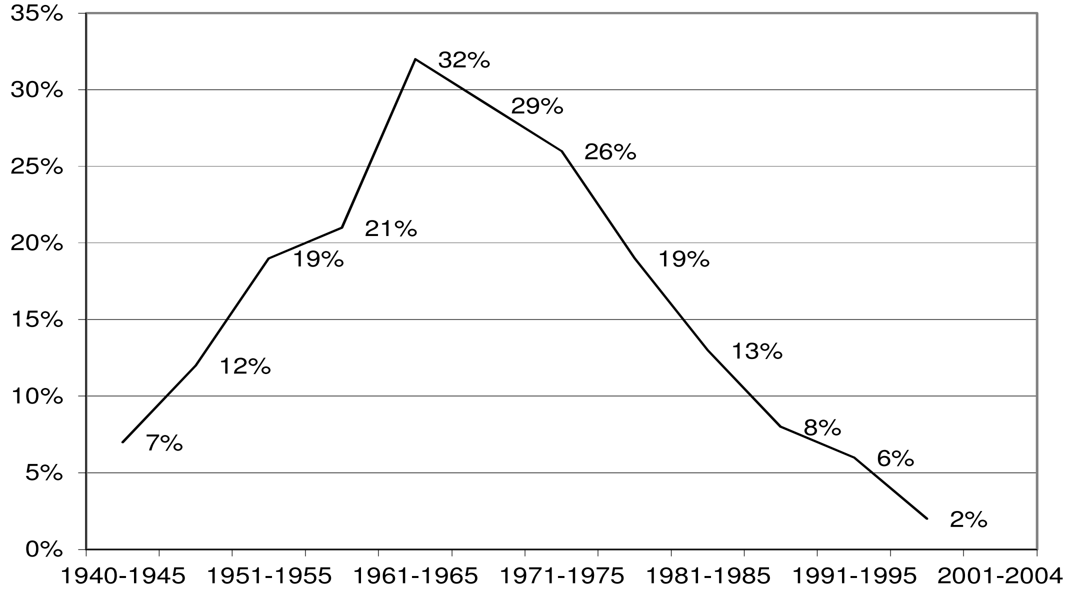

Figure 8-2**.** Dynamics of increasing brucellosis in cattle breeding farms

8.3.5 Vaccine quality

From 1950 to 1960, the scientists proposed to use live vaccines for the active prevention of brucellosis (Vyshelessky, Angeloff et al. 1956), and at the beginning of this century they proposed the use of inactivated (killed) adjuvant vaccines (Kim, 2004) Since then major researches have been focused on the development and improvement of vaccines with adjuvant (Denisov et al., 2010, Ivanov et al., 2011).

Preventing animal infection with vaccines has been rather ineffective, and long-term work brought insignificant success, thus cattle vaccination with S19 was ceased in 1998.

S19 vaccine was used for cattle in 1949 in the USSR and since 1954 it was also applied in Kyrgyzstan (Vozhdaev et al., 1971, Avila-Calderon et al., 2013). During the Soviet regime in Kyrgyzstan different vaccines have been used for cattle: *B. abortus* S19, S82, 104M. Small ruminants were also vaccinated using *B. melitensis* Rev-1, 38/59, Nevsky-12 (Ivanov et al., 2010, Ivanov et al., 2011, Shumilov et al., 2010, Sklyarov et al., 2010).

Suspected infected farms were vaccinated with S19 vaccine following this scheme: first vaccination of calves at 3-5 months of age and revaccination (with 80×10^9 Colony forming units (CFU)) at the age of 10-12 months (1-2 months before onset of sexual maternity). Cattle were revaccinated every two years (Ivanov et al., 2010, Kim, 2004, Salmakov et al., 2010, Shumilov et al., 2010).

In parallel to the described brucellosis prevention measures, mass immunization by B. *abortus* S19 was used in 1975 for small ruminants. The following vaccination scheme was used: adult and young females from potentially positive brucellosis herds were examined by serology (AT, Complement Fixation Test (CFT), and Rose Bengal Test (RBT)) after separation of the lambs. Positive animals were isolated for slaughter and negative ones immunized. Females were re-immunized by the same vaccine without prior testing for brucellosis 1-2 months prior to artificial insemination. Vaccination was conducted annually until the absence of clinical signs and positive tested animals. All herds have been slaughtered if brucellosis positive animals were found.

In order to reduce brucellosis, immunized animals were grouped by flocks. All abortion and/or birth of weak or dead lambs were registered. Particular attention was paid to the quick isolation and removal of aborted ewes from flocks. Disinfection was conducted regularly- planed and emergent. Rams were not vaccinated but only tested for brucellosis by serological methods.

It should be noted that after vaccination with S19 vaccine animals were not examined serologically and therefore the question rose whether the infection’s foci were removed or infected animals remained in herds as sources of infection. That’s why it is believed that widespread application of vaccine in sheep husbandry did not achieved brucellosis eradication. Observations of the expression of immunological reactions in vaccinated sheep of various ages showed that they remained seropositive for a long time (Kim, 2004).

8.3.6 Causes of disease prevalence

Apparently, the reason for the growing incidence of brucellosis in Kyrgyzstan is the use of locally produced vaccines. Here one can indicate two main causes: first is the use of S19, *B. abortus* for vaccination of small ruminants against *B. melitensis* which is recognized to have much severe virulence; second is the quality of the vaccine, as the vaccines go through the internal control only, in other words, the private company checks their products with no involvement of independent control.

The initial indicators of the vaccine titers were not the best even back in time (1998-1999).

Besides the outbreaks of infectious diseases in those regions where the locally produced vaccines were used testify to that, for example, except for the brucellosis vaccine the vaccines against FMD, sheep and goat pox, anthrax and rabies were used, and these infections are recognized as endemic in Kyrgyzstan.

Many foreign experts who worked in Kyrgyzstan used to ship the vaccine samples back home for testing the quality, however, the findings were never reported officially instead of that there were verbal statements indicating that the vaccines do not meet the quality standard.

In 2008, on the invitation of the State Veterinary Department, OIE experts checked the bio-factory status and concluded that the factory poses a threat not only to the country but also to the entire neighbouring region. The official OIE expert’s opinions were submitted to the KR Government and Parliament. Later this issue was considered by the Parliament in 2010 whereby they approved the closure of this enterprise unless it is adequately equipped according to the required international standards; however, the company is still running at the same capacity, meaning that the produced vaccines are sold outside the country. Mainly they are exported to neighbouring countries.

In general, it can be concluded that the use of locally produced vaccine did not enable either the prevention or eradication of infectious diseases. Over the past 17 years prior to 2009 the bio-factory was a monopolist in terms of vaccine production and tended to win all annual vaccines procurement tenders of the SVD and during that time the incidence in animals went up that affected the human health and the country’s economy.

8.3.7 Serological test

In Kyrgyzstan, as in many other republics of former USSR, serological diagnostic methods (AT and CFT) combined with veterinary-sanitary measures could eliminate the infection only in weakly infected farms and stables, which were isolated during the whole year (tab.3) (Ashepa et al., 1973, Vozhdaev et al., 1971). An agglutination test (AT) was used for diagnostic purposes (Kim, 2004).

Table 3. Lab diagnosis of sheep

Year	Positive sheep (thousand)	Positive Ram (thousand)	Abortion	Human cases
1977	0.6	0.1	142	191
1978	1	0.1	169	215
1979	1.2	0.2	233	209
1980	1.3	0.2	269	221
1981	1.6	0.2	288	249
1982	1.9	0.4	407	292
1983	1.9	0.6	404	301
1984	1.9	1	409	327
1985	2.1	1.4	432	368
1986	2.3	1.5	555	411
1987	2.9	1.6	583	427
1988	3.3	1.6	599	489
1989	4	1.7	970	508

Standardized diagnostic tools and techniques are very important but their implementation in the field might not be adequate in the lacking gold standard and proper validation which requires the confirmation by bacteriological tests (tab.4).

Table 4. Lab diagnosis of cattle

Year	Investigation serology	Number of Positive	%	Investigation bacteriology	Positive confirmed	%
1987	626385	11342	1,8	2081	131	6,3
1988	620697	11468	1,8	2138	124	5,8
1989	817526	5739	0,7	2277	78	3,4
1990	796700	4963	0,7	1482	43	2,9

Basic tests such as CFT and AT were used. AT was used to identify acute brucellosis and CFT was used to identify chronic brucellosis. There were no rapid tests as Rose Bengal Test (RBT). RBT was first used in 1986 (Kim, 2004), and ELISA and PCR are not used to study brucellosis because they are too costly.

8.3.8 *Brucella* characterization

Studies on typing *Brucella* species were done in 1960. Initially the studies on typing were conducted in the 60s under the leadership of A.A. Volkova and four types of *Brucella* were identified : *B. abortus bovis, B. melitensis, B. suis* and later on *B. ovis* was identified (Kim, 2004).

During the past years and up to 2008 bacteriological tests were used to differentiate brucellosis field strains from vaccine strains. In the framework of the Swiss TPH project in close collaboration with Institute of Veterinary Bacteriology Vetsuisse of the Bern University and the Cantonal Microbiological Laboratory (Istituto di Microbiologia Cantonale, IMC), Bellinzona, Switzerland *B. melitensis* was confirmed by biochemical and PCR tests. It enabled to collect about 250 aborted fetuses from sheep and cows from Naryn rayon of Naryn oblast. The cultures were isolated and 36 of them were shipped to Switzerland for typing at different times. *Brucella melitensis* was isolated from an aborted bovine fetus. The first molecular study of Kyrgyz brucellosis strains attempted to place them in the global phylogeny.

This study verified the findings of the previous serological studies on brucellosis incidence and its endemicity in the Naryn region and interspecies transmission. (Kasymbekov et al., 2013).

8.3.9 Political change and public health systems

Most of human brucellosis cases (88.5%) were registered in the areas where brucellosis is endemic in small ruminants, whereas less people were infected (11.5%) in cattle endemic territories. Human infections were more benign where cattle brucellosis occured. Besides a high human incidence in the areas with numerous small ruminant infections, human mortality was also higher in these regions (Vozhdaev et al., 1971).

Year by year thousands cases of human brucellosis were registered. Fig 8-3 shows the incidence of human brucellosis from 1996-2009. Since that time, human incidence has increased year by year and Kyrgyzstan has now one of the highest brucellosis incidence worldwide (Annual Incidence: 78 per 100 000 in 2007) (NatStatCom, 2013) (Fig. 8-3).

8.3.10 Epidemiology of brucellosis in animals and humans before, during and after the political transition

The epidemiology of brucellosis in Kyrgyzstan is influenced by numerous factors. Brucellosis in small ruminanats being the most important of them. On remote mountainous pastures common control measures in small ruminant species were mostly ineffective due to the complexity of their full implementation. To some extent the spread of brucellosis among animals could be restrained.

Despite the long-lasting vaccination period (40 years) using *B. abortus* S19, complete eradication of brucellosis among sheep and goat was not successful.

Over the last 5-6 years, immunization of sheep was hampered because of numerous reasons, including economic and institutional issues (treatment or sale of infected animals instead of culling), leading to a deterioration of the epidemiological situation on brucellosis. This was later confirmed by the incidence of brucellosis in the human population in Kyrgyzstan, which has raised sharply Fig 8-3.

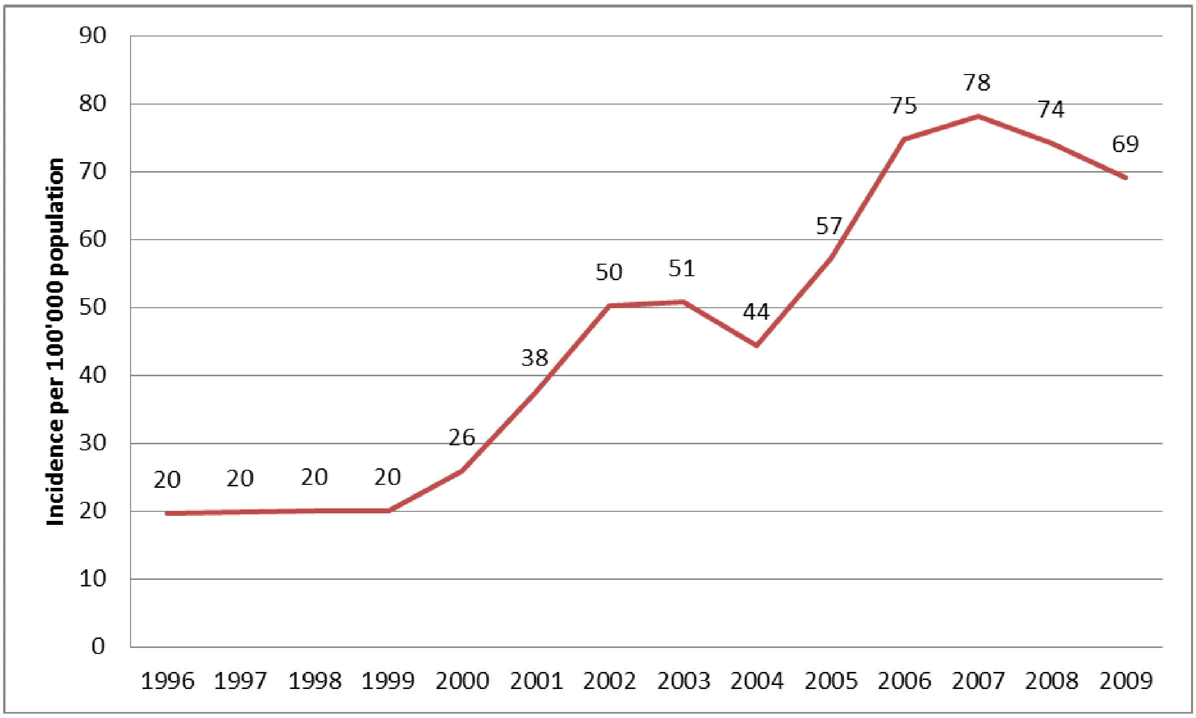

Figure 8-3. Human brucellosis incidence 1996-2009

The southern regions of the country reported the highest human brucellosis incidence over 1996-2009. Only for the past 5 years 2007-2011, 19887 infected people were registered, which are correlated with the high brucellosis incidence of small ruminants in the same region. The correlation of human and animal brucellosis was confirmed by a representative serological study for the whole country (Bonfoh et al., 2012).

In farms where brucellosis affects sheep and goats, the majority of people are infected at lambing and abortion periods. In most areas of the country the highest incidence of human brucellosis is recorded from April and June and is mostly linked with people conducting small ruminant livestock or persons processing raw materials from these animals.

Based on these observations it appears that the main source of human brucellosis in Kyrgyzstan is the contact with sheep and goat especially during the spring lambing period.

A possible source of infection could be found in dogs that are eating/carrying the aborted foetuses, thus spreading the bacteria around and contaminating the environment (Studentsov 1975). There is a possibility of infection from secondary carriers of brucellosis infection - particularly from cattle, pigs, yaks, horses and dogs, as well as from wild animals and blood-sucking arthropods (Shumilov et al., 2010).

8.4 *Discussion*

8.4.1 Analysis of the effects of past and current policies and interventions

Brucellosis represents a major issue for veterinary and public health systems, leading to economic losses and simultaneously endangering human population and animal species. In countries where brucellosis has been eradicated brucellosis control programs were generally started, financed and coordinated by the government itself (Zinsstag et al., 2007).

In Central Asia the activities on brucellosis elimination were not enough intensified probably also because of the newly organised farming system and political instability and political changes. Partly this is also due to poor public and private veterinary services and new (small-holder) livestock owners without sufficient knowledge on livestock production. One of the important reasons is payment for private veterinarians for their services. The reason for that is the lack of information on the state-of-the-art scientific achievements, non-use of advanced control technology, non-use of high-tech equipment, methods of diagnostics and specific preventive interventions. The information on these matters is hardly available to the wide range of professionals. These important topics are covered in international scientific articles and other publications that are not available and not translated to local languages. Therefore, valuable information on brucellosis control often remains unclaimed by professionals and livestock producers at the village, rayon and even the oblast level. Communication between veterinarians and physicians is absent (Zinsstag et al., 2009)

Issues of the implemented control programs of brucellosis in Kyrgyzstan revealed the lack of efficiency and highlighted the needs of an interdisciplinary approach combined with high quality vaccines in order to achieve the disease elimination. Interdisciplinary approaches should provide capacity building, functional laboratory systems, enlightenment and comprehensive studies supervised by efforts of the veterinary and public health sectors.

8.5 *Recommendations for brucellosis control policy and interventions in Kyrgyzstan and Central Asia*

The present historical review showed that control of brucellosis in Kyrgyzstan was and is still facing major political, cultural and economic challenges. The actual prevalence of brucellosis in Kyrgyzstan suggests that an effective mass vaccination of sheep, goats and cattle, following the guidelines of the World Animal Health Organization (OIE) is critical in order to control human brucellosis.

The recommendations suggest annual mass vaccination campaigns of sheep and goats (achieving immunity coverage of at least 80%) during 5 to 10 years, before moving on to vaccination of young replacement subjects along with testing and slaughtering.

Mass vaccination should be carefully monitored on an annual basis to assess the proportion of vaccinated animals. Otherwise, if not at least 80% of small ruminants are immunized, the risk of disease re-emergence will increase immediately as has been seen in other Asian countries and probability of increasing of disease like in Syria an Mongolia(Roth et al., 2003, Zinsstag et al., 2005, Pappas et al., 2006). The recording of new human cases, conducted at regular intervals (2 to 4 years) after animal vaccination should provide additional information on the effectiveness of mass livestock vaccination.

A test-and-slaughter strategy can be implemented only if public funds are available to compensate farmers for culled livestock and if other enabling conditions are in place. Of course, both interventions require well-functioning veterinary field and diagnostic laboratory capacity.

As a novel approach, we recommend simultaneous assessment of human and livestock disease incidence, which provides a good overall picture of the disease distribution and transmission.

The reasons for that a plight are inadequate counter brucellosis interventions, inappropriate selection of vaccines and their use, non-compliance with the cold chain, minimum vaccination coverage, minimum use of diagnostic tools and low public awareness. The awareness on the type and prevalence of the circulating brucellosis is equally important. It is necessary to define the brucellosis control strategy and approve brucellosis control programme at the national or regional levels.

9. Representative seroprevalences of brucellosis in humans and livestock in Kyrgyzstan

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Abstract: Kyrgyzstan reported 77.5 new human brucellosis cases per 100,000 people in 2007, which is one of the highest incidences worldwide. The Rose Bengal Test and the Huddleson test are currently used diagnostic tests in humans and animals in Kyrgyzstan. A national representative cross-sectional study using cluster sampling proportional to size in humans, cattle, sheep, and goats was undertaken to assess the apparent seroprevalence in humans and animals. A total of 4,936 livestock sera and 1,774 human sera were tested in Naryn, Chuy, and Osh Oblasts. The overall apparent seroprevalences of brucellosis were 8.8% in humans (95% CI 4.5–16.5), 2.8% (95% CI 1.6–4.9%) in cattle, 3.3% (95% CI 1.5–6.9%) in sheep, and 2.5% (95% CI 1.4–4.5%) in goats. Naryn Oblast had the highest seroprevalences in humans and sheep. More men than women were seropositive (OR = 1.96; $P < 0.001$). Human seroprevalence was significantly associated with small ruminant seroprevalence but not with cattle seroprevalence. Annual incidence of human brucellosis exposure, measured by serological tests, was more than ten times higher than the annual incidence of reported clinical brucellosis cases. This indicates an under-reporting of human brucellosis cases, even if only a fraction of seropositive people have clinical symptoms. In conclusion, this study confirms the high seroprevalence of brucellosis in Kyrgyzstan and warrants rapid effective intervention, among others, by mass vaccination of sheep and goats but also of cattle.

Keywords: apparent prevalence, incidence, brucellosis, human, livestock, serology, Kyrgyzstan.

9.1 *Introduction*

Brucellosis is a bacterial disease of livestock with a high zoonotic potential. Its transmission from livestock to humans occurs mainly by consumption of raw dairy products and by direct contact during delivery and abortion. *Brucella abortus* is mainly found in cattle and *B. melitensis* and *B. ovis* in goats and sheep. Humans are susceptible to both *B. abortus* and *B. melitensis*, the latter being most frequently reported in humans (Corbel, 2006).

Brucellosis occurs worldwide, particularly in developing and transition countries, but it is well controlled in industrialised countries. Kyrgyzstan has one of the highest brucellosis incidences worldwide with 36 reported annual human cases per 100,000 people in 2002 (Pappas et al., 2006) and 77.5 per 100,000 people in 2007 (promedmail.org, Archive Number 20090201.0449, published Feb 1, 2009).

However, it is not known if the Kyrgyz health system coverage is exhaustive and if all patients have access to care. It is likely that the true incidence is underestimated. An earlier study by Kozukeev et al. (Kozukeev et al., 2006) indicated the importance of cattle for the transmission of brucellosis in Kyrgyzstan, but the role of small ruminants in brucellosis transmission to humans is not clear. Brucellosis can be diagnosed by isolation (culture) of the bacteria, direct PCR of *Brucella* spp. genome in contaminated specimens or indirectly by antibody detection either in serum or milk (Corbel, 2006). The culturing of *Brucella* spp. from animal samples is complicated and dangerous and requires biosafety level 3, which is not currently available in Kyrgyzstan. Hence, we rely on available and more recent serological methods for diagnosis. The objective of this study was to assess representative brucellosis seroprevalences of livestock and humans for Kyrgyzstan and the association between human and livestock brucellosis seroprevalence. Representative estimates should be related to official reports and inform Kyrgyz public health and veterinary policy. The test characteristics of the Huddleson test in humans and Rose Bengal Test in livestock, which are currently used in Kyrgyzstan, are not known and there is no gold standard.

9.2 *Materials and methods*

9.2.1 Partnership between public health and the veterinary sector

In the fall of 2006, an integrated assessment of human and livestock brucellosis seroprevalence was undertaken jointly by the Republican State Center for Veterinary Diagnostics, the Republican Centre for Quarantine and especially dangerous diseases, the State Sanitary Epidemiological Department of the Kyrgyz Republic and the Swiss Tropical and Public Health Institute. Complementary Support of Community Action for Health facilitated this partnership(Zinsstag et al., 2009).

9.2.2 Study design

For this survey, the national census data on sheep and goat populations was used. A multistage cluster sampling proportional to size was determined by levels of Oblast (province), Rayon (district), and village (Bennett et al., 1991, Schelling et al., 2003). Three out of seven Oblasts were sampled. Naryn Oblast was selected by convenience (availability of previous serological studies), and the two others were sampled randomly in proportion to their size. In every Oblast, three Rayons, and in every Rayon, ten villages, were selected randomly in proportion to their size as shown in Fig 9-1 (Bennett et al., 1991)

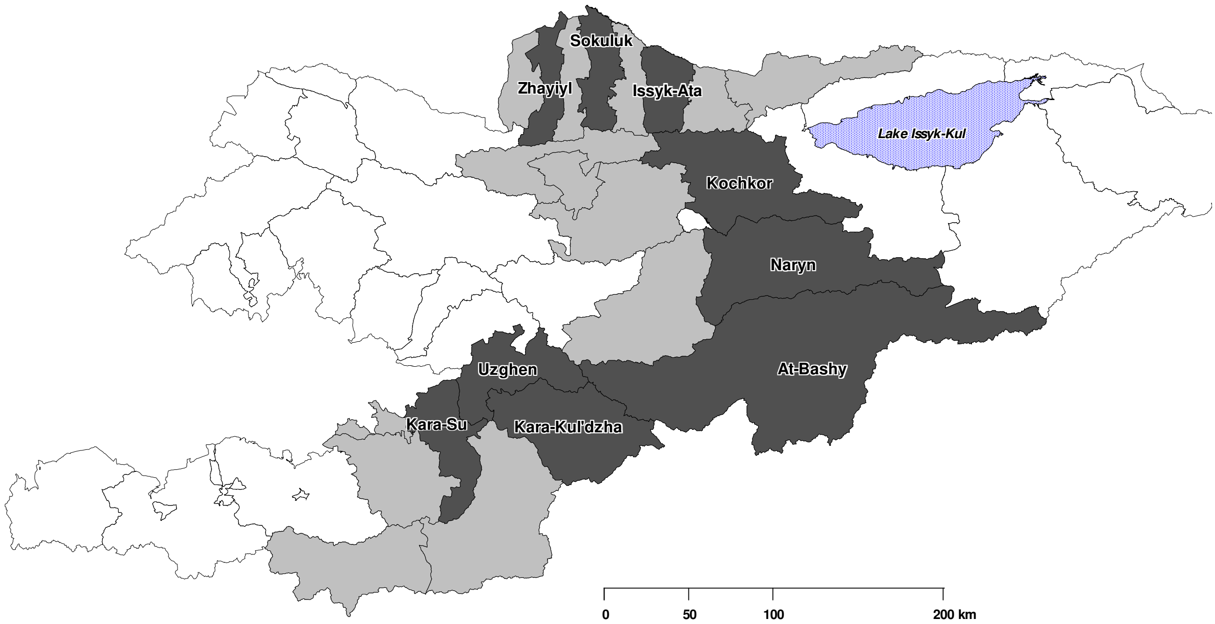

Figure 9-1. Map of Kyrgyzstan with selected Oblasts and Rayons
Light grey Rayons belong to selected Oblasts but not selected Rayons, *dark grey* Rayons are the nine in this study selected Rayons *(oblasts = regions and rayon = district)*

In this way, a total of 90 villages were selected randomly and they were used as cluster units. We assumed an intraclass correlation roh of 0.2 between clusters and a design effect of 4.8. Sampling 20 humans or livestock in every cluster provided a total sample size of 1,800 per species and 95% confidence limits of the estimate of <3% below and above the estimated seroprevalence, which is representative for the whole country.
This study was approved by the Ethical committee of the Cantons of Basel and the Kyrgyz Health Authorities. Informed written consent was provided by all persons participating in the study or of young children's mothers, after they had received detailed patient information. Overall, the proportion of sheep to goats was estimated at 6:1. Due to the lack of more detailed information, we assumed that this proportion is true for all Rayons. In the year of study (2006) very few animals were vaccinated against brucellosis in Kyrgyzstan and their influence on the serological results of this study were considered negligible.

9.2.3 Human and livestock sample collection

The study was conducted by three field teams composed of one veterinarian and one physician in the spring of 2006. A total of 103 villages in nine selected Rayons were visited. Venous blood was taken with 5 ml Vacutainer tubes and the age, sex, and names were recorded for all participants.

Blood of livestock (cattle, sheep, and goats) was obtained by venipuncture with 10 ml Vacutainer tubes. Livestock and human samples were not necessarily collected from the same households as the participation was voluntary. Human blood was transported to the Rayon Health Center and animal blood was transported to the Veterinary laboratory and centrifuged. All sera were shipped to Bishkek either to the Centre for Quarantine for testing human sera with the Huddleson agglutination test or to the Central Veterinary Laboratory in Bishkek for other tests described below.

9.2.4 Diagnostic tests

In Kyrgyzstan, the most common test is the Huddleson agglutination test for humans (official test) and the Rose Bengal Test (RBT) for animals. Human sera were subjected to the Huddleson test, the RBT (Bio-Rad Laboratories®), and an IgG and IgM ELISA (Chekit® IDEXX Laboratories Inc.) with anti-human-goat IgG and anti-human-goat IgM conjugates (Sigma-Aldrich Co®). The latter IgM test was only done for sera that were Huddleson test or RBT positive and IgG ELISA negative. Classification of ELISA was then positive if either the IgG ELISA and/or the IgM ELISA were positive. Livestock sera was tested with a RBT from the Kherson Bio-Factory, Ukraine, an indirect ELISA for ruminants (Checkit® IDEXX Laboratories Inc.) and the Fluorescence polarization assay (FPA) (*Brucella* FPA®, Diachemix, LLC). 2 ml tubes with sera were kept for further testing and were simaltaneously tested with the RBT at the Rayon veterinary laboratory.

Serological test results were interpreted according to the manufacturers' recommendations. Cut-off values were determined by a titration curve analogous to Bonfoh and Steinmann (Bonfoh et al., 2002, Steinmann et al., 2005). With the exception of FPA, all values were recorded as negative, doubtful, and positive. The cut-off value of FPA was at 90 mP. Since, agreements of pair wise comparison of tests (Kappa statistics) within species were generally better when all doubtful results were classified negative rather than positives, for binary classification of results, doubtful sera were classified as negative for all tests, although the best agreement between two serological tests was only a moderate agreement (Kappa < 0.6). For livestock sera, agreements between tests were better for cattle than for sheep and goats. For further statistical analyses, we used the Huddleson agglutination test for human sera and ELISA results for livestock sera.

9.2.5 Data analysis

Serological results were converted into dichotomous outcomes (1 = seropositive, 0 = seronegative), depending on the cut-off value of each test. Logistic regression, modelling for the outcome of seropositive humans and livestock (SP) included random effects (re) at various levels as follows: (1) at the level of rayon for the national representative estimate and for Oblast level, SP * re(Rayon) (Table 2). (2) For the analysis of human sera the level of village was used as random effect. Univariable models related SP individually with sex, age, and Oblast (Table 3). (3) Assessing a possible relationship between human and livestock seropositivity we have regressed SP * proportions of human and livestock seropositivity at rayon level (Table 4).

9.2.6 Analyses of human sera

Participants with the same family name within a village were regrouped in a unique family code. Age in years was categorized in steps of 10 years (0–10 years up to 80–90 years) and in three categories that represent the life stage: 0–18 years; 19–45 years; and 46 years. A unique code was created for all villages. We have tested if age, sex, and Oblast were associated with seropositivity in humans using logistic regression model (with a random effect at the village level) based on the likelihood-ratio test (LRT) (xtlogit procedure in STATA 10). The variances of the Huddleson test (humans) and ELISA (livestock) were the highest at the Rayon level (district) when compared to the levels of family, village, and Oblast (province). For the logistic regression of human sera, we considered village as a random effect. Age, sex, and Oblast were tested as univariable variables. The LRT test was used to test the significance of a variable (Table 3). Apparent seroprevalences are presented, because the test characteristics were not known for the different species.

9.2.7 Correlation between human and livestock seropositivity

Regressions have been done with SP* proportions of human and livestock seropositivity (all livestock, small ruminants [sheep and goats together] and the single species cattle, sheep, and goats) at a rayon level. Not more than one livestock category was tested in a model due to strong correlation between sheep and goat seropositivity at a Rayon level. The confidence intervals were constructed using a bootstrap re-sampling technique and the information on total number of individuals tested per rayon.

9.2.8 Estimation of incidence of apparent brucellosis seropositivity in humans

The age-specific apparent seroprevalence of the Huddleson tests was used to estimate the incidence of brucellosis seropositivity of a catalytic two way model under equilibrium conditions(Muench, 1959). We used data between the 2nd and 8th decade of life because data on younger or older patients were too sparse.

$$dS / dt = -aS + bI \quad (1)$$

$$dI/dt = aS - bI \quad (2)$$

where *S* is the susceptible population and *I* is the seropositive population. Parameter *a* is the incidence of seroconversion and *b* - the rate of loss of sero-positivity. Under equilibrium conditions the apparent seroprevalence *P* is related to *a* and *b* (Eq. 3).

$$P = a / (a + b) \quad (3)$$

We estimated the seroconversion rate *a* and the loss rate *b* simultaneously from the data using Vensim (Ventana Inc.) software, using a Powell algorithm analogous to Zinsstag et al., (Zinsstag et al., 2005).

9.3 *Results*

A total of 4,936 different livestock sera (1659, 1642, and 1635 from Naryn, Chuy, and Osh Oblast, respectively) and a total of 1,774 human sera (564, 606 and 604 from Naryn, Chuy, and Osh Oblast, respectively) were tested with at least one serological test (Table 5).

Table 5. Total sample size by species and number of samples examined with different diagnostic test

Species	Total	RBT (Biorad)[1]	RBT (Ukraine)[2]	ELISA (ruminant)[3]	ELISA IgG (human)[4]	ELISA IgM (human)[4]	FPA [5]	Huddleson [6]	Cf[7]
Cattle	1813	737	1560	1698	0	0	1691	0	21
Sheep	2076	761	1855	2029	0	0	2029	0	49
Goats	1286	764	1082	1209	0	0	1176	0	37
Humans	1775	644	0	0	1762	369	0	1774	0

[1]Rose-Bengal Biorad
[2]Rose-Bengal Ukraine
[3]indirect enzyme-linked immunosorbent assay detecting IgG in ruminants
[4]indirect enzyme-linked immunosorbent assay detecting IgG and IgM, respectively, in humans
[5]Fluorescence polarization assay
[6]Huddleson test
[7]Complement fixation test, was not further used in the analysis because the low number of available results

9.3.1 Representative apparent seroprevalences and human incidences

In Table 6, human and livestock seroprevalences are presented for every Oblast. The highest human brucellosis seroprevalence is found in Naryn Oblast and the lowest in Osh Oblast. Using Rayon as a random effect, the overall representative apparent seroprevalences of brucellosis for Kyrgyzstan were 8.8% in humans (95% CI 4.5–16.5), 2.8% (95% CI 1.6–4.9%) in cattle, 3.3% (95% CI 1.5–6.9%) in sheep, and 2.5% (95% CI 1.4–4.5%) in goats. The average duration of brucellosis seropositivity (1/b) was estimated at 10.9 years. Keeping this constant, human incidence of apparent sero-conversion is estimated at 0.88% (95% CI 0.43–1.77%) per year for the Huddleson test. This means that on average 880 (95% CI 400–1,770) persons per 100,000 are exposed to brucellosis per year. Extrapolated to the total Kyrgyz population of 5.2 million on average 45,882 persons per year get exposed to brucellosis.

9.3.2 Analysis of human sera for risk factors of brucellosis seropositivity

Additional data on age, sex, and village was available for 1,761 people. The proportion of sampled female participants varied between 0.42 and 0.78 in 9 Rayons and was 0.57 over the whole sample. The median age was 39 years (5–95% percentiles 17–66). More male participants were seropositive compared to females (in all three age classes) (OR = 1.96; p(LRT) < 0.001) (Table 7).

Table 6. Apparent sero prevalence estimates of brucellosis in Kyrgyzstan for human, cattle, sheep and goats per oblast, 2006

Oblast Species	*n*	Seroprevalence[a]	95% CI
Naryn Oblast			
Humans	564	18.3	14.0-23.7
Cattle	536	2.2	0.8-6.0
Sheep	562	8.9	5.8-13.5
Goats	561	2.5	0.9-7.0
Chuy Oblast			
Humans	606	8.9	6.9-11.5
Cattle	598	5.7	4.1-7.9
Sheep	610	3.0	0.0-9.7
Goats	434	2.7	1.3-5.7
Osh Oblast			
Humans	604	2.2	0.1-27.6
Cattle	564	1.6	0.5-4.5
Sheep	857	1.3	0.4-3.9
Goats	214	2.8	1.3-6.1

[a] Seroprevalences (and 95% CI) calculated with a logistic regression model specifying Rayon as a random effect.

9.3.3 Correlation between human and livestock seropositivity

At the Rayon level, both pooled small ruminant and sheep seroprevalences were correlated positively with human seroprevalences: an increase of 1% of small ruminant and sheep seroprevalences, increased human seroprevalence by 0.97 and 0.83%, respectively (Table 8).

Table 7. Risk factor of human seropositivity determined with the Huddleson test as outcome variable

Variable	Huddleson Test		Univariable logistic regression model	
	n	Pos %	OR	*p*(LRT)
Sex				
Female	1011	8.4	1	<0.001
Male	750	14.9	1.96 (1.4-2.7)	
Age category				
0-18	128	14.1	1	0.60
19-45	1049	11.7	1.2 (0.6-2.3)	
>45	584	9.6	1.03 (0.5-2)	
Oblast				
Naryn	560	18.4	1	<0.001
Chuy	600	8.3	0.4 (0.2-0.7)	
Osh	601	7.3	0.2 (0.1-0.5)	

The uni- and multivariable models used a random-effect on village level. P-values of the log likelihood ratio test (LRT) are presented

Table 8. Regression coefficient for human and livestock seropositivity

	Intercept (95% CI)	Slope (95% CI)
All livestock species	0.09 (0.05–0.12)	0.81 (-0.1 to 1.7)
Small ruminants	0.08 (0.05–0.11)	0.97 (0.3 to 1.7)
Cattle	0.15 (0.11–0.18)	-0.88 (-1.8 to 0.2)
Sheep	0.07 (0.05–0.10)	0.83 (0.3 to 1.4)
Goats	0.09 (0.07–0.12)	0.99 (-0.06 to 2.1)

9.4 *Discussion*

This study was designed as a representative cross-sectional assessment of brucellosis prevalence in humans and livestock in Kyrgyzstan in 2006. Because of the highly endemic epidemiological situation, the true incidence of clinical brucellosis cannot be stated. The estimated incidence of apparent seropositivity using the Huddleson test of 880 (95% CI 400–1,770) per 100,000 is 11 times higher than the officially reported number of brucellosis cases. There is no reference data indicating the proportion of seroconversion that leads to clinically manifested brucellosis in endemic areas. If 10% of seroconverted people showed clinical symptoms, the incidence of clinical brucellosis would be in agreement with the reported data. However, if 50% of seroconverted people showed clinical symptoms, the level of under-reporting would be 5.6 (95% CI 2.5–11.4). Studies in Saudi Arabia indicate a high proportion of clinical illness among seropositive family members of acute brucellosis cases (Almuneef et al., 2004, Alsubaie et al., 2005). Further studies require the assessment of the true incidence of human cases. The estimated duration of seropositivity of 10.9 years is in agreement with Beklemishev in Kazakhstan (cited v.Oldershausen, 1968) (v.Oldershausen, 1968), but we were not able to estimate a confidence limit, without time series data.

In our study, the apparent seroprevalences between the Oblasts ranged from 2.2 to 18.3% in humans and between 1.3 and 8.9% in livestock species with the highest variance for both at the Rayon level. Although, the Oblast seroprevalences of three livestock species were comparable, we have seen a significant correlation between human and small ruminant seropositivity at the Rayon level, thus suggesting Rayon specific clustering. This likely reflects the relatively localized contact networks and marketing system of Kyrgyz livestock. A sheep–cattle relationship has been confirmed by detecting *B. melitensis* strains in sheep and in two cattle (data not shown). In humans, male participants were more frequently seropositive than female participants but no difference between age classes was found. More data needs the understanding of the frequency of brucellosis in children, where transmission may be due to both direct exposure to contaminated livestock material and consumption of raw livestock products. The higher risk of male seropositivity in human adults indicates that exposure in these rural villages may more likely be due to direct (professional) close contact with infected livestock, rather than because of the exposure through contaminated livestock products, but this hypothesis requires further investigation. In a study about risk factors of human brucellosis in Kyrgyzstan, Kozukeev et al. (Kozukeev et al., 2006) reported a higher, however both non-significant, odds ratio (OR) for keeping cattle (adj. OR = 4.5) followed by goats (adj. OR = 1.6). However, we find no correlation between cattle and human seropositivity (indeed, we found a negative correlation) and we only find a significant correlation with sheep, but not for goats. Such contradictory results, also due to different study designs, reflect the difficulty in determining an association of infection between humans and animals for zoonotic diseases. Confirmation of transmission chains by molecular analysis of strains isolated from humans and different livestock species is warranted.

This study was intended to inform Kyrgyz policy on brucellosis epidemiology by providing representative data using existing and new diagnostic tests. As it is important to adapt assessments to local health policy decision pathways(Habicht et al., 1999), our analysis of human apparent seroprevalence is based on the Huddleson agglutination test, which is the officially recognized diagnostic test in Kyrgyzstan.

In a complementary analysis, we assessed the test characteristics of all used tests through a Bayesian model for the estimation of true seroprevalence in the absence of a gold standard, which are published separately. The results will be further used to estimate the cost of brucellosis to the Kyrgyz economy analogous to Roth (Roth et al., 2003).

The presented study is an example of an integrated human and animal study design under a ‘‘One Health’’ paradigm, facilitating the identificating of the source of zoonosis in the animal reservoir in a single step because of a connected study design and the ability to assess the impact of the disease in multiple sectors, notably the health and livestock production sectors. It is thus an example of the added value of a closer cooperation between human and animal health practitioners (Zinsstag et al., 2009).

9.5 *Conclusion*

Our study confirms the high seroprevalence of brucellosis in Kyrgyzstan and suggests that the annual incidence of human brucellosis exposure, measured by serological tests, is ten times higher than the annual incidence of reported clinical brucellosis cases. This indicates the underreporting of human brucellosis cases even if only a fraction of the seropositive persons have clinical symptoms. Human brucellosis seroprevalence was most closely associated with brucellosis seroprevalence in sheep. Effective mass vaccination of sheep, goats, and cattle, following the World Animal Health Organization (OIE) guidelines are warranted to control human brucellosis at its source. Further research is needed to further confirm the human–livestock linkages by molecular typing of *Brucella* strains from humans and livestock, to relate human Brucellosis seropositivity and clinical symptoms in a highly endemic area like Kyrgyzstan and to monitor control policies on their effectiveness.

9.6 *Acknowledgments*

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9.7 *Open access*

10. Molecular epidemiology and antibiotic susceptibility of livestock *Brucella melitensis* isolates from Naryn oblast, Kyrgyzstan

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Key words
Sheep, cattle, *Brucella melitensis*, aborted fetus, culture, antibiotic susceptibility, variable number tandem repeat (VNTR), MALDI-TOF MS, and Naryn, Kyrgyzstan

10.1 *Abstract*

The incidence of human brucellosis in Kyrgyzstan has been increasing in the last years and was identified as a priority disease needing most urgent control measures in the livestock population. The latest species identification of *Brucella* isolates in Kyrgyzstan was carried out in the 1960s and investigated the circulation of *Brucella abortus*, *B. melitensis*, *B. ovis*, and *B. Suis.* However, supporting data and documentation of that experience are lacking. Therefore, typing of *Brucella* spp. and identification of the most important host species are necessary for the understanding of the main transmission routes and adoption of the effective brucellosis control policy in Kyrgyzstan. Overall, 17 *B. melitensis* strains from aborted fetuses of sheep and cattle isolated in the province of Naryn were studied. All strains were susceptible to trime thoprimsulfamethoxazole, gentamicin, rifampin, ofloxacin, streptomycin, doxycycline, and ciprofloxacin. Multilocus variable number tandem repeat analysis showed low genetic diversity. Kyrgyz strains seem to be genetically associated with the Eastern Mediterranean group of *Brucella* global phylogeny. We identified and confirmed transmission of *B. melitensis* to cattle and a close genetic relationship between *B. melitensis* strains isolated from sheep sharing the same pasture.

10.2 *Introduction*

Agriculture is a key component of Kyrgyzstan's economy and livestock plays a major role in the daily lives of the population. Sixty four percents of the population live in rural areas and rely on agriculture for their livelihoods. Up to 76% of the rural population of the country is classified as poor.(FAO, 2010)

Since independence in 1991, veterinary support ceased then largely and the incidence of diseases transmitted from animals to humans (zoonoses) has increased dramatically in many regions in Kyrgyzstan. Brucellosis, anthrax, rabies and echinococcosis are of public health concerns and present serious risk to the human and livestock health. The incidence of brucellosis has increased steadily and Kyrgyzstan has now one of the highest human brucellosis incidences worldwide (annual incidence: 77.5 new cases per 100,000 people in 2007) (NatStatCom, 2009).

Currently, Kyrgyz communities are concerned about the effective reduction of brucellosis burden for people and livestock.

The latest species identification of *Brucella* spp. cultures in Kyrgyzstan was done in the 1960ies. Both *B. abortus* and *B. melitensis* were isolated from cattle. *B. melitensis* infections in cattle were thought to be a spill-over from sheep. Smirnov and Tretyakova noted that abortions in cows after immunization with S19 were most often seen in herds infected with *Brucella* spp. *B. melitensis* was isolated from vaccinated and non-vaccinated sheep 3,4 . The authors concluded that *B.* melitensis steadily adapted to sheep (Kim, 2004).

At present, the circulating genotypes of *Brucella* spp. are not known. This is true for virtually all Central Asian regions. Bacteriological confirmation of *Brucella* spp.-induced abortions is almost absent, because the owners do not report suspected abortions to the veterinary services. Here we report recently isolated *Brucella* spp. strains from sheep and cattle, which were collected in addition to a representative national study on brucellosis sero-prevalence in humans and livestock (Bonfoh et al., 2012) and to cost of disease studies in Kyrgyzstan (data not shown). The results contribute to the understanding of the main transmission routes and effectively inform brucellosis control policy in Kyrgyzstan.

10.3 *Material and methods*

10.3.1 Sampling sites and survey

The study site was in Naryn oblast, which has the highest human brucellosis incidence in Kyrgyzstan and most of its population has an income through selling of animals and animal products. First primary isolations of *Brucella* strains from aborted fetuses were done at the veterinary laboratory of the Naryn province in November 2008. All public and private veterinarians were informed about the on-going project on brucellosis. The farmers were informed beforehand and were asked to report abortions through local village veterinarians; leaflets with information were distributed through veterinarians and the announcement was published in the province newspaper. Abortions from sheep and cattle were collected during the lambing seasons of 2009 and 2010. In general, the lambing season starts in January and continues till March and April and thus first abortions can occur in late November/December. Veterinarians brought the collected specimens – aborted sheep and cattle fetuses - dissected on site – to the Naryn laboratory. Stomach content was collected in tubes and liver, spleen, kidney, lung, heart and other tissues were collected in plastic bags. Veterinarians collected accompanying basic information on the animals and farms such as geographic position and keeping of other than affected animals. Two weeks after the abortion, a visit to the affected farm allowed blood sampling of farm animals for serology (data not shown) and interviewing livestock holders with a questionnaire to obtain epidemiological data. Total number of fetuses collected by the veterinarians was 125 from the whole district and positive isolates by the Urease and Oxidase were selected for further study.

10.3.2 Cultures

Primary cultures were done at the Naryn zone Center for Veterinary Diagnostic and specimens were frozen. When culture was negative, frozen specimens were re-cultured at the Republican Center for Veterinary Diagnostic in Bishkek.

Stomach content and organs of the aborted fetuses were cultured onto *Brucella* selective agar (bioMe´rieux, Switzerland) and onto own produced *Brucella* selective agar (with agar, horse serum and antibiotics from Oxoid, Switzerland). Strains were cultured on *Brucella* agar at 37uC with 10% CO2 for 2 days (Marianelli et al., 2007).

10.3.3 Antibiotic resistance testing

For the investigation of sensitivity of the cultures to phenotypic antibiotic resistance to 7 different drugs was assessed by the standard E-tests (bioMe´rieux, Switzerland) on Mueller-Hinton blood agar (MHS2, bioMe´rieux SA, France) and their minimum inhibitory concentrations (MIC) were determined additionally. The following antibiotics were tested: trimethoprimsulfamethoxazole (SXT) (1.25+23.75 mg), gentamicin (GM) (10 mg), rifampicin (RA) (30 mg), ofloxacin (OFX) (1 mg), streptomycin (S), (15 mg), doxycyclin (D), (30 mg), and ciprofloxacin (CIP) (5 mg). Inducible clindamycin resistance test (“D-zone” test) was also carried out for all isolates. Results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines; for the purpose of this study, intermediate results were classified as resistant.

10.3.4 DNA extraction and genotyping

DNA was extracted from one loopful of bacterial cells grown for 48 h on chocolate agar, and single colonies were isolated by using the tissue protocol of the QIAamp DNA minikit (Qiagen, Germany). DNA concentrations were measured by UV spectrophotometry (Shimadzu, Japan). Multiple Loci Variable Number of Tandem Repeat Analysis (16 locus MLVA) typing was performed with the 17 isolates according to the protocol initially proposed by Le Fle`che et al. (Le Flèche et al., 2006) and modified by Al Dahouk (Al Dahouk et al., 2007). To include 1 additional locus, bruce19 the protocols are available online on the MLVA-NET for *Brucella* (http://mlva.u-psud.fr). In brief, the assay comprised the typing of eight mini-satellites of the so-called panel 1 (bruce06, bruce08, bruce11, bruce12, bruce42, bruce43, bruce45, and bruce55), three micro-satellites of the panel 2A (bruce18, bruce19, and bruce21), and five micro-satellites of the panel 2B (bruce04, bruce07, bruce09, bruce16, and bruce30).

Sixteen published VNTR loci were PCR-amplified in parallel and the numbers of tandem repeats determined after electrophoresis on agarose gel. DNA extracts of *B. melitensis* 16MT and vaccine strain Rev1 were used as positive controls. The obtained MLVA patterns of each sample were then matched with an online database (http://minisatellites.u-psud.fr/MLVAnet/querypub1.php) for identification.

10.3.5 MALDI-TOF MS analysis

A small amount of a colony of each pure culture was transferred to a FlexiMass target well using a disposable loop and overlaid with 1.0 ml alpha-cyano matrix solution (CHCA; 40 mg alphacyano in 33% acetonitrile, 33% ethanol, 33% ddH2O and 1% trifluoroacetic acid). The spotted solution was air-dehydrated during 1–2 min at room temperature and analysed with MALDI-TOF MS Axima Confidence spectrometer (Shimadzu-Biotech Corp., Kyoto, Japan). The reference strain Escherichia coli K12 (GM48 genotype) was used as a standard for calibration and as reference measurement for quality control. Mass spectrometry (MS) analyses were performed in positive linear mode in the range of 2,000–20,000 mass-to-charge ratio (m/z) with delayed, positive ion extraction (delay time: 104 ns with a scale factor of 800) and an acceleration voltage of 20 kV. For each sample, 2650 averaged profile spectra were stored and used for analysis. All spectra were processed by the MALDI MS Launchpad 2.8 software (Shimadzu Biotech) with baseline correction, peak filtering and smoothing. A minimum of 20 laser shots per sample were used to generate each ion spectrum. For each bacterial sample, 50 protein mass fingerprints were averaged and processed. Spectra were analysed using SARAMIS (Spectral Archive and Microbial Identification System, AnagnosTec GmbH) at default settings. Cladistic analysis were based on the peak patterns of all analysed strains submitted to single-link clustering analysis using SARAMIS with an error of 0.08% and a m/z range of 2,000 to 20,000 Daltons.

10.3.6 Data analysis

Allelic diversity was calculated using the formula below, where xi is the

$$h= 1-\sum x_i^{\ 2}\,[n\,/\,(n\text{-}1)]$$

relative frequency of the *i*- the allele at the locus, n- the number of isolates in the sample and (n/(n-1) is a correction for bias in small samples (Selander et al., 1986). VNTR data was the basis for the phylogenetic analysis using SAS (Statistical Analysis Systems Inc. Cary, USA) proc cluster using the unweighted pair-group method with arithmetic averages, (UPGMA). For the assessment of the phylogenetic place of the Kyrgyz isolates, strains were selected from the online database by Maquart (Bricker et al., 2003, Maquart et al., 2009a). (1471-2180-9-145-S1.xls; http://www.biomedcentral.com/1471-2180/9/145). Isolates were selected to reflect the diversity of geographical origin and the different biovars. Phylogenetic trees were drawn using SAS proc tree.

10.3.7 Ethics statement

The Ethics Committee of the University and the state of Basel has approved this study without restrictions at the meeting of January 11, 2007 (Reference number 02/07). The project conforms to the ethics requirements on animal testing (Published in Schweiz. Ärztezeitung, 2006, Band 87, S. 832–837) of the Swiss Academy of Medical Sciences and the Swiss Academy of Natural Sciences. Animal owners were asked for consent to test aborted fetuses of their livestock for brucellosis.

10.4 *Results*

Livestock systems and management of herds from which *B. melitensis* were isolated varied between owners. Livestock owners kept cattle and small ruminants together and practiced seasonal transhumance to high-altitude pastures. They sometimes also kept entrusted animals from several owners and actively traded animals. During the lambing seasons 2009 and 2010 in Naryn, 125 aborted fetuses (112 from sheep and 13 from cattle) were collected in 4 villages and in Naryn city (Figure 10-1). The isolation rate for sheep was 8.9% and 15% for cattle but the difference is not statistically significant. Urease and oxidase positive cultures were selected and for 17 out of 23 isolates *B. melitensis* were confirmed by MALDI-ToF MS and MLVA-16 (Figure 2).

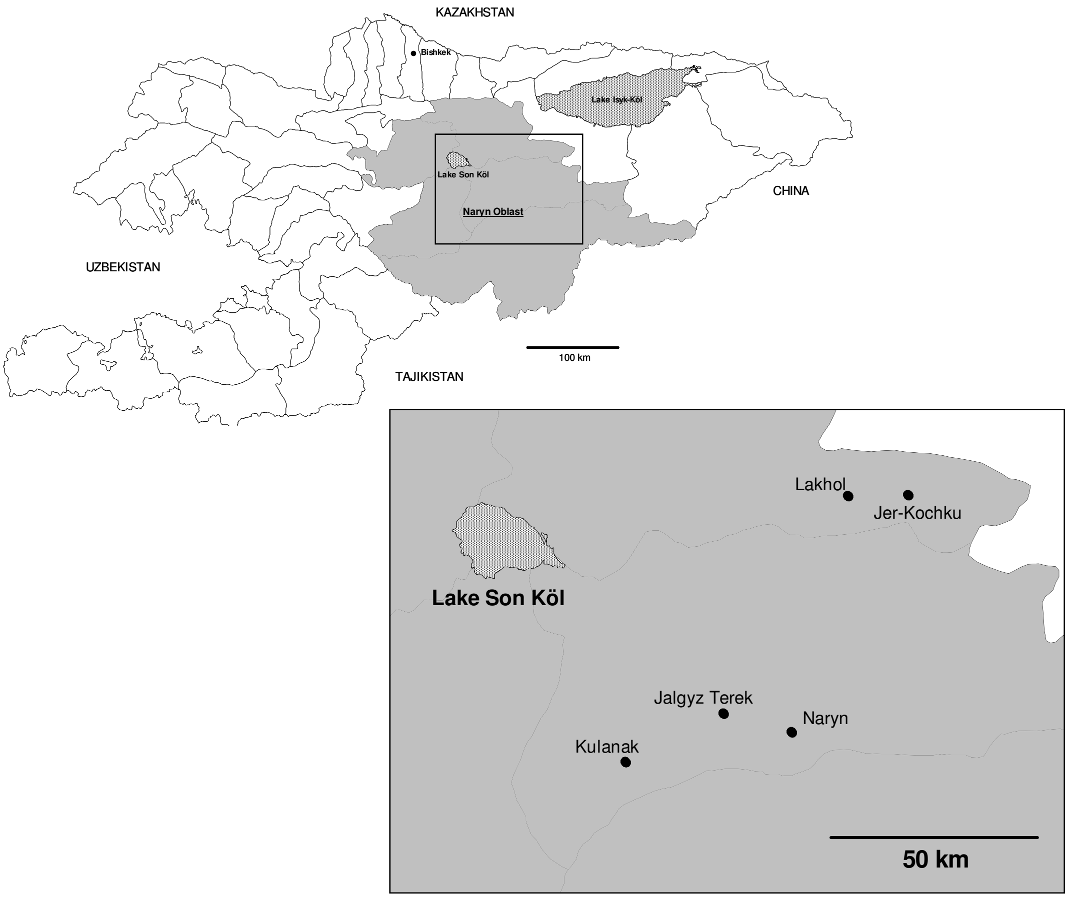

Figure 10-1. Geographic location of the Naryn oblast and the villages from where *Brucella melitensis* was isolated.
doi:10.1371/journal.pntd.g001

The dendrogram is based on MLVA-16 genotyping assay showing the relationship of 15 sheep and two cattle isolates of *Brucella melitensis*. For each locus showing variability, the number of tandem repeats is presented. Additional information is provided on the type of sample, the local strain designation, and the serial number of the animal owner and the name of the village in Naryn oblast. Numbers in brackets indicate repeated isolates from the same animal. Isolates not indicated as primary were frozen prior to cultivation.

Sample	year of sampling	Owner	Village	Species	nimal I	Isolated from	Bruce04	Bruce16	Bruce30	Strain No.
102A01C2F BC8	2009	1	Jalgyz-Terek	Sheep	4(1)	Liver aborted fetus	4	5	5	1
102A01C2E BC9	2009	1	Jalgyz-Terek	Sheep	4(2)	Spleen aborted fetus	4	5	5	2
102A01C3F BC10	2009	1	Jalgyz-Terek	Sheep	5	Liver aborted fetus	4	5	6	3
102A01C5A BC11	2009	1	Jalgyz-Terek	Sheep	9	Primary isolate abortus fetus	4	5	6	4
102A02E1F BC14	2009	2	Naryn	Cattle	7	Liver aborted fetus	4	5	6	5
102A02E2A BC15	2009	2	Naryn	Cattle	8	Primary isolate aborted fetus	4	5	6	6
102A01C6A BC13	2009	1	Jalgyz-Terek	Sheep	10	Primary isolate aborted fetus	5	5	6	7
JF4886	2010	6	Kulanak	Sheep	30	Lung aborted fetus	5	6	6	8
102A03C3F BC12	2009	3	Kulanak	Sheep	29B	Liver aborted fetus	5	6	7	9
JF4883	2010	4	Jer-Kochku	Sheep	48	Liver aborted fetus	7	5	5	10
JF4884	2010	4	Jer-Kochku	Sheep	48 (2)	Spleen aborted fetus	7	5	5	11
JF4887	2010	4	Jer-Kochku	Sheep	48 (3)	Liver aborted fetus	7	5	5	12
JF4891	2010	4	Jer-Kochku	Sheep	47	Spleen aborted fetus	7	5	5	13
JF4889	2010	4	Jer-Kochku	Sheep	47 (2)	Heart aborted fetus	7	5	5	14
JF4890	2010	5	Lakhol	Sheep	49	Spleen aborted fetus	7	5	5	15
JF4888	2010	6	Kulanak	Sheep	19	Liver aborted fetus	7	5	5	16
JF4885	2010	6	Kulanak	Sheep	19 (2)	Spleen aborted fetus	7	5	5	17

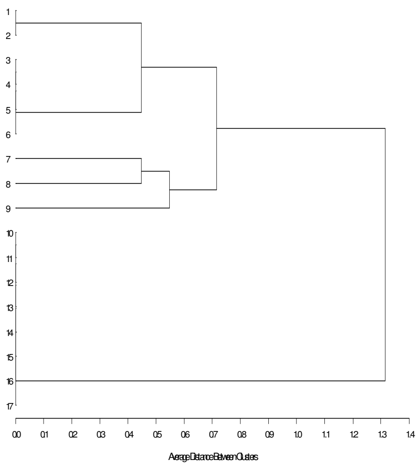

Figure 10-2. Dendogram showing the relationship of 15 sheep and two cattle isolates of *Brucella melitensis*

Of 17 isolates, 15 ones were isolated from sheep and two from cattle. All strains were susceptible to the tested antibiotics. The allelic diversity of VNTR (h) was low, with only three loci showing variation in the numbers of repeats. For locus 4 it was 0.6, for locus 16 - 0.16 and 0.49 for locus 30 (Table 9).

Table 9. Allelic diversity of VNTR loci (all other loci were equal)

reference # of copies	Number of repeats 4	5	6	7	allelic diversities
bruce 4	0,35/6	0,18/3		0,47/8	0,601
bruce 16		0,88/15	0,12/2		0,162
bruce 30		0,59/10	0,35/6	0,06/1	0,496

Nominator – allelic diversity index
Denominator – number of repeats
doi:10.1371/journal.pntd.0002047.t001

All other loci did not show any variation. Eight out of 17 strains were grouped into 6 different clusters. However, it should be noted that more than one isolate was obtained from four animals. Isolates of cluster 2 were found in sheep and cattle herds of two different owners. With regard to the geographical location, the Kyrgyz isolates are the closest to strains from Kazakhstan, Israel and Iraq which are all biovar3 (Figure 10-3)(Maquart et al., 2009a).

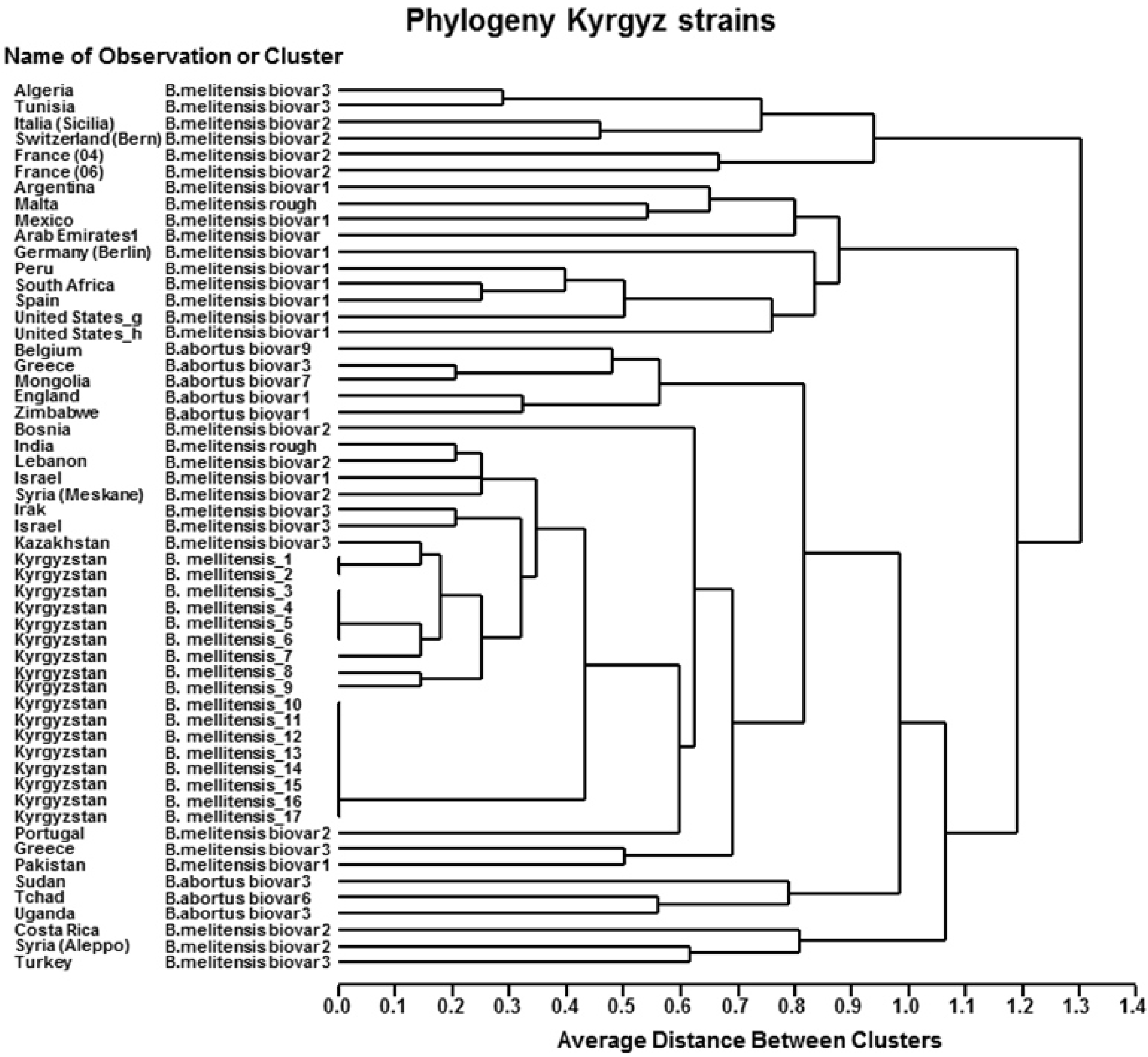

Figure 10-3. Dendogram of the relationship of the Kyrgyz isolates when compared to global database 11.

10.5 *Discussion*

B. melitensis isolates from Kyrgyzstan appear to be close to the so-called Eastern Mediterranean group (Figure 10-3) (Maquart et al., 2009a), but a more detailed analysis and more isolates are required to conclusively determine the position of Kyrgyz *Brucella* in the global phylogeny. All *B. melitensis* isolates from Naryn Oblast were closely related according to VNTR patterns. Isolates belonging to the second cluster from the top (Strain No. 3–6) (Figure 10-2) were found in sheep and cattle herd of two owners, indicating that the strains circulated between farms and were transmitted between small ruminants and likely to cattle during communal grazing. These two owners live 45–50 km apart. The owner of the cattle lives in the city of

Naryn and his cattle graze is on a summer pasture with several other animals suggesting rural/urban spill over through sharing common pasture. Eight isolates (sixth cluster from the Top in Figure 2) from sheep stem from Jer-Kochku and Lakhol, two villages 10 km apart. The animals from which they originated use the same pasture for grazing, except for the two strains from Kulanak located more than 80 km far from Jer-Kochku and Lakhol. This may indicate a contact relationship between Kulanak, Jer-kochku and Lakhol (Figure 1). Owner 1 has sheep in which three *B. melitensis* genotypes are present (Three B.melitensis genotypes are available in sheep of Owner 1). A better understanding of the contact network of each animal's owner could possibly further explain the genetic diversity.

Multiple strains were isolated from liver, spleen and heart in three animals (Figure 2). Isolates from different organs of the same animal had always the same VNTR pattern, hinting to a likely mono infection. The isolation of *B. melitensis* in sheep and cattle is the first recent confirmation by culture since the 1960ies in Kyrgyzstan. It was expected because brucellosis in cattle was not a problem a decade ago and increasing sero-prevalences and brucellosis abortions in cattle were observed during the past years. It was therefore speculated that cattle may be a spill-over host of *B. melitensis* from small ruminants. More isolates are needed to further consolidate this finding. If confirmed, this may have policy implications for on-going pilot mass livestock vaccination campaigns, considering cattle vaccination. We found no antibiotic resistance and therefore the standard regimen used in Kyrgyzstan (i.e., Gentamicin plus Doxicycline) is likely to be adequate for humans. However, human isolates should be tested as well. The use of antibiotics in livestock is not clearly recommended.

This study confirms on-going transmission of *B. melitensis* in sheep and likely to cattle in the province of Naryn in Kyrgyzstan. The high genetic homogeneity indicates rather clonal expansion and on-going transmission, confirming serological observations (Bonfoh et al., 2012). The role of cattle in the transmission of *B. melitensis* should be examined more specifically. Further studies on human brucellosis strain characteristics are needed to confirm sheep as the suspected principal source of livestock to human transmission (Bonfoh et al., 2012). For this purpose more discriminatory methods than VNTR may be needed. Further collection of isolates from aborted fetuses including information on contact networks are needed to monitor the success of the ongoing mass vaccination campaign and to allow calibrating VNTR dynamics in space and time.

10.6 *Conclusion*

We conclude that *B. melitensis* is endemic in Naryn oblast and sheep are apparently the main host. *B. melitensis* is also transmitted to cattle. In the study period we observed no abortions in goats and hence consider them less important for brucellosis transmission in Naryn oblast. Our findings confirm an earlier serological study, which related human brucellosis sero-prevalence to sheep but not to goat and cattle (Bonfoh et al., 2012).

10.7 *Acknowledgments*

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10.8 *Author contributions*

The experiments were conceived and designed by: JK ES JZ. The experiments were performed by: JK JI MB NS SP PP MT CB KA ZJ. The data was analysed by: JK MB MT ES JZ. Contributed reagents/materials/analysis tools: JK NS SP PP MT CB KA. The paper was written by: the JK MT ES JZ.

11. Societal cost of brucellosis to Kyrgyzstan

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Abstract

Objective: Estimate societal cost of brucellosis for Kyrgyzstan

Materials: Cost data were collected from public health authorities and 95 brucellosis patients. Herd composition and livestock productivity were collected from the Ministry of Agriculture and the National Statistical Committee farmer surveys.

Methods: Cost of disease for human health and livestock sectors to Kyrgyzstan was modelled using a cross-sector stochastic simulation of livestock production and estimated for 2006-2011. We developed a demographic model for livestock to estimate cost of disease with and without brucellosis. Societal cost and number of disability adjusted life years (DALYs) lost were the primary outcomes.

Findings: Net present cost of brucellosis to the public health sector (2006 – 2011) was estimated at 1.38 million USD (95% CI 1.22–1.55) and the private net present health cost was 6.02 million USD (5.5- 6.5). The overall net present health cost was 23.0% of the societal net present cost of 32.5 million USD (25.7– 39.6). For 2006-2011, losses of the net present value were 13.7 million USD (7.1 – 20.7) for cattle, 0.78 million (0.49 – 2.05) for sheep and 0.75 million (0.08 – 1.43) for goat products. The incremental asset value was estimated at 2.66, 1.63 and 0.11 million USD for cattle, sheep and goats, respectively. Human brucellosis caused 14,520 DALYs (12,496-19,901)

Conclusion: The societal cost of brucellosis is very likely higher than the cost of livestock mass vaccination, justifying investments for elimination of brucellosis in Kyrgyzstan.

Key words

Brucellosis, public and private health cost, income loss, cattle, sheep, goats, Kyrgyzstan

11.1 *Introduction*

Brucellosis in Kyrgyzstan was estimated from reports and literature as having one of the highest annual human incidences among the countries of the Former Soviet Union (FSU) and worldwide in 2002 (Pappas et al., 2006). Brucellosis control became a national priority due to high prevalence observed in livestock and humans (Kozukeev et al., 2006, Näscher, 2009, Bonfoh et al., 2012, Kasymbekov et al., 2013). Official data indicated an increase in human incidence since the end of the socialist rule, up to 80 per 100,000 in 2011 (NatStatCom, 2013) (Figure 1). Although there is currently rather an increased awareness of the impact and importance of the disease (Wolfram et al., 2010) and despite continuous progress in brucellosis control, it still remains a major public health concern

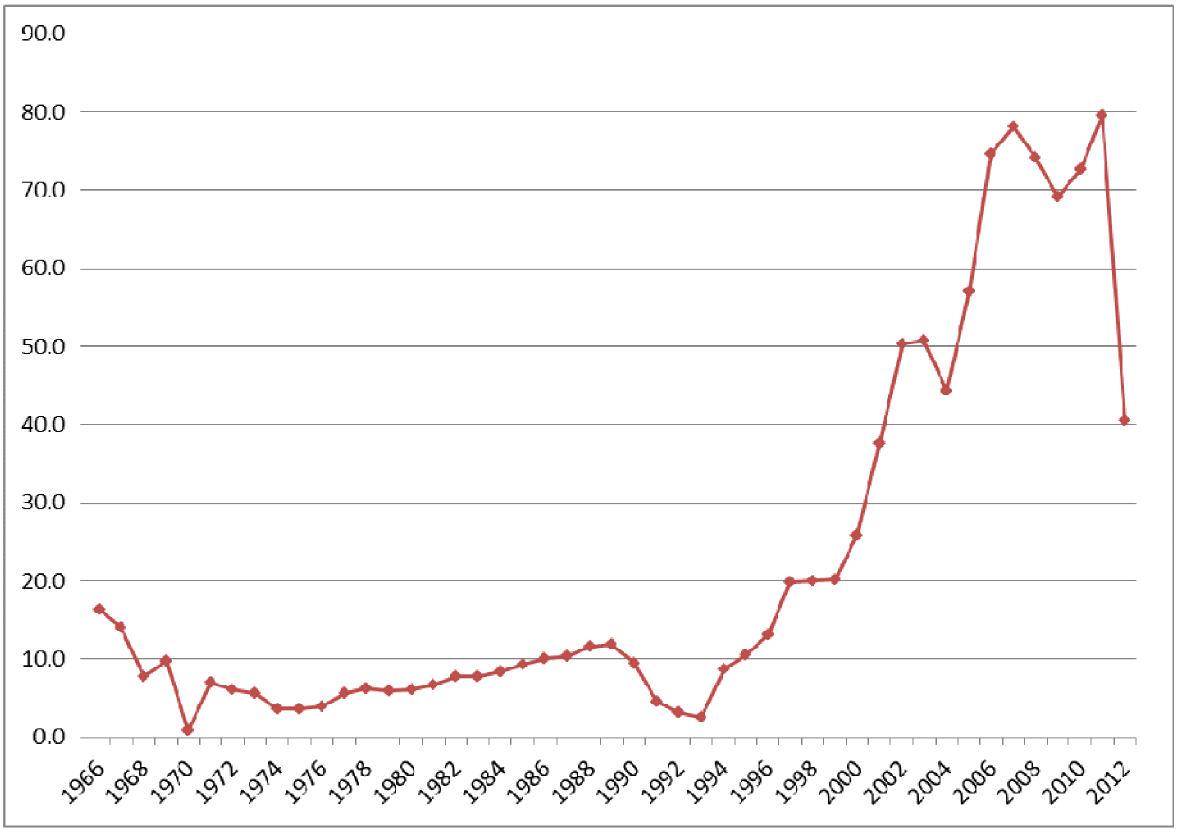


Figure 11-1. Human brucellosis incidence 1966-2012

A representative population-based serological survey in Kyrgyzstanwas estimated up to five times higher than that listed in official reports (Bonfoh et al., 2012) and *B. melitensis* has been characterized using molecular typing methods which showed that sheep appear to be the main host of *B. melitensis* (Kasymbekov et al., 2013).

Livestock production is one of the most important economic activities in Kyrgyzstan. The livelihood of two thirds (2/3) of the Kyrgyz population depends on livestock production (FAO, 2010). The pastoral resources in Kyrgyzstan are limited and endangered by degradation processes in some areas (Näscher, 2009). Infected livestock (including foetuses and retained placenta) and contaminated livestock products are the sources of human infection. The appreciation of the demographic trends of the Kyrgyz livestock population is critical for natural resource management and animal disease control (Zinsstag et al., 2005). The latter is particularly important for planning and monitoring of animal disease interventions(Racloz et al., 2013). Because there is almost no human to human transmission, brucellosis can only be eliminated in humans through control in livestock. However, the cost-effectiveness of control programmes in livestock for human health must be established (Zinsstag et al., 2005). A livestock demographic model allows for simulation of national population dynamics providing a precise tool for comparison of disease frequency in populations with and without control measures (Caldow et al., 2001).

Preliminary estimates for Kyrgyzstan showed that the annual losses in livestock production due to brucellosis and the annual costs of human brucellosis treatment totalled between 5 and 15 million USD (Bonfoh et al., 2012). In Mongolia, the benefit-cost ratio of livestock brucellosis mass vaccination was estimated at 3.2 from a multi-sectoral perspective including the agriculture and health sector. Using a cost-sharing scenario based on the separable cost method, the public health sector should contribute 11% to the intervention costs resulting in a cost-effectiveness of 19 USD per disability adjusted life year (DALY) averted (95% CI 5.3 – 486.8), whereas including private economic gain due to improved health should increase the public health share to 42% and decrease the cost-effectiveness to 71.4 USD per DALY averted (95% CI 19.7 – 1824.1) (Roth et al., 2003). Brucellosis prevention and control poses several challenges to national authorities, particularly to the Veterinary and Public Health services of FSU countries. For the Government of Kyrgyzstan it is important to get better insight of the true losses to the livestock industry, the cost created in the public health sector, and the cost-effectiveness of interventions. The objective of this study was to estimate the societal cost of brucellosis to inform Kyrgyz health policy on options for control.

11.2 *Materials and methods*

11.2.1 Cross-sector cost analysis

We conducted an incremental cross-sector cost analysis for brucellosis to the public and private health sectors as well as income loss and losses to sheep, goat and cattle production for the period 2006 to 2011. We applied a framework considering human health and animal production similar to Roth et al. (Roth et al., 2003, Narrod et al., 2012). The data of the representative population-based serological survey in 2006 an apparent seroprevalence of 8.8% in humans (95% CI 4.5-16.5), 2.8% (95% CI 1.6-4.9) in cattle, 3.3% (95% CI 1.5-6.9) in sheep and 2.5% (95% CI 1.4-4.5) in goats were used for analysis (Bonfoh et al., 2012). The sum of all described costs was considered as the cost of brucellosis to the Kyrgyz society. For each sector the net present value of the cost was computed. The overall burden of disease was expressed in terms of discounted disability adjusted live years (DALY).

11.2.2 Human brucellosis burden and cost

The overall burden of human disease was expressed in terms of discounted DALYs - health with time reflects the social preference of a healthy year now.

Data on the number of reported human brucellosis cases from 2006 to 2011 stratified by age and sex was provided by the Ministry of Health (MoHKR) (Table 1). Data on public health costs was collected from interviews with physicians and from official information of the MoHKR. Data on private health costs and income loss was collected during a patient-based survey with 95 clinically diagnosed brucellosis patients who attended a public health facility in 2013, similar to Roth et al. (Roth et al., 2003). This study was approved by the Ethics Commission of the Cantons of Basel-Stadt and Basel-Land and the Kyrgyz health authorities.

Table 10. Numbers of patients used for the calculation of exposure constants*

	2006	2007	2008	2009	2010	2011
Women	1652	1735	1649	1561	1711	1898
Men	1612	1694	1610	1524	1670	1853
Children 5-15 years	499	525	498	472	517	574
Children <5 years	77	81	77	73	80	88
Total no. of cases	3840	4035	3834	3629	3977	4412

*Source of information - www.stat.kg

11.2.3 Valuation and benefit measurement

DALYs were used as a measure of health outcome. Based on the systematic review by Dean et al. (2012), disability weights of 0.15 for chronic and 0.19 for acute brucellosis were used (Dean et al., 2012a). The average age at onset was estimated for each age group. For duration of disease, the Ersatz (EpiGear International, Australia) exponential function was used, with β=3.11 years (Roth et al., 2003). The economic evaluation included the impact on human health cost and income loss including coping cost. The coping cost is included transport cost, lab fee, doctor fee, drug cost and hospital cost. The quantities and unit cost for the health sector and the opportunity cost of human brucellosis cases were derived through the patient-based survey and data of MoHKR. All model calculations were in USD. The Kyrgyz currency was converted to USD with an exchange rate of 1 USD = 41.3 KGS in 2006 as the baseline year (http://www.oanda.com/currency/converter).

11.2.4 Cross-sector economic model (ECOZOO)

The treatment parameters included both inpatient and outpatient data. The human health parameters, population demographic structure and age and sex distributions of brucellosis patients were obtained from the reports of the MoHKR (2007 – 2008) and the National Statistical Committee (NSC) (NatStatCom, 2009) (Table 2). The cross-sector health economic model (ECOZOO) was populated with all retrieved data. ECOZOO is composed of a spread sheet backbone in Microsoft Excel, which is linked to Ersatz stochastic simulation and a *de novo* matrix model of the livestock population (Roth et al., 2003).

Table 11. Human health input variables

Disease characteristics	**Central Value**	**Minimum**	**Maximum**	**Distribution, Source or basis of calculation**
Proportion of chronic cases	0.70	0.5	3.3	(H)
Duration of illness (years)	3.00	1	4.5	(H)
Proportion of inpatients in chronic cases	0.40	0.22 (22.6%)	0.77 (77.4%)	(H)
Outpatient visits	4	St. dev. 1		Normal
Unit Transport cost (USD)	13.92	13.32	14.53	Pert
Average age at onset (for DALYs)				
Women	42.3	36.3	48.3	(H)
Men	37.5	34.0	40.9	(H)
Children 5-15	13.0	10.7	15.2	(H)
Children <5	3.20			(C)

Inpatient days				
Women	14.00	3	16	(H)
Men	14.00	3	50	(H)
Children 5-15	14.00	3	20	(H)
Children <5	14.00			(C)
Proportion of hospitalisation	0.50			(H)
Hospital drug cost (USD)	16.3	15.74	16.83	Pert
Rate of non-formal treatment	0.45		16.2	(H)
Proportion of cases reporting loss of income	0.42	1		(H)
Coping cost per case (USD)	227.4	171.06	227.58	(P)
Disability adjusted life years				
Disability weight for acute (*D*)	0.190	0.172	0.211	(S)
Disability weight for chronic (*D*)	0.150			(S)
Discount rate (*r*)	0.05			(C)
Age weighing (*C*)	0.16			(C)
Parameter of age weighting (*beta*)	0.04			(C)
Duration of disability in years (*L*)	4.50			(C)

(H) Ministry of Health of Kyrgyz Republic
(C) Roth F, Zinsstag J, Orkhon D, Chimed-Ochir G, Hutton G, et al. (2003) Human health benefits from livestock vaccination for brucellosis: case study. Bulletin of the World Health Organization 81: 867-876. The frequency distribution of clinical disease duration fits best with an exponential function for an average duration of 4.5 years. For cost effectiveness, we used the median of the cumulated discounted DALYs, which corresponds to a median duration of brucellosis of 3.11 years
(P) Patient survey
(S) Dean AS, Crump L, Greter H, Hattendorf J, Schelling E, et al. (2012) Clinical manifestations of human brucellosis: a systematic review and meta-analysis. PLoS Negl Trop Dis 6: e1929

11.2.5 Herd composition

Herd composition data were collected from farm surveys and the Ministry of Agriculture (MoA) and NSC (NatStatCom, 2013). These data provided the herd composition vector for a *de novo* developed matrix model using a stable state vector of the herd composition. The data used to compare the model simulations of sex disaggregated population had three age classes for cattle and sheep: juveniles - age between 0-1 years old; sub-adults between 1-2 years and adults above 3 years for cattle and 2 years old for sheep. For goats only two age classes were retained, juveniles and adults, due to the earlier sexual maturity of female goats (Table 3).

Table 3. Equilibrium herd structure (Eigenvector) used for the simulation from 2006-2011

Livestock species	**Population numbers**	**Relative proportions of the Eigenvector**
Cattle		

Female calves	130’409	0.116777
Male calves	130’409	0.116777
Heifers	207’328	0.185656
Replacement males	50’264	0.045010
Cows	552’398	0.494655
Bulls	45’925	0.041124
Total cattle	1’116’733	
Sheep		
Female lambs	594’689	0.18601
Male lambs	594’689	0.18601
Replacement females	462’371	0.144623
Replacement males	156’943	0.04909
Adult sheep	1’330’446	0.416145
Rams	57’937	0.018122
Total sheep	3’197’075	
Goats		
Female lambs	204’827	0.241009
Male lambs	204’827	0.241009
Female goats	365’298	0.429827
Male goats	74’921	0.088156
Total goats	849’873	

11.2.6 Matrix model

The basic structure of the model included a population vector N which was multiplied with a projection matrix P to establish the population vector for the next generation at time t+1 in years. A matrix model $N_{t+1} = PN_t$ (Vandermeer and Goldberg, 2003) was used to simulate the demographic process of the national cattle, sheep and goat populations in Kyrgyzstan with no resource constraints. Livestock populations of future years were evolved through the multiplication of the projection matrix (Table.4, a-c) with a vector of the age and sex stratified population (Table 3).

Population vectors were adjusted by the respective normed Eigenvector to simulate a population in equilibrium. We adapted the demographic model to the official data of the national cattle, sheep and goat population 2006 – 2011 (Figure 2) using an equilibrium population structure obtained from 20’000 iterations of the matrix model (Table.3). This resulted in an overall growth rate (Eigenvalue) of 3.7% in cattle, 5.4% in sheep and 6.5% in goats.

Table 4a: Cattle productivity parameters and their variability (projection matrix)

Parameter/(Unit)	**Central Value**	**Min**	**Max**	**Distribution**
Fertility rate for female offspring (calves per year)	0.245	0.244	0.246	Pert
Fertility rate for male offspring (calves per year)	0.245	0.244	0.246	Pert
Survival female calves	0.85	0.849	0.51	Pert
Survival male calves	0.4	0.39	0.41	Pert
Survival heifers	0.9	0.899	0.901	Pert
Inverse years as heifers	0.5			Fixed value
Survival bulls	0.4	0.39	0.41	Pert
Inverse years bulls	0.66	0.65	0.67	Pert
Inverse years cows	0.7	0.699	0.701	Pert
Slaughter female calves	0.085	0.0849	0.0851	Pert
Slaughter male calves	0.5	0.49	0.51	Pert
Slaughter heifers	0.03	0.029	0.031	Pert
Slaughter young bulls	0.5	0.49	0.51	Pert
Slaughter cows	0.2	0.199	0.201	Pert
Slaughter bulls	0.3	0.29	0.31	Pert

Table 4b: Sheep productivity parameters and their variability (projection matrix)

Parameter/(Unit)	**Central Value**	**Min**	**Max**	**Distribution**
Fertility rate for female offspring (female lambs per year)	0.4725	0.471	0.473	Pert
Fertility rate for male offspring (male lambs per year)	0.4725	0.471	0.473	Pert
Survival female lambs	0.82	SD0.0005		Normal
Survival male lambs	0.28	0.279	0.281	Pert
Survival female sheep replacements	0.88	SD0.0001		Normal
Survival male sheep replacements	0.15	0.14	0.16	Pert
Inverse years as adult female sheep	0.75	0.7499	0.7501	Pert
Inverse years as adult male sheep	0.66	0.65	0.67	Pert
Slaughter female lambs	0.1	0.099	0.101	Pert
Slaughter male lambs	0.64	0.639	0.641	Pert
Slaughter female replacement sheep	0.1	0.099	0.101	Pert
Slaughter male replacement sheep	0.83	0.828	0.832	Pert
Slaughter adult sheep	0.25	0.2499	0.2501	Pert
Slaughter rams	0.33	0.32	0.34	Pert

Table 4c: Goat productivity parameters and their variability (projection matrix)

Parameter/(Unit)	**Central Value**	**Min**	**Max**	**Distribution**
Fertility rate for female offspring (female kids/young goat per year)	0.6	0.597	0.603	Pert
Fertility rate for male offspring (male kids/young goat per year)	0.6	0.597	0.603	Pert
Survival female kids	0.66			
Survival male kids	0.15			
Inverse years as adult goat	0.7	0.699	0.701	Pert
Inverse years as adult male goat	0.66	0.65	0.67	Pert
Slaughter female young goats	0.1	0.099	0.101	Pert
Slaughter male young goats	0.64	0.635	0.645	Pert
Slaughter female adult goats	0.25	0.24	0.26	Pert
Slaughter male adult goats	0.33	0.32	0.34	Pert

11.2.7 Effect of brucellosis on livestock production

For the productivity losses from brucellosis we considered a prevalence related reduction in fertility in terms of number of offspring per fertile female and milk production in terms of liters of milk per year (Equation 1). Brucellosis does not cause additional mortality in general (Table.4, a-c) (Roth et al., 2003).

Equation 1

$$F_b=F*(1-(P_v*R_b))$$

Whereby F_b is fertility with brucellosis, F is baseline fertility, P_v is brucellosis seroprevalence and R_b is the reduction of fertility as a proportion depending on the specificities of cattle, sheep and goats (Table 5).

Table 5. Slaughter parameters of Kyrgyz livestock

Items	**Slaughtering coefficient**	**carcass yield (kg)**	**Distribution Source**
Cattle			
Females of the respective birth year	0.085	90	(A, D)
Males of the respective birth year	0.5	100	(A, D)
Female replacements	0.03	170	(A, D)
Male replacements	0.5	180	(A, D)
Adult females	0.2	200	(A, D)
Adult males	0.3	220	(A, D)
Sheep			
Females of the respective birth year	0.1	15	(A, D)
Males of the respective birth year	0.64	15	(A, D)
Female replacements	0.1	18	(A, D)
Male replacements	0.83	20	(A, D)
Adult females	0.25	20	(A, D)
Adult males	0.33	25	(A, D)
Goats			
Females of the respective birth year	0.1	15	(A, D)
Males of the respective birth year	0.64	15	(A, D)
Adult females	0.25	20	(A, D)
Adult males	0.33	25	(A, D)

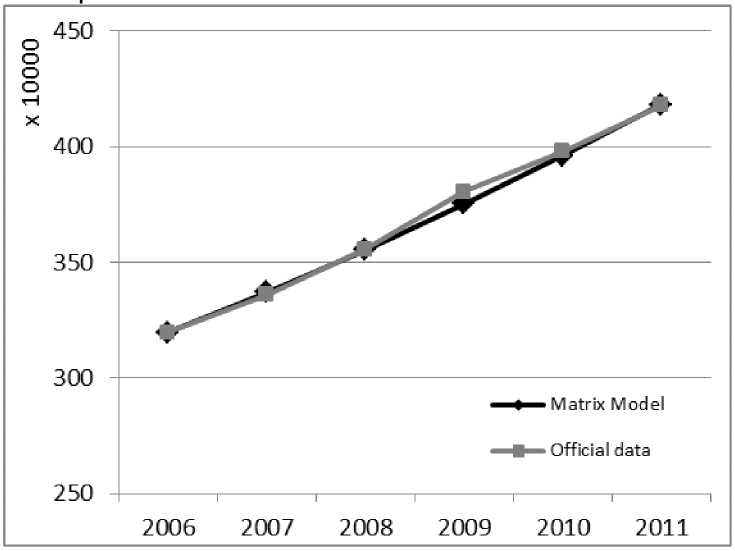
(A) Ministry of Agriculture of Kyrgyz Republic
(D) Delphi Panel

11.2.8 Consideration of uncertainty

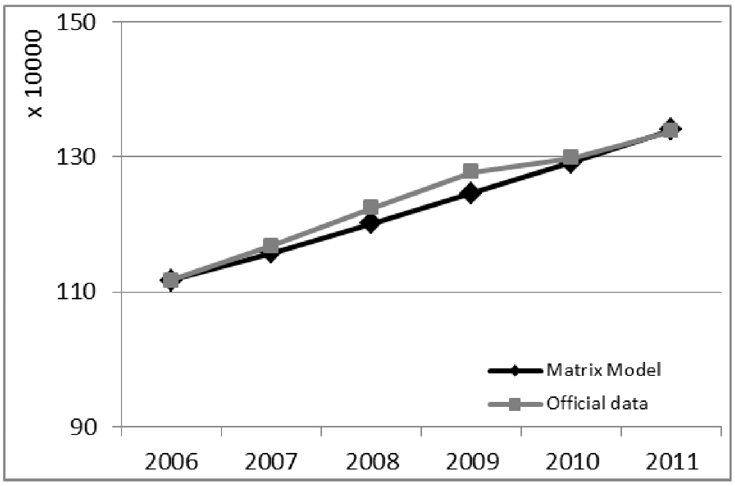
The livestock numbers, product prices and production parameters were expressed as probability distributions using Ersatz. The relative contributions of the different variables were explored during the automatic sensitivity analysis in Ersatz. The variability of the parameters is based on expert opinions within a Delphi on the effects of brucellosis on livestock productivity and, for cattle, the fit of parameters from the official livestock production data was also used (Figure 2).

Figure 2. Comparison of data of matrix model with official data

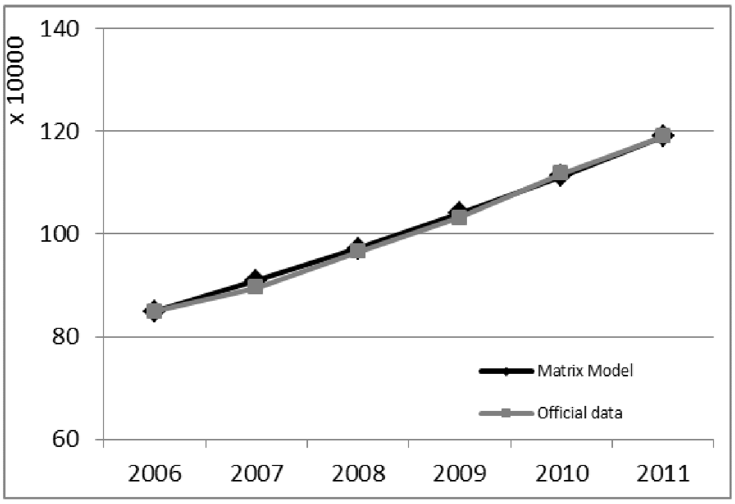
Sheep



Cattle



Goats



Ersatz software extends Excel with a range of functions that offer statistical distributions and therefore the ability to draw randomly from these distributions as a Monte Carlo simulation. This was done for the demographic and economic calculations using 20,000 iterations for each scenario, and net present values (NPV) were output functions. The convergence of the Monte Carlo simulation was tested by the inbuilt convergence graph.

11.2.9 Economic evaluation

For the incremental cost analysis scenario we considered the endemic brucellosis seroprevalence, as presented in Table 6. For the six years of simulation, the annual asset value of the live animals was estimated as the sum of all incremental live animals between the scenarios with and without disease multiplied by their market price in each year. Livestock production was composed of the amount of meat and milk produced in a given year multiplied by the price (Roth et al., 2003, Tschopp et al., 2012). The net present value of livestock meat product was calculated using the Excel function NPV and a discount rate of 5%. The prices of livestock and livestock products were collected in 2006 (baseline year) (Table 7).

Table 6. Decreasing effect of brucellosis (RBT positivity) on livestock productivity parameters

Parameters	Functions	LCL	UCL	Source
Cattle				
Fertility	0.49	0.38	0.42	(A, D)
Seroprevalence	2.8	1.6	4.9	(B)
Reduction of calving rate among brucellosis positive	0.33	0.15	0.5	(C)
Reduction in milk production brucellosis positive	2.1	0.73	4.95	(A, C, D)
Sheep				
Fertility	0.943	0.85	1.035	(A, D)
Seroprevalence	3.3	1.5	6.9	(B)
Reduction in lambing rate brucellosis positive	0.325	0.5	1.5	(C)
Goat				
Fertility	1.215	1.0	1.51	(A, D)
Seroprevalence	2.5	1.4	4.5	(B)
Reduction in lambing rate brucellosis positive	0.015	0.01	0.03	(C)
Reduction in milk production brucellosis positive	0.319	0.78	1.42	(A, C, D)

(A) Ministry of Agriculture of Kyrgyz Republic
(B) Bonfoh B, Kasymbekov J, Dürr S, Toktobaev N, Doherr MG, et al. (2012) Representative seroprevalences of brucellosis in humans and livestock in Kyrgyzstan. EcoHealth 9: 132-138
(C) Roth F, Zinsstag J, Orkhon D, Chimed-Ochir G, Hutton G, et al. (2003) Human health benefits from livestock vaccination for brucellosis: case study. Bulletin of the World Health Organization 81: 867-876
(D) Delphi panel

Table 7. Price in US Dollars in 2006 of live animals and livestock products used for the production system

Value	Average (USD)	Minimum (USD)	Maximum (USD)	Distribution source
Cattle				
Breeders	1029.1	605.3	1452.8	(A, D)
Replacements	605.3	363.2	847.5	(A, D)
Other stock (e.g. castrated males)	544.8	363.2	726.4	(A, D)
Young stock	109.0	48.4	169.5	(A, D)
Meat price / ton - off farm	2905.0	2179.2	3632	Normal (A, D)
Milk price / ton - off farm	363.2	242.1	484.3	Normal (A, D)
Hide price - off farm	20.6	12.1	29.1	Normal (A, D)
Hide weight (tons)	0.015	0.012	0.018	FAO Stat
Draft power price	30	25	35	
Proportion of draft animals	0.22			
Discount rate (%)	5			
Sheep				
Breeders	96.9	48.4	145.3	(A, D)
Replacements	48.4	84.7	121.1	(A, D)
Other stock	72.6	48.4	96.9	(A, D)
Young	48.4	24.2	72.6	(A, D)
Meat price / ton - off farm	3389.8	2421.3	4358.4	Normal (A, D)
Hide price - off farm	4.8	2.4	7.3	Normal (A, D)
Hide weight (Tons)	0.003	0.001	0.005	FAO Stat

Draft power price	30	25	35	
Proportion of draft animals	0.22			
Discount rate (%)	5			
Goats				
Breeders	29.1	24.2	48.4	(A, D)
Female goats	32.7	16.9	48.4	(A, D)
Young females	31.5	14.5	48.4	(A, D)
Young males	18.2	12.1	24.2	(A, D)
Meat price / ton - off farm	2905.6	2179.2	3632	Normal (A, D)
Milk price / to - off farm	169.5	121.1	242.1	Normal (A, D)
Hide price off farm	4.8	3.6	8.5	Normal (A, D)
Hide weight (Tons)	0.003	0.001	0.005	FAO Stat
Draft power	30			
Proportion of draft animals	0.22			
Discount rate (%)	5			

(A) Ministry of Agriculture of Kyrgyz Republic
(D) Delphi panel

11.2.10 Sensitivity Analysis

The sensitivity analysis of the demographic model and cost of disease simulation was done using the Monte Carlo simulation in Ersatz with and without the disease scenario of apparent seroprevalences. Ersatz provides a multivariate sensitivity analysis producing a list with the most sensitive parameters, expressed as Spearman correlation coefficients. All simulations were then summarized by calculating mean values and 95% confidence limits (Zinsstag et al., 2005).

11.3 *Results*

11.3.1 Cost to the health sector

The overall net present health cost was 23% of the societal net present cost of 32.5 million USD (95% CI 25.7– 39.6). The net present cost of brucellosis to the public health sector of Kyrgyzstan between 2006 and 2011 was estimated at 1.38 million USD (95% CI 1.22–1.55), and the private net present health cost was 6 million USD (95% CI 5.5- 6.5). The private health cost includes treatment cost of chronic patients (41%), travel costs (19%), inpatient hotel cost (11%) and private doctor costs (11%), along with drug cost (9%), informal treatment cost (6%) and private costs for laboratory tests and food (3%) (Figure 3). The income loss was primarily the coping cost (80%) (Table 8). In the six year period, human brucellosis caused a loss of 14’520 DALYs (95% CI 12,496-19,901).

Figure 3. Breakdown of private health cost

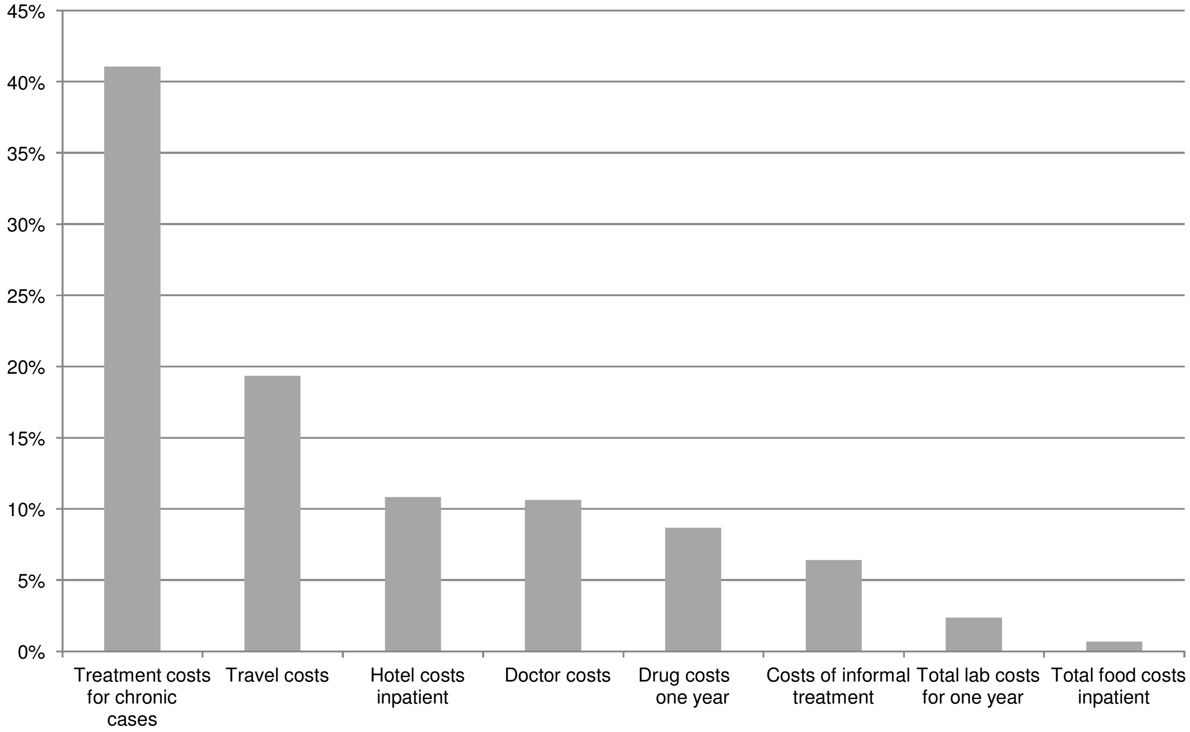


Table 8. Cross-sector cumulative cost of disease for brucellosis to the Kyrgyz society

NPV losses	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2011 95% LCL**	**2011 95% UCL**
USD								
Public health sector cost (A)	253'583	507'355	737'004	944'022	1'160'088	1'388'374	1'222'118	1'551'044
Private health sector (B)	1'099'881	2'200'579	3'196'645	4'094'556	5'031'714	6'021'869	5'544'708	6'491'980
Household income (C)	1'703'558	3'408348	4'951'148	6'341'884	7'793'408	9'327'018	9'228'750	9'443'267
A+B	1'353'464	2'707'935	3'933'648	5'038'578	6'191'802	7'410'243	6'769'646	8'046'266
Human health cost (A+B+C)	3'057'022	6'116'319	8'884'796	11'380'462	13'985'210	16'737'261	8'895'241	22'816'280
Cattle (D)	4'949'437	6'532'384	8'193'533	9'901'111	11'779'117	13'857'658	7'049'416	20'765'216
Sheep (E)	1	82'871	205'865	354'279	545'171	778'260	488'480	2'054'701
Goats (F)	72'447	162'315	275'722	411'591	571'405	755'776	83'864	1'428'881
A+B+C+D+E+F	**8'078'907**	**12'893'889**	**17'559'915**	**22'047'443**	**26'880'903**	**32'128'955**	**25'648'490**	**39'578'898**
Kyrgyz Som (KGS)								
Public health sector cost (A)	10'472'992	20'953'777	30'438'274	38'988'099	47'911'655	57'339'850	50'473'472	64'058'113
Private health sector (B)	45'425'067	90'883'932	132'021'429	169'105'160	207'809'774	248'703'187	228'996'453	268'118'791
Household income (C)	70'356'961	140'766'272	204'482'398	261'919'817	312'867'755	385'205'829	381'147'369	390'006'931
A+B	55'898'059	118'837'709	162'459'675	208'093'259	255'721'429	306'043'037	279'586'385	332'310'805
Cattle (D)	204'411'751	269'787'460	338'392'895	408'915'897	486'477'528	572'321'280	291'140'885	857'603'410
Sheep (E)	27	3'422'583	8'502'206	14'631'707	22'515'561	32'142'152	20'174'216	84'859'163
Goat (F)	2992'074	6'703'591	11'387'327	16'998'713	23'599'020	31'213'550	3'463'573	59'012'803
Cost of brucellosis (A+B+C+D+E+F)	333'658'872	532'517'615	725'224'501	910'559'393	1'110'181'293	1'326'925'849	1'059'282'649	1'634'608'489

11.3.2 Net present value of livestock productivity and cost of disease

The present value of cattle products (meat, milk, hide) in 2006 was estimated at 1.78 billion USD and sheep products (meat) at 376 million USD while goat products (meat, milk) were 197 million USD. In the period from 2006 to 2011, the cumulated net present losses caused by brucellosis to cattle production were 13.79 million USD, to sheep production 0.77 million USD and to goat production 0.756 million USD.

The relative contributions of the public and private health cost of brucellosis to the societal cost were 22%, with the bulk being borne by private health costs, amounting to 81% of the total health costs. The losses to livestock production were 48.6% of the societal cost of brucellosis and the losses to household income were 28%.

11.3.3 Sensitivity analysis

The effect of the variability of the parameters was assessed on the most important outcomes, which were the overall cost of disease, the household health cost, the income loss and the cost to cattle production. The overall cost of disease depended most on the cattle milk price (Spearman's rank correlation coefficient [RCC] of 0.32), cattle fertility rate (RCC of 0.259) and slaughter rate of young male cattle (RCC of 0.254). No human health cost parameter significantly influenced the overall costs of disease. The household health costs were most sensitive to the number of outpatient visits (RCC of 0.991), followed by transport cost (RCC of 0.089) and hospital food cost (RCC of 0.078). The cost to cattle production was most sensitive to the same parameters as for the overall cost. The DALY estimate was highly sensitive to the duration of disease (RCC of 1).

11.4 *Discussion*

This study presents the across-the-board estimation of the cost of brucellosis to the Kyrgyz society. This estimate includes human health costs, income losses and costs to livestock production. The human health cost is 22% (one fifth) of the total cost of brucellosis, of which private households bear the most. Surprisingly, the private income losses were higher than out-of-pocket health costs. The reported income loss by patients is related to losses of out of pocket payments during the illness of patients to maintain the needed work and is not related to livestock or livestock production losses. Note that income losses and livestock production were not counted twice. Hence, the total losses to Kyrgyz households amount to 25 million USD. This may not seem like a large amount, however, importantly, most of countries which have eliminated brucellosis considered freedom from brucellosis as a public good.

Losses to the cattle production were 42% of total costs. Within these, cattle milk contributed to 62% of losses, which reflects the importance and high prices of cattle milk. The study was limited by the lack of empirical livestock productivity data in general and the lack of concordance of official Kyrgyz data. The biggest limitation was that losses due to international trade which resulted from brucellosis could not be included.

The estimates of the costs of brucellosis to Kyrgyzstan were comparable to similar studies done in Mongolia (Roth et al., 2003). The Mongolian study analysed the profitability of brucellosis livestock mass vaccination over ten years, whereas in the Kyrgyz study we estimated the cost of disease without intervention over a six year period. The overall costs cannot be directly compared, but the distribution of costs shows that relative private income losses seem to be higher in Kyrgyzstan. Also, private health costs were proportionally higher in Kyrgyzstan than in Mongolia. The costs to the livestock sector were about half of the costs to the Kyrgyz society. The new strategy of brucellosis control (vaccination of livestock) implemented in Kyrgyzstan since 2012 has not yet shown effects. The tentative vaccination cost for small ruminants during the six years from 2016-2020 was estimated at 0.52 million USD annually. At present, the health sector must bear the costs of 2,296 human brucellosis cases (total number of officially reported new cases in 2012) because of the lack of any effective control programme in the livestock sector. As human brucellosis originates from livestock and livestock products, the health sector is expected to benefit if brucellosis is controlled in livestock. Similar to the Mongolian study (9), it would not be cost-effective for the health sector alone to cover the full cost of a livestock mass vaccination programme and a cost-sharing between the public and animal health sectors should be considered (9). Allowing for annual average losses of approximately 5.3

million USD (95% CI 4.2-6.5) to Kyrgyz society, brucellosis control is likely to be profitable with a benefit-cost ratio of 3 - 5.

11.5 *Conclusions*

Our study shows a sizeable societal cost of brucellosis to the Kyrgyz society which, when compared to existing livestock mass vaccination schemes, is very likely to be higher than the current livestock mass vaccination cost and thus benefit-cost-efficient for the society and cost-effective to the public and private health sectors which are only proportions of the societal cost. Monitoring costs of vaccination efforts should be added to the intervention costs, and vice versa, and livestock export losses due to brucellosis could be added to overall economic analysis. Further research is needed to estimate the benefit-cost ratio and cost-effectiveness of brucellosis control in Kyrgyzstan.

11.6 *Acknowledgements*

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12. Abattoir surveillance of infectious disease in Kyrgyzstan

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[3] This manuscript has to be submitted to Preventive Veterinary Medicine

12.1 *Abstract*

Representative active surveys based on random selection provide accurate estimates of disease prevalence, but may be more costly than abattoir surveillance. However surveillance at slaughterhouses in Kyrgyzstan is poor. In addition, monitoring of vaccination coverage is lacking. The goal of this study was to compare the data of the abattoir with findings of a field survey and to see if slaughterhouse surveillance could be recommended in Kyrgyzstan.

To estimate the achieved brucellosis immunisation coverage, we have computed the demographic composition through adjustment of the eigenvalues. Then the values of seroprevalence at slaughterhouses were adjusted to the values of brucellosis seroprevalence obtained through the active surveillance.

The field seroprevalence was in the same range of the abattoir seroprevalence. Abattoir seroprevalence was 9.8% (95% CI 8.0 -11.5%) and field seroprevalence was 10.7% (95% CI 8.9 -12.6%) as well. When the abattoir seroprevalence was adjusted to the national population structure, the brucellosis seroprevalence became 10.4% (95% CI 8.6 – 12.2%).

However, the peste des petits ruminant (PPR) seroprevalence was significantly lower in the field than at the abattoir and could not be corrected with the inputs of a demographic model.

For brucellosis vaccination monitoring, the abattoir surveillance seems predictive for field prevalence when adjusted to the national demographic composition, but it can hardly be used to estimate vaccination coverage without good individual identification system of animals.

Incremental field surveillance is more expensive than abattoir surveillance. Abattoir surveillance is feasible, but was only accurate for one of the two diseases. We will further analyse our questionnaire data to conclude if the demographic model can be improved and thus good predictive values can be obtained for more diseases or not.

Key words: brucellosis, PPR, abattoir surveillance, demography, matrix model, seroprevalence, Kyrgyzstan,

12.2 *Introduction*

Efficient and reliable surveillance systems are needed in order to know the disease status of a population and to provide reliable information on the absence of diseases for trade partners (Cameron and Martin, 2006, Martin et al., 2007, Hadorn et al., 2008).

In the countries of the former Soviet Union (FSU), mass screening and testing, managed by laboratory practitioners, have been used for many decades and, indeed, has led to public and private industries specialized on such mass screening events. Currently, FSU countries seek more pragmatic approaches to random sampling and less expensive approaches than mass screening to monitor their disease control efforts (e.g. brucellosis and FMD vaccination coverage monitoring) and the presence/distribution of other diseases (new diseases in the region like peste des petitis ruminant (PPR) and endemic diseases such as rabies and echinococcosis). However, active field surveillance and activities of veterinary services mainly do outbreak investigations than surveillance to prevent new outbreaks.

In resource-poor countries, abattoir surveillance could play an important role because of the high costs of active field surveillance; however, its usefulness must first be assessed. Representative and randomized on-farm surveys provide more accurate estimates on achieved immunization coverage and disease prevalence, but may be more costly than abattoir sampling and particularly they also require training of a sufficient number of people in epidemiological methods.

Abattoirs can provide important information on livestock demography and health and abattoir -based disease surveillance is widely used in the pig and poultry industry (Alawneh et al., 2014, Lund et al., 2013, Lynch and Silva, 2013, Vial and Reist, 2014, Kidie et al., 2013). However, routine meat inspection procedures are not always sensitive enough to detect disease (Biffa et al., 2010).
Abattoir populations do not necessarily reflect the composition of the total livestock population. Nevertheless, the combination of information on animal origin (transport certificates) and adequate meat inspection of large numbers of

slaughtered animals could be sufficient to replace field surveillance and, hence, reduce the cost of surveillance (Caldow et al., 2001).

livestock markets and abattoir pose a risk for disease spread because animals from different farms intermix and increase risk of spread infection to humans also through livestock products.Brucellosis remains a major preventable zoonosis that causes nonetheless significant public health concerns and livestock production losses in different regions worldwide. Its overall burden is underestimated and the disease often neglected (Dean et al., 2012b, Pappas, 2010)

In Kyrgyzstan, in the past two decades since the breakdown of the Soviet regime, brucellosis control policy was based on test and slaughter, but this strategy did not lead to tangible results because it was only geared to cattle and did not include compensation scheme for livestock owners. Failure was partly also due to weak public and private veterinary services and new (small-holder) livestock owners without sufficient knowledge on livestock production. Until recently, there was no consistent livestock vaccination program. Vaccination campaigns were suspended due to general poor control of interventions in the livestock production sector, economic instability and inefficient use of S19 for small ruminants.

A representative serological study found brucellosis sero-prevalence of 8.8% in humans (95%CI 4.5-16.5), 2.8% (95%CI 1.6-4.9%) in cattle, 3.3% (95% CI 1.5-6.9%) in sheep and 2.5% (95%CI 1.4-4.5%) among goats in Kyrgyzstan (Bonfoh et al., 2012). The Naryn oblast (one of the 7 provinces in Kyrgyzstan) had the highest seroprevalence in sheep than other species. In this study, sheep were associated with human brucellosis (Bonfoh et al., 2012, Näscher, 2009). Recently, B. *melitensis* isolated from Naryn oblast has been characterized with molecular typing method. The study confirmed that in the Naryn oblast B. *melitensis* is endemic and sheep are apparently the main host of infection for cattle (Kasymbekov et al., 2013).

Kyrgyzstan implements a new livestock mass vaccination scheme (“Strategy of mass vaccination of small ruminants in Kyrgyzstan, 2008-2013”) with alternating vaccination of the entire herd and annual vaccination of young animals. The strategy includes the shift from S19 to Rev-1 for small ruminants (with imported vaccines since the locally produced vaccines did not comply with international standards) and a shift away from subcutaneous to conjunctival vaccination. The World Bank funded this scheme for small ruminants and the vaccinations started in fall 2008. The implementation was incremental with new oblasts being added each year. In 2012, all 7 oblasts were included. With on-going livestock vaccination it is important to ensure continued awareness on preventive measures and that already infected humans have access to diagnostic and treatment. Key of study is also the monitoring of the brucellosis vaccination campaigns results to see if the needed minimum immunization coverage was reached and, if not, corrective measures can be implemented.

In the neighbouring countries Tajikistan and China, outbreaks of peste des petits ruminants (PPR) are reported, and in other countries of the region such as India, Afghanistan and Pakistan, PPR is endemic (Kwiatek et al., 2007, Zhang et al., 2012, Malik et al., 2011, Abubakar and Munir, 2014, Munir et al., 2013, Zahur et al., 2008, Albina et al., 2013) and is present in Kazakhstan (Lundervold et al., 2004). The status of PPR in Kyrgyzstan was unknown. PPR foci existed in 2013 in Tajikistan near the border to Kyrgyzstan (http://web.oie.int/wahid/public.php) and there are frequent cross-border movements of people, livestock and commercial goods between Kyrgyzstan and Tajikistan as well as China. Strategic vaccination was implemented along the border with Tajikistan until 2009, but then was no longer practiced to keep the status quo of absence of disease. To better counter the risk of spreading across the country or to neighbouring countries; it is necessary to conduct risk assessments and disease communication to enhance the surveillance systems at different levels including the improvement of diagnostic capacity for the detection of infection and standard laboratory procedures. When planning this study, it was assumed that PPR already existed in Kyrgyzstan, however, at a very low prevalence.

Livestock census data is not reliable in Kyrgyzstan, thus it is even more important to monitor vaccination campaigns and adjust vaccine numbers when needed. Various modelling techniques have been developed to close gaps of knowledge on population demographics. Using a matrix model to simulate population dynamics and estimate demographic parameters, allows to disease frequency estimates. Such adjusted abattoir surveillance data may provide sufficient information for disease surveillance needs in scarce resource settings (Ridley, 2004).

The pivotal question of this study was to decide if the disease monitoring and surveillance can be done at abattoirs instead of field sampling. The aim was to demonstrate the potential for surveillance of abattoir and livestock markets for epidemiological intelligence and, thus, promoting viable animal production and improving the livelihoods of those living from livestock production. The hypothesis is that the abattoir surveillance is sufficiently representative and sensitive for monitoring of disease surveillance and is more cost-effective than on-farm testing.

12.3 *Materials and methods*

12.3.1 Study design

The study included both a random sample at farm and abattoir levels between July and November 2012 in the Naryn oblast and Osh oblast (convenience sampling including some of the areas previously sampled by Bonfoh et al., 2012). On-farm level, surveillance was conducted based on random selection of animals in the catchment areas of randomly selected slaughterhouses. Both livestock owners who slaughter sheep, goats and cattle at home and those who use the slaughterhouse services were surveyed. The proportions of animals slaughtered at home and at accessible abattoirs were recorded.

The timing of the farm visits was critical, because anti-body titers in livestock are below the detection threshold 3-4 months after the conjunctival vaccination (Stournara et al., 2007). (Vaccination started in June and continued till October 2012). In each of two provinces, three districts, and in every selected district, ten villages, were selected randomly with the selection probability proportional to the size (Bennett et al., 1991). In this way, a total of 60 villages (the animals of one village were considered belonging to one herd) were selected. We assumed an intracluster correlation coefficient roh = of 0.2 and a design effect of 4.2.(Bonfoh et al., 2012) Sampling 17 livestock older than 3 months was planned for each village cluster. This led to a total sample size of 500 per species and province. The 95% confidence limits of the estimate (assumed to be 10%) would be +/-<3% (Bonfoh et al., 2012). The proportion of sheep to goats was estimated at 6:1. Due to the lack of more detailed information, we assumed that this proportion is true for all districts. In the study year, mostly young animals (new-borns and until 3 months of age) and very few adult animals were vaccinated against brucellosis. Their influences on the serological results of this study were considered negligible. When the animal moved to summer pasture from June till September and sampling was done on pastures it was important to keep time of vaccination. We started sampling only three or four weeks after the vaccination campaign.

The slaughterhouses in Kyrgyzstan are rather small and private. The catchment areas were matched to the slaughterhouse and defined together with the local veterinary authorities. In Naryn province, there were only two slaughterhouses and both were enrolled. In Osh province, we have selected eight out of thirty-three slaughterhouses. Selection criteria were accessibility and districts selected randomly for active farm survey. Unfortunately, during the sampling period, the number of slaughtered cattle in Naryn and of goats in Osh was limited and the the wanted numbers were not always obtained. At the slaughterhouses, blood samples of each third or each fifth animal was taken for serological testing.

As to the animal health and slaughterhouse professionals in the study area, we conducted individual interviews and focus group discussions with them to record their opinions on anticipated outcomes of the sero-surveys on farm and slaughterhouse levels.

12.3.2 Sample collection

The study was conducted by two field teams and total of 95 villages in six selected districts were enrolled. With informed consent of livestock owners blood samples of each fifth or tenth (depending on the herd size) sheep, goat and cattle was taken for serological testing of brucellosis and PPR at the local veterinary laboratories. Blood of livestock was obtained by venipuncture with 10 ml Vacutainer tubes. Samples were identified by the name and code of the village, the owner's name, livestock species and age. All collected sera were transported to the provincial Veterinary laboratory, centrifuged and stored until further serological testing (Rose Bengal Test). All sera from Naryn area were shipped to Osh for ELISA tests. The selected districts and samples per species are shown in Table 18 (village sampling) and Table 19 (slaughterhouse sampling).

Table 12. Total sample size of village sampling by species and districts

Species	Total	Aktalaa	Atbashy	Naryn	Karakulja	Uzgen	Nookat	Karasuu
Cattle	1106	170	172	154	165	194	200	51
Sheep	1087	170	170	143	164	185	182	73
Goat	1055	170	164	154	163	185	186	33
Total	3248	510	506	451	492	567	567	157

Table 13. Total sample size of slaughterhouse sampling by species and number of slaughterhouse

Species	Total	Naryn Oblast		Osh Oblast							
		1	**2**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Cattle	582	7	9	109	28	16	58	69	80	75	131
Sheep	1111	509	15	453	-	2	4	17	0	111	-
Goat	96	85	11	-	-	-	-	-	-	-	-
Total	1789	601	35	562	28	18	62	86	80	186	131

Names of Naryn slaughterhouses
1 – Naryn 1
2 - Naryn 2
Names of Osh slaughterhouses
1 – Sadykov (Osh city);
2 – Kara-Suu;
3 – Abdullaev (Osh city);
4 – Kashgar-Kyshtak-1;
5 – Plodovosh;
6 – Kashgar-Kyshtak-2;
7 – Rosibaev;
8 – Erkin SUP#2;

12.3.3 Questionnaire

The questionnaire included the proportions of animals slaughtered at home and at accessible abattoirs, the livestock owner's experiences in the recent vaccination campaigns, the information they have received and their knowledge on brucellosis in general. Since we wanted to explain the brucellosis seropositivity - given that the vaccination campaigns were on-going when sampling - we have collected the needed farm-level data for the seemingly most important explanatory variables. As to PPR, livestock owners were asked about symptoms and their spontaneous associations with PPR.

12.3.4 Serological tests

Serological testing for brucellosis was done at the Naryn and Osh State zonal Centres for veterinary diagnostic (provincial level). The sera were tested with the Rose Bengal test (RBT), whereby the modified test with increased sensitivity of three parts of sera to one part (3:1) of RBT reactive was used for small ruminants and one to one (1:1) part for cattle. Positive results of cattle were confirmed with the CFT. For each sample the strength of the reaction was recorded as (++) positive, (+) weak positive or doubtful, and negative (-).

Post vaccination titers were established to estimate vaccination coverage of vaccinated animals. We sampled villages from three to four weeks after vaccination and no longer than four months. Seropositive sheep and goats vaccinated four months earlier were identified as "negative" and counted as vaccination titer. Seropositive sheep and goats vaccinated more than four months ago were considered as infected with field strain. In parallel to the positive serological test, the availability of an ear-notch was recorded and the owners asked when brucellosis vaccination was done for their animals. All seropositive cattle were tested with CFT and identified as infected if the test was positive. The owners of seropositive animals have been informed through district veterinary department and local veterinarians.

For PPR, a cELISA (ID Screen® PPR Competition, ID vet, Montpellier, France) was used at Osh State Center for Veterinary Diagnostic (Osh oblast Veterinary Laboratory). The cELISA is specific at 99.4% and has a sensitivity of 94.5% (Libeau et al., 1995).

For each sample the competition percentage was calculated using the following formula:

$$\text{Competition \%} = \frac{OD_{\text{sample}}}{OD_{\text{negative control}}} \times 100$$

According to the manufacturer, the cut-off value for positive samples was PI ≤ 35 per cent and the mean value of the OD of the Negative Control was greater than 0.7 ($OD_{NC}>0.7$) and the mean value of the Positive Control was less than 30 per cent of the OD_{NC} ($OD_{PC}/OD_{NC}<0.3$);. The cut off for seropositivity used was: the samples having competition values between 35 and 45 per cent were considered doubtful and these samples were tested again for confirmative purposes as recommended by the manufacturer.

12.3.5 Demographic model

Under the assumption of geographic representativity (knowing the abattoir catchment area in a given area), knowing the composition of an abattoir population can be used for comparison to the overall livestock population. A livestock demographic model was developed for sheep and optimized on national livestock data in Excel. The basic structure of the model was a population vector N which is multiplied with a projection matrix P to establish the population vector for the next generation (Vandermeer and Goldberg, 2003). $N_{t+1}=PN_t$ (Table 20). Saying briefly, the sheep population was subdivided into three age classes lambs, sub adults and adult sheep for male and female animals. Population vectors were adjusted by the respective normed Eigenvector to simulate a population in equilibrium. The equilibrium herd structure was used to adjust the population structure in the abattoir by an adjusting factor Rc (see below) for each age and sex class.

Livestock populations of future years evolve through the multiplication of the projection matrix (Table 3) with a vector of the age and sex stratified population. This provides the number of animal units in age groups through the defined unit. The transition matrix corresponding to the graph of livestock life cycle consists of three age classes, each of which is divided into two sexes as described below (Table 20). Projection matrix showed the probability of the animal unit of i- class to move to the next year.

Table 14. Projection matrix

Population Vector				
Female calves	0	birth rate female	0	0.395
Male calves	0	birth rate male	0	0.395
Heifers	1/years as heifer	survival female calves	0.50	0.855
Replacement male	survival male calves	0	0.4	0
Cows	Survival heifers	1/years as cow	0.9	0.491
Bulls	survival bulls	3/years as bull	0.416	0.648
Female calve slaughter	survival or mortality	0	0.085	0
Male calve slaughter	slaughter mail calves	0	0.5	0
Young female slaughter	0	home slaughter calves	0	0.03
Young male slaughter	slaughter young mail	0	0.5	0
Cow slaughter	0	slaughter cows	0	0.2
Bull slaughter	0	bull slaughter	0	0.3

12.3.6 Correction of abattoir data to national demographics’

Based on composition of the livestock population at equilibrium from the livestock demographic model, we estimated the age specific population / abattoir ratio and we showed that the weighted population prevalence is similar to the weighted abattoir prevalence and we multiplied the abattoir data for every age class using the population / abattoir ratio. We inferred the population disease prevalence from the abattoir prevalence. Although abattoir prevalence estimates were corrected for field prevalence, there was a remaining bias from the animal selections for slaughter.

Based on the empirical data this bias was estimated and established the proportions of animals at farm and slaughtered and integrated in the demographic model.

12.3.7 Model of seroprevalence estimates versus measured seroprevalences at slaughterhouses and households

The model describing immunisation coverage of sheep based on coefficients of the matrix based model is a ratio of RBT positive samples to the total number of sampled animals. Also, it was necessary to determine the age and sex composition ratio by dividing the number of particular sex and age group to the total number of tested animals. The following formulas were used to estimate the seroprevalences of slaughterhouse and households surveillance.

$$Pvi=Pp/n_i \qquad (1)$$

$$Pvtot=\sum Pp/\sum n_i \qquad (2)$$

$$Sa= (n_i/\sum n1+n2+\ldots n_i) \qquad (3)$$

$$Rv = Pvsl / Pvfl \qquad (4)$$

$$Rc=Sab/San \qquad (5)$$

$$Ajp=Pab*Sab/Rc \qquad (6)$$

Where –
Pv – *seroprevalence*
Pp – *positive animals*
n$_i$ – *number of tested animals in* i*-th age class*
Sa – *Age-sex composition (proportions)*
R_v – *ratio of slaughterhouse prevalence and field prevalence*
R_c=Sab/San – slaughterhouse and national comparison ratio
Sab – abattoir
San - national
Pab= Abattoir (age and sex slaughtered)
Ajp – adjusted seroprevalence

12.4 *Results*

12.4.1 Demographic model

We adapted the demographic model to the official data of the national sheep population 2006 – 2011 (Tab. 21 and Figure 12-1) using an equilibrium population structure obtained from 20 iterations of the matrix model. The overall growth rate (Eigenvalue) in cattle was 3.7%; in sheep 5.4% and in goats 6.5%.

Table 15. Equilibrium cattle herd structure following 20 iterations

Sex	Age	Proportion herd structure
Male	young	0.186
Male	sub adult	0.049
Male	adult	0.018
Female	young	0.185
Female	sub adult	0.144
Female	adult	0.415

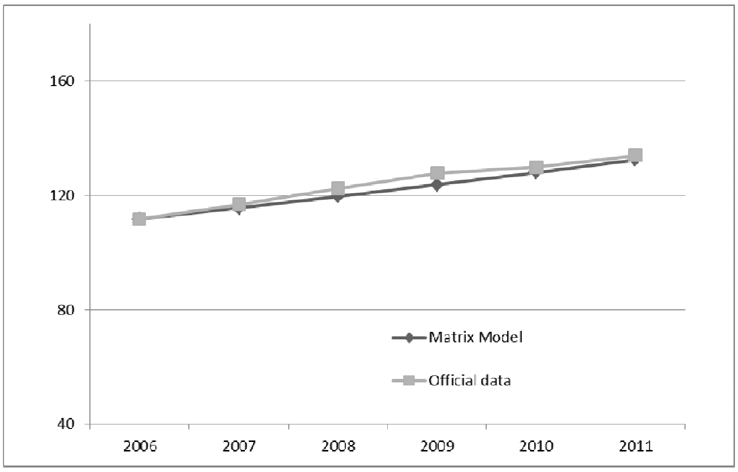


Figure 12-1. Comparison of sheep data of matrix model with official data

12.4.2 Cost of samples at abattoir and active monitoring

Based on the actual study expenditures, the cost of field surveillance was estimated at 92.7 KGS per sample of which transport cost was 46.3 KGS, human resources - 19.9 KGS and accommodation - 26.4 KGS. Overall, the transport cost of one sample for the abattoir surveillance was estimated at 16.0 KGS.

12.4.3 Estimates of seroprevalence due to vaccination using matrix-based models

Disease frequency data from abattoir surveillance is presented as uncorrected sero-prevalence for brucellosis and PPR, the corrected seroprevalence uses the adjustment factor Rc to the national herd structure and the seroprevalence from the representative field survey in the catchment area of the slaughterhouses (Figures 12-2 and 12- 3)

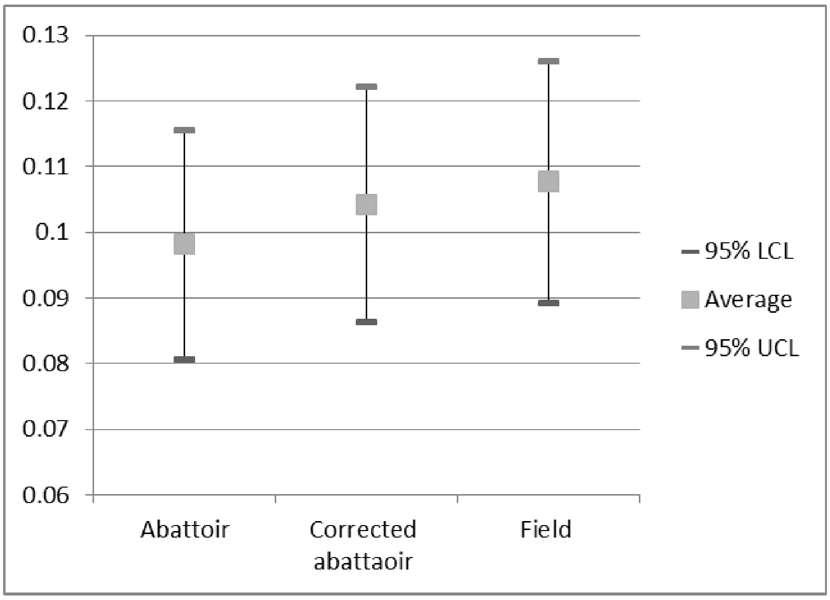


Figure 12-2. Abattoir, corrected abattoir and field brucellosis sero-prevalence in sheep in Naryn and Osh oblast.

Thus, we estimated the brucellosis sero-prevalence and brucellosis vaccination coverage and the PPR sero-prevalence of sheep when sampling and compared the prevalence of livestock disease with the sero-prevalence at slaughtering. We have found that PPRV circulates all over the country. Moreover adult sheep more affected with PPR (male 19% and female 28%) and seropositivity of young sheep was less (10.2% and 11.8%). We have found high seropositivity of PPR at slaughterhouse and we assume that the animals were slaughtered visually because of PPR.

The proportion of brucellosis seroprevalence is high among male animals at slaughterhouse and female at farm level.

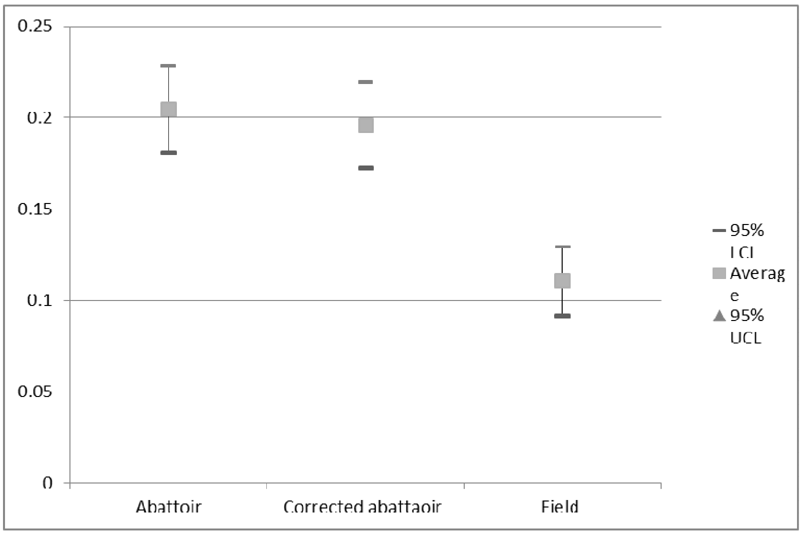


Figure 12-3. Abattoir, corrected abattoir and field PPR seroprevalence of sheep in Naryn and Osh oblast.

12.5 *Discussion*

Abattoir seroprevalence of sheep with a correcting factor for the relationship of the abattoir and overall population structure results in a comparable estimate to the observed field sero-prevalence in the catchment area of the abattoirs. Hence for the case of brucellosis, the abattoir sero-prevalence corrected for the overall population structure predicts reasonably well the observed seroprevalence in the field. However this is not the case for PPR sero-prevalence. The abattoir values are much higher than the field seroprevalence and the correction for the herd structure does not adjust it. The surveillance of PPR at abattoirs overestimated the field prevalence. This could be attributed to earlier slaughtering of ill animals and prevalence obtained at the slaughterhouse appeared to be higher than the reported number of infected animals. On the other hand this information could indicate also a higher sensitivity of the abattoir to detect PPR cases. More research is needed to establish comparative surveillance sensitivity in abattoirs as compared to active field surveillance. The findings of the survey of the public and the veterinarians on vaccination coverage were substantially higher than the antibodies titers tested in the laboratory. According to the veterinarians and their reported data the vaccination coverage makes up around 80-100%. According to the questionnaire based survey of the public and the animals' owners the total vaccination coverage of all animals has made up 67%, whereas 33% of sheep and goats were left unvaccinated.

From the field survey we could divide the sero-prevalence into two groups of sheep vaccinated less than five months ago and those vaccinated more than 6 months ago, however we could not clearly distinguish the time between vaccination and slaughter at the abattoirs and, hence, failed to estimate the vaccination immunity and natural infection rates in the abattoir. We should bear in mind that the quality of the demographic and seroprevalence data is unknown at the time of vaccination, owners and origin of animal at the slaughterhouses. During the sampling period the number of slaughtered cattle in Naryn and goat in Osh was limited. We could cover neither the cattle nor the goats. Overall, abattoir brucellosis seroprevalence is predictive when it comes to field prevalence once adjusted with the national demographic composition but cannot be used to estimate the vaccination coverage without good traceability (identification) system at the slaughterhouse. And it seems not useful for establishing PPR seroprevalences at abattoirs. The difference of prediction between brucellosis and PPR may be due to visibility of disease and owners strive to kick out sick animals and brucellosis gain latent form.

It was assumed that in developed countries the slaughtered animals are mainly healthy (Vial and Reist, 2014) and ill animals in developing countries. In our study, we did not do meat inspection ourselves, but we have likely found echinococcosis and mycobacterial infections and nevertheless further study needed to confirm this assumption.

The correction of the abattoir data with the national herd structure can be used to predict population level seroprevalence for brucellosis. Abattoir surveillance could be used to assess the total prevalence of other zoonoses to estimate disease frequency in the overall population.

The cost of field sampling of small ruminants in terms of human resources is twice higher and the transport costs are much higher compared to abattoir sampling. The cost-effectiveness in terms of cost per collected sample is currently calculated and is reported elsewhere. The cost-effectiveness of abattoir sampling is likely higher than the field sampling. The potential of abattoir surveillance for certain diseases in Kyrgyzstan requires further studies in view of its adoption with regard to selected diseases in a prevalence range of 5-10%. This could save substantial resources for the Kyrgyz Government and could be used to control other infectious diseases.

12.6 *Conclusion*

If the abattoir surveillance proves to be a cheaper and equally sensitive way for brucellosis immunization surveillance compared to on-farm surveillance, the Kyrgyz Government could save substantial resources making it more feasible to implement across all oblasts given the lack of trained field epidemiologists. Finally, the analysis of the abattoir surveillance and assessment of needs with regard to diagnostic and treatment of patients with brucellosis can generate further evidences in Kyrgyzstan.

12.7 *Acknowledgements*

We would like to thank the Swiss Federal Veterinary Office, the NCCR North-South JACS ALP, and the International Graduate School for financial support of this study. We would like to extend our acknowledgments to farmers and veterinarians of Aktalaa, At-Bashy, Karakulzha, Naryn, Nookat districts and the cities of Osh and Naryn for their assistance in the collection of materials and data. Our special thanks go to Julen Cuasabon, Islam Abdymomunov, Asamidin Ashimov, Bumariya Alimzhanova, Gulzhamal Bekmamatova, Joldoshbek Imanseitov and Rebekka Ott for their assistance and support throughout the field surveillance. Jyldyz Bekimbaeva and Gulnur Anashevna helped with the English language editing.

12.8 *Author contributions*

Conceived and designed the experiments: JK ES JZ. Performed the experiments: JK JZ. Analysed the data: JK ES JH JZ. Contributed reagents / materials / analysis tools: JK ES JZ. Wrote the paper: JK ES JZ.

13. General discussion

This research project was conducted in multidisciplinary partnership between the Swiss Tropical Public Health Institute (Swiss TPH) and the Veterinary Services, Ministry of Agriculture, Ministry of Health of the Kyrgyz Republic. Close collaboration was maintained with the Institute of Veterinary Bacteriology of the University of Berne in Switzerland.

The study was conducted within the research of the Human and Animal Health Unit at the Department of Epidemiology and Public Health (EPH) at Swiss TPH. It benefited from in-house support for statistical and epidemiological analysis of field data. Specifically an interdisciplinary approach considering the interconnectedness of human and animal health could be pursued. I further involved molecular biological, statistical and economic methods, benefiting from collaboration across EPH units.

The current test and slaughter program in Kyrgyzstan is an inefficient strategy to control brucellosis as the prevalence is high. In order to propose a modern Rev-1 vaccination programme for all the livestock, cost-effectiveness of the control programme has to be estimated in a systematic way including all involved sectors.

Results from the representative sero-prevalence and molecular study showed that sheep are the main infection source and could also transmit brucellosis to cattle. It is critical to know the scope of infection in animals as well as in humans to establish a transmission model. Based on the current data a transmission model could be parameterised which could serve as a basis to simulate control options and thus to make evidence-based recommendations to the authorities. Eventually, this should lead to more a effective brucellosis control programme.

13.1 *Relevance*

The outcomes of brucellosis control in animals in Kyrgyzstan varied during different periods. In particular, the required interventions under the brucellosis control programme were not implemented in full. It was not always feasible to ensure rapid and reliable recovery or replacement of infected animals to maintain well-being as well as the on-going counter brucellosis interventions often generating minimum effects. In other words brucellosis tends to re-emerge after a certain time span following implemented interventions. The reasons behind this are lack of information on the state-of-the-art scientific achievements, non-use of advanced control technology, non-use of high-tech equipment, methods of diagnostics and specific preventive interventions (Kim, 2004). The information on these matters is hardly available to the wide range of professionals. These important topics are covered in international scientific articles and other publications that are not available for several reasons, including language barrier or access to internet. Therefore, valuable information on control of brucellosis often remains unutilised by professionals and livestock producers at the village, rayon and even the oblast level.

13.2 *Brucellosis background in Kyrgyzstan*

Over the last century, scientists of Kyrgyzstan have concluded that the infection was imported to the country. However, back in my childhood my grand-parents very often used to say that "one shouldn't drink raw goat's milk" or "drinking raw milk may cause a disease". Perhaps, this was due to the effective ban on consumption of raw goat's milk owing to brucellosis. Could it be related to brucellosis or was it some other infection? Currently it is difficult to answer this question, but it is not unlikely that brucellosis already existed earlier in Kyrgyzstan. The place of the Kyrgyz strains in the global phylogeny needs to be further analysed using full sequencing of strains at this stage. It appears that Kyrgyz strains may be related to strains found in the Middle East. This could indicate much earlier spread, likely associated with the spread of domesticated livestock.

The use of classical diagnosis methods has also played important role in preventing full identification of infected animals. The basic tests such as CFT and tube agglutination test (AT) were used. AT was used to identify acute brucellosis while CFT was used to identify chronic brucellosis. RBT was first used only 26-28 years ago and such tests as ELISA and PCR were not used to study brucellosis due to costly diagnostic tests and huge scope of research work and the lack of manpower to process 1.5 - 2 thousand samples per 3 lab technicians a day at the central laboratory. Considering the issue of classical methods of brucellosis diagnostics it should be noted that not all existing laboratories can apply AT and CFT. This is due to lack of diagnostic equipment or loss of the laboratory technicians practicing such methods who left their jobs in search of better salaries. According to the statistics in Kyrgyzstan there are 27 rayons, area based (regional) laboratories and branches including the central laboratory, but not all laboratories are equipped with diagnostic tools and there are insufficient personnel, although the position of the Director in such laboratories is never vacant.

Until recently, the official incidence rate in cattle and small ruminants did not exceed 1%, the data of laboratory tests and the State Veterinary Department did not correspond, and in cattle the rate was 0.6 and 0.8% and in small ruminants 0.8 and 1% accordingly. Due to unknown reasons, SVD tended to underestimate the incidence of brucellosis in animals. Only after the rapid growth of brucellosis prevalence in humans was attention paid to the disease control.

Also there is a trend of frequent change of management of the veterinary services as well as continuous veterinary service restructuring that affects the zoonosis control in the country. According to Anton van Engelen, the international expert, the post of the chief veterinarian is "politicized" and management without proper knowledge about the veterinary service system comes to power.

Despite this factor, substantial efforts were made with the assistance and support of the international donors and projects.

13.3 *Sero-surveillance*

It should be noted that for the first time in the history of veterinary and healthcare services, under the financial support of the Swiss Agency for Development and Cooperation (SDC), the Swiss Tropical Institute and the Swiss Red Cross in Kyrgyzstan, a joint sero monitoring of incidence and prevalence of brucellosis in humans and animals was conducted, whereby three teams were established and each team involved one health worker and one veterinarian. It was the first step towards a "One Health" approach in Kyrgyzstan.

The findings of the Swiss-Kyrgyz research were presented at the workshop in the village of Koi-Tash in June 2008 with the participation of leading experts from Switzerland, Mali, the USA, Mongolia and neighbouring countries including Kazakhstan, Tajikistan and Uzbekistan. The workshop participants suggested practicing mass vaccination of sheep and goats and continuing molecular study.

The research findings served as the basis for developing the brucellosis control strategy in Kyrgyzstan, which was approved by the Prime Minister in 2008. The same year, the World Bank launched the project in Aktala rayon of Naryn oblast and in autumn of the same year the mass vaccination of cattle was initiated. In subsequent years the mass vaccination was implemented in Naryn oblast and further in other oblasts of the country. The full vaccination coverage of small ruminants across the country was completed in 2012.

13.4 *The potential of abattoir surveillance*

A comparative study of field and abattoir surveillance combined with a correction of the demographic composition showed that abattoir surveillance can reflect field prevalence at the example of sheep brucellosis sero-prevalence. However abattoir prevalence cannot be used to estimate brucellosis vaccination coverage. Abattoir surveillance results were higher than field PPR seroprevalence. However, this could indicate a higher surveillance sensitivity of abattoir surveillance. Overall abattoir surveillance cost make up at least half of field surveillance and definitely has a potential for use in Kyrgyzstan. More research is needed to further validate the usefulness of abattoir surveillance in Kyrgyzstan.

It was planned to collect 6000 samples; however, it was not possible to collect cattle blood serum due to non-use of abattoirs for slaughtering cattle in Naryn oblast, and goat sampling was not done in the abattoirs in Osh oblast due to lack or non-use of abattoirs for slaughtering the goats. In total, 5035 blood samples and 170 questionnaires were collected in two oblasts.

13.5 *Demographic model*

In order to get comprehensive estimates and design the demographic model we computed the estimates in two different calculations, the LDPS FAO and the Matrix models.

All the data for estimates were obtained from the National Statistical Committee, reported documents of the Ministry of Agriculture and the Ministry of Health as well as through personal communication, interviews and the Delphi panel.

Initially, the official data on the livestock population and animal productivity were processed through LDPS for ten years, then the livestock population data of 2006 were processed through the Matrix model for twenty-one years in advance, as during this period of time there is the probability to reduce brucellosis incidence on the whole. The derived coefficients of the population composition were adjusted in their “own” vector through the correlation of coefficients of the last year to the first year until a straight line was achieved.

The Matrix and LDPS models reproduce the official data with adequate compatibility. The LDPS has even a slightly lower Root SSD. We replaced the Matrix herd structure of year 21 and multiplied it with the start-up population of year 2006. The main difference between the LDPS and the Matrix model is that we do not have a category of other stock in the Matrix model.

We compared the results of two calculations in vector graphics. Upon achieving the uniform line, the adjusted Matrix model was used to estimate the sero-prevalence of brucellosis at slaughterhouses and field surveillance, for the costing (cost effectiveness) of brucellosis.

13.6 *Vaccination coverage*

To estimate the vaccination coverage we have drawn the mathematical model. This kind of model was developed in Kyrgyzstan for the first time. The mathematical model allows for the estimation of the infection prevalence at slaughterhouses and during active surveillance and to compare the results at the national level. In order to estimate we obtained the data by demographic composition through adjusting the eigenvalues. Then the prevalence values at slaughterhouses were adjusted in compliance with the values of brucellosis prevalence obtained through the active surveillance.

When the values of the active surveillance corresponded to the values of the abattoir, the data were compared with the demographic values at the national level.

Also during the active surveillance we have surveyed the owners of cattle and the veterinarians involved in the process of mass vaccination. The findings of the survey of the public and the private veterinarians’ data on vaccination coverage were substantially higher than the antibodies titers tested in the laboratory

The goal of this study was to compare the data of the abattoir with the findings of the active surveillance and to propose the replacement of the active surveillance by the surveillance at slaughterhouses. As this method of surveillance of the prevalence and animals’ vaccination coverage enables to save travel and per diem costs, less resources are required for blood sampling from animals (less workload for veterinarians).

However, it appeared that this method is good only for identifying the overall of availability and presence of any infection in general. The difficulty was that we could not estimate the coverage of vaccinated sheep due to lack of identification of animals; it was often impossible to find information about the time of vaccination and animal origin, as prior to the slaughterhouse the animals are resold several times whereby the original documentation (veterinarian's certificate) is lost.

Also during the research, caprine contagious pleura pneumonia (CCPP) was detected, the ELISA digital results were sent to the OIE Reference Laboratory in France and the results obtained from them confirmed the presence of infection. The veterinary authorities of the rayon and the oblast were informed about the incident, however, the Central Veterinary Department did not report to relevant authorities and the OIE respectively on the presence of infection. Apparently the information remained at the oblast or central level. During that time there was the murrain of more than three thousand sheep in Jalalabad oblast due to the use of substandard vaccine. Possibly one of the reasons behind the murrain was the negligence to minor murrain of goats in different districts. At the meeting with veterinarians in different districts of Osh and Jalal-Abad oblasts it was reported that the outbreak of CCPP took place everywhere, by autumn it had spread to other oblasts of the country.

13.7 *Cost of brucellosis in animals and humans*

The estimates of the costs of brucellosis to Kyrgyzstan are similar to the studies and economic estimates of Mongolia, F. Roth (2003). The Mongolian study analysed the profitability of brucellosis livestock mass vaccination, whereas in the Kyrgyz study we estimated the cost of disease. The private income loss seems to be higher in Mongolia. Also private health cost was proportionally higher in Kyrgyzstan than in Mongolia. The cost to the livestock sector is about half of the average annual total cost of approximately 3 million USD to the Kyrgyz society. Compared to Mongolia, the national Kyrgyz brucellosis mass vaccination program should not exceed one million USD per year which would make brucellosis control largely profitable. More research is needed, using a livestock human transmission model to assess the profitability of brucellosis mass vaccination to Kyrgyzstan in more detail.

13.8 *Estimates of livestock productivity*

The livestock productivity data of 2006 were used in calculating the productivity of livestock. The main difficulty was a discrepancy of official data and the findings of the official report. According to official figures 72% of sheep are slaughtered, based on the estimated number of lambs born in the current year at the same fertility rate which creates a deficit of 370 000 young stock. Annually 46% of total cattle population is slaughtered that is hardly compatible with the official data. Kyrgyzstan exports animals to neighbouring countries such as Kazakhstan and Iran and imports buffalo meat from India and China, but there are no official records on this information at all.

Inaccurate data on the size of cattle population and livestock productivity presented to the central level create difficulties in making decisions on control measures, planning and procurement of vaccines. It is obvious that the official contribution of the livestock sector to the national economy is highly distorted and it causes constraints in determining the volume of gross domestic income (net present value and asset value). No data on export of animals (for selling) are available, thus the importance of livestock investments is underestimated in Kyrgyzstan.

13.9 *Declining transmission of brucellosis in humans*

The peak incidence of human brucellosis was in 2011 with 80 cases per 100,000 people per year and since 2012 the incidence was declining likely owing to the implemented mass vaccination of small ruminants.

This trend should be in place all over the country. It is quite apparent, that preventive measures to control brucellosis affect the incidence of brucellosis in humans.

According to the Chief of the Veterinary Department of Aktala rayon in Naryn oblast where the mass vaccination was started in 2008, the incidence of brucellosis in small ruminants and humans tended to decline; even the incidence of brucellosis in cattle appeared to decrease (personal communication).

Undoubtedly we assume that if cattle vaccination strategy was in place the incidence in humans might be even lower.

It should be noted that where there are village health committees (VHC) there is much lower incidence of brucellosis in comparison to communities without VHCs

(Tobias Schüth, personal communication). Local health committees are continuously outreaching to the farmers as well as promoting proper use of means of protection during lambing and handling the placenta of new-born animals.
It should be noted that success of mass vaccination is due to comprehensive and joint activities of VHCs and Pasture commities at the village level.

14. Recommendation

1. These studies include basic research on brucellosis prevalence in humans and animals, and the economic costs of brucellosis in general; we believe that these studies can serve as an important line of reasoning for politicians and officials in taking drastic measures to control both brucellosis and other zoonoses.
2. The molecular tests were conducted in one oblast and the findings have proved the presence and interspecific migration of *B. melitensis*; it is necessary to continue such tests in other oblasts of the country and across the Central Asian region on the whole.
3. It is recommended to continue the mass vaccination of small ruminants until the minimum (0.1-0.2%) prevalence in humans is achieved and the strategy of testing and slaughtering should be addressed with further compensation to the owners of animals.
4. It is recommended to vaccinate the cattle, at least 3-6 month old calves should be vaccinated once.
5. It is necessary to raise, as much as possible, the awareness of people on the importance of vaccination and its effects and promote personal hygiene measures during the delivery of new-borns.
6. It is recommended to conduct annual, independent sero-monitoring, using modern epidemiological cross-sectional study designs proportional to size.
7. It is recommended to translate and publish scientific articles in clear, plain language and design a website for veterinarians to enable access to information.
8. In order to improve the veterinary diagnostic capability, it is necessary to strengthen the oblast laboratories and, depending on the distance, to reduce the number of branches and rayon laboratories and restructure the collection points where the staff could make a preliminary diagnosis and ship samples for further tests to the oblast laboratory. Laboratory staffing should be accordingly revised.
9. It is recommended to reconsider the role of veterinarians' assistants and expand their ultisation in vaccination programmes. Students of veterinary schools could be invited for internships during the mass vaccination campaigns.
10. It is recommended to reduce the number of veterinarians engaged in administration of the central management, with a focus on establishing a sound data base of specialists at the oblast and rayon levels.

7. General discussion and recommendation

11. It is recommended to improve the recording and reporting of statistical data of the Ministry of Agriculture and the National Statistical Committee

12. It is recommended to ensure transparent tenders for procurement of veterinary vaccines and diagnostic tools.

15. Appendixes

Central Asia

research evidence for policy

Regional edition Central Asia No. 2 January 2012

(Left) Sheep and goats are often herded together and can be infected with Brucella melitensis: small ruminant pen in Mongolia. Photo: J. Zinsstag (Right) Milk cream separator in Kyrgyzstan. Unpasteurised dairy products are a health risk in brucellosis-endemic areas. Photo E. Schelling

NCCR north south

It's time to control brucellosis in Central Asia

Case studies featured here were conducted in: Kyrgyzstan

Policy message

- Brucellosis is a highly contagious livestock disease that can be transmitted to humans through direct contact and the consumption of unpasteurised milk and milk products. In Central Asia, it is profitable for society as a whole to control it by mass vaccination of sheep, goats, cattle, and yaks.
- At least 80% of the animals should be vaccinated each year. If less than 1% of livestock are affected, vaccinations can be restricted to young replacement animals only.
- Treatment of humans should be supported by education campaigns and by ensuring that diagnosis and treatment services are available at the district level.
- Vaccinating animals is the best way to reduce human infections. Education on safe animal handling and the boiling of milk can also considerably decrease the number of cases in humans.

Brucellosis is a livestock disease that is transmissible to humans, so is of major public health concern. Industrialised countries have eliminated brucellosis with massive financial and technical interventions, but these are currently not feasible in Central Asian countries. Brucellosis has re-surfaced as a major health risk in the region after the end of the socialist period (1990). Can brucellosis be controlled under current financial, technical, and political constraints? Epidemiological and economic studies in people and livestock show promising options for the effective control and elimination of brucellosis in the region, provided appropriate knowledge and technology are available and there is political will for change.

Re-emergence of brucellosis in Central Asia

During the socialist period, public health and veterinary medical services in Central Asian countries were entirely state-led and effectively kept zoonoses (diseases transmissible between animals and humans) under control. But after the end of the socialist period at the beginning of the 1990s, public and animal health systems collapsed and livestock production was privatised. Surveillance of animal diseases was limited, and controls were ineffective. In the following decade, many different zoonoses, including tapeworm and rabies, re-emerged. Brucellosis is one of the most important of these.

Alarmingly high numbers of human brucellosis cases prompted the World Health Organization to assess options for controlling the disease. International experts recommended interventions in livestock to reduce human health risks. An economic assessment of a ten-year livestock mass-vaccination campaign in Mongolia showed that brucellosis control is profitable and cost-effective for society as a whole, including the public-health and animal-production sectors. If costs are shared between the livestock and public-health sectors proportionally to their benefits, the intervention is in the most cost-effective band of public-health interventions.

Featured case studies

Joint human and animal brucellosis studies

Blood testing for brucellosis was done simultaneously in nomadic pastoralists and their livestock in Chad. When medical doctors and veterinarians worked together directly, the sources of human brucellosis could be identified. This study was used as a model for the work in Central Asia (Schelling et al. 2003).

A model of animal-human brucellosis transmission in Mongolia

A mathematical model of livestock-human brucellosis transmission showed how human brucellosis can be reduced by interventions in animals. This model was used for the economic assessment mentioned below (Zinsstag et al. 2005).

Human health benefits of livestock vaccination for brucellosis

A case study of cross-sector societal economic assessment of the profitability of brucellosis mass vaccination showed that the societal benefits were three times higher than the cost of the intervention (Roth et al. 2003).

Towards a "one health" research-and-application toolbox

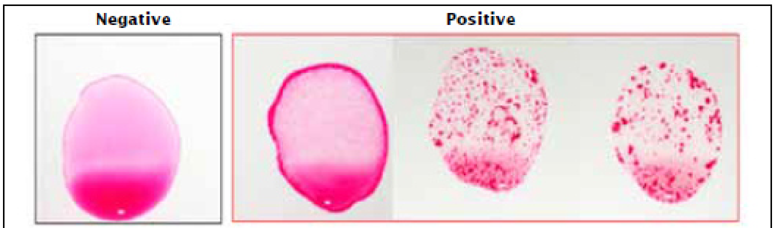
Integrated human and animal disease monitoring and surveillance was tested in Kyrgyzstan. This method can serve public health and veterinary services to join efforts for monitoring and surveillance and save scarce logistic and human resources (Zinsstag et al. 2009).

Obstacles to controlling brucellosis in humans

People with brucellosis suffer from long-term recurring fever, joint pain, weakness and fatigue. But there are no specific symptoms that make the disease easy to identify. That means that unless adequate laboratory tests are available, brucellosis is often under-diagnosed and hence under-reported. Curing brucellosis takes years – even a decade – of treatment, and its control is often not considered profitable because the disease is only seen either in humans or livestock, but not together – and doctors and veterinarians often fail to communicate. It is not possible to vaccinate humans against the disease.

Stepwise brucellosis elimination: Choosing the right approach

To choose the best option of control, detailed information is needed on the distribution and the number of new cases in a country. However, if the disease is present (independent of its prevalence in animals), it is recommended to start annual mass vaccination campaigns of animals (covering over 80% of animals every year) for 5–10 years, before moving on to vaccinating young replacement stock along with testing and slaughtering. The test-and-slaughter strategy can be advised only if public funds are available to compensate farmers for culled stock and if other enabling conditions are in place (see below). Both interventions require well-functioning veterinary field and laboratory capacity.



A blood test for brucellosis using the Rose Bengal Plate agglutination test: On the left side is a negative result. On the right are positive results. Photo: I. Moriyon


Cattle in peri-urban dairy production in Bishkek, Kyrgyzstan. Cattle are usually infected by Brucella abortus, but can also be infected by Brucella melitensis. Photo: J. Zinsstag

Integrated baseline assessment

Almost everyone who is infected with brucellosis has come into contact with infected livestock or livestock products. Detecting brucellosis in humans depends on suitably equipped laboratories in health centres, and access to such centres, especially in rural areas. A lack of such conditions leads to the under-reporting of human cases. Studies in Kyrgyzstan and Mongolia show only 5 to 20% of cases are ever reported officially. As a novel approach, we recommend the simultaneous assessment of human and livestock disease frequency, which provides a good overall picture of the distribution and transmission of the disease (see case studies).

Enabling environment

Effective interventions against brucellosis rely on important enabling conditions:

- Public or private veterinary services that cover fully the area of intervention and have sufficient equipment and staff.
- Human and veterinary laboratory capacity at district and provincial levels with sufficient capacity to handle the testing of blood samples, and at the central level a laboratory that can grow and identify the pathogen.
- Electricity to produce vaccines and to keep the vaccines cooled until they reach the animals to be vaccinated.
- If test-and-slaughter systems are implemented, sufficient public funds to compensate farmers for culled stock and a relatively corruption-free environment (if farmers are not compensated, they may sell infected animals illegally, which contributes to continued transmission of the disease).
- Animal registration and movement traceability.
- Appropriate management and monitoring of the control programme.

Choice of vaccine for livestock mass vaccination

If brucellosis is detected in livestock, mass vaccination of livestock should be the first option, regardless of the number of animals infected. It is safer to vaccinate using eye drops than the more usual syringe. Different species need different types of vaccine: sheep and goats should be vaccinated with *B. melitensis* Rev-1, and cattle and yaks with an attenuated *B. abortus* S19 strain. Sheep and goats of any age and sex can be vaccinated, including lactating animals, but vaccination should take place before the mating season, as vaccinating pregnant sheep can provoke abortion. In cattle, female animals of all ages can be vaccinated, but never males. More than 80% of the animals in an area should be vaccinated every year to reduce the risk of disease transmission.

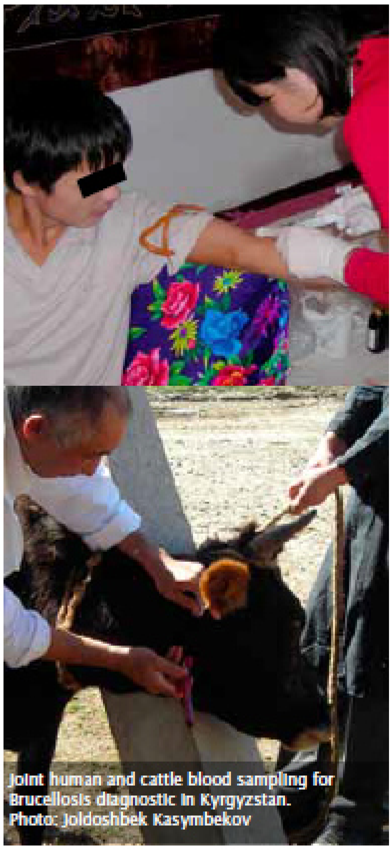
Mass vaccination should be followed immediately by an annual monitoring programme to assess the proportion of vaccinated animals. The recording of new human cases, conducted at adequate intervals (2–4 years) after vaccination, provides additional information on how effective the vaccination has been. The number of new human cases should drop, although not immediately, as infected animals are not culled during a mass vaccination and will remain for several years until they are eliminated by natural replacement. The vaccine quality should be tested prior to the annual campaign.

From mass vaccination to test and slaughter

Once annual mass vaccinations have begun, it is necessary to identify individual animals (e.g., though ear tags) and control livestock movements to prevent infected stock from entering the vaccinated area. After 5–10 years of livestock mass vaccination, once less than 1% of the flocks are infected and the disease is restricted to certain areas, it is possible to change the control policy: only young replacement animals need be vaccinated, and the adult animals should be blood-tested. Animals that test positive must be culled and destroyed. Their owners should be compensated adequately, depending on the market value of the animals. Failure to compensate farmers effectively will lead to illegal sales, jeopardising efforts to stop further transmission and to eliminate the disease. Once no new clinical livestock and human cases occur, and no blood samples test positive, the country can apply to the World Organisation for Animal Health (OIE) for certification of freedom from the disease. This certification requires annual representative surveys demonstrating the absence of positive animals.

Prevention of human exposure

At the same time as the interventions in livestock, humans should be provided with access to care and adequate treatment free of charge. Efforts should be made to prevent human exposure through direct contact with livestock and the consumption of unpasteurised milk and milk products. Information, education, and communication campaigns using the mass media, new information and communication technologies, schools, and engagement with communities, herders, and opinion leaders should be used to create awareness, motivate people to change their behaviour, and seek health care if necessary.



Joint human and cattle blood sampling for Brucellosis diagnostic in Kyrgyzstan.
Photo: Joldoshbek Kasymbekov

Definitions

Brucellosis: A bacterial disease causing abortions in sheep, goats, and cattle. Humans get infected by direct contact with livestock and the consumption of raw milk and milk products.

Disease incidence: The number of newly reported cases per time and population at risk. The incidence of human brucellosis was 78 cases per 100,000 per year in Kyrgyzstan in 2007.

Human incidence of disease: The number of infected people who applied for medical assistance as a proportion of the total population.

Prevalence: The proportion of humans or animals reacting to a serological test. Prevalence is not time-dependent and is often reported as percentage.

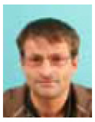
Sentinel: An animal or human that is monitored for a disease in order to indicate its presence. A sentinel can be used to predict the presence of a disease in another species.

Rose Bengal Test: A test of blood serum used to diagnose brucellosis. It can be used for both animals and humans. A drop of serum is mixed with a reagent; if it forms granules, it indicates the presence of the disease.

Test and slaughter: A control strategy in which animals are tested for brucellosis. The animals that test positive are culled.

Vaccination coverage: The proportion of animals effectively vaccinated among all animals during a vaccination campaign. For effective brucellosis control, vaccination coverage should be at least 80%, optimally 100%.

Vaccination route: Brucellosis vaccines can be applied under the skin with a syringe and needle ("subcutaneous") or with a dropper into the eye ("conjunctival application"). This second method is safer than the former, so is preferable.



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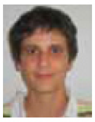

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Policy implications of NCCR North-South research

Adapt technologies to the local context

NCCR North–South research shows that brucellosis is massively under-reported, and that its control would be profitable in Central Asian countries. Simple laboratory tests, which can be implemented at the district level, enable it to be diagnosed in humans and animals. Well-monitored mass-vaccination campaigns of livestock would reduce the number of newly infected animals until it is possible to start a regime of testing animals and slaughtering those that are infected. The goal is to eliminate the disease.

Close cooperation between human and animal health services

Closer cooperation between public and animal health services would increase the benefits of interventions against brucellosis in livestock to prevent risks to human health.

Enabling conditions

Effective interventions against brucellosis rely on adequate public and private veterinary services and laboratory capacity. There should be sufficient electricity and storage facilities to maintain a cold chain. The application of a test-and-slaughter campaign requires the adequate development of veterinary services, the ability to identify all animals individually, and the effective control of animal movements. It also depends on sufficient public funds to compensate farmers for culled stock. Animal registration is an essential factor for successful animal disease control.

Further reading

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Мал ээлери үчүн суроолор

15.1 *Questionnaire for farmer survey (2006)*
Анкетный опрос для владельцев животных

(Бруцеллездун чарбага тийгизген таасири
Impact of Brucellosis on households
Влияние бруцеллеза на домашние хозяйства)

Final version from 3 June 2006

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1. *Интервью боюнча жалпы маалымат*
General information on interview
Общая информация об интервью

[1.1.] Интервьюнун N
 # Interview: ____________________________
Интервью N:

[1.2.] Репортердун аты жөнү:
Initials interviewer: ____________________________
Инициалы репортера:

[1.3.] Интервью алынуучу күн: Жыл Ай Күн
Date of interview: *Year:* _____ *Month:* _________ *Day*______
Дата интервью: *Год* *Месяц* *День:*

[1.4.] Интервьюнун башталышы:
Time of beginning of interview: ____________________________
Время начала интервью:

[1.5.] Интервью алган жер: (айыл)
Location of interview : ______________________ (village)_________________
Местоположение интервью: (село)

көчө, үй №
steet ________________________________ # of home _________________
улица дом №

---

2. **Фермерге карата жалпы маалымат**
General information on farmer
Общая информация относительно фермера

[2.1.] Интервью алынды:
*Initials interviewed*____
Интервью получено от:

[2.2.] Жашы: жаш паспортунун №
Age of the: _______________ *years old* *passport #* ________________
Возраст пациента: *лет* *№ паспорта*

[2.3.] Жынысы: *Sex: Пол:*
[2.3.1.] ❐ Эркек? *Male? Мужчина?*
[2.3.2.] ❐ Аял? *Femal ? Женщина?*

---

3. **Мүнөздөмө жана чарбага стратификациялоо чарасы**
Характеристики и меры для стратификации домашнего хозяйства
Household characteristics and measures for stratification of households

[3.1.] Үй ээсинин негизги кызматы:
Main occupation of head of household:
Основное занятие главы семьи:
[3.1.1]. ❒ Мамлекеттик кызматкер? State employee? *Государственный служащий?*
[3.1.2]. ❒ Жекече ишмер? Private employer? *Частный предприниматель?*
[3.1.3]. ❒ Малчы? Livestock herder? *Животновод?*
[3.1.4]. ❒ Башка? Other? *Другое*? ___________

[3.2.] Сиздин короодогу малдын саны?
(керектүүсүн белгилеп толтургула:)
Which number of animals do you have in your household?
(please cross and fill in a number if there are any:)
Количество животных в Вашем хозяйстве?
(пожалуйста подчеркните и заполните нужное:)

[3.2.1.] ❒ Бодо мал? (2006 чейин туулган) — 2006 туулган торпок
Cattle ? *(adults, born before 2006)* __________ *(juveniles, born in 2006)* _______
КРС (взрослые, рожд. до 2006) — *телки, рожд. в 2006*

[3.2.2.] ❒ Жылкы? *(2006 чейин туулган)* — *2006 туулган кулун*
*Horses ? (adults, born before 2006)*________ *(juveniles, born in 2006)* _______
Лошади? (взрослые, рожденные до 2006) — *жеребята, рожденные в 2006*

[3.2.3.] ❒ Эчки? *(2006 чейин туулган)* — *2006 туулган улак*
Goat? *(adults, born before 2006)* ____________ *(juveniles, born in 2006)* _______
Козы? *(взрослые, рожд. до 2006)* — *козлята, рожд. в 2006*

[3.2.4.] ❒ Кой? *(2006 чейин туулган)* — *2006 туулган козу*
Sheep? *(adults, born before 2006* ________ *(juveniles, born in 2006)* _______
Овцы? *(взрослые, рожд. до 2006)* — *ягнята, рожденные в 2006*

[3.2.5.] ❒ Чочко? *(2006 чейин туулган)* — *2006 туулган торопой*
Pigs ? *(adults, born before 2006)* _________ *(juveniles, born in 2006)* _______
Свиньи? *(взрослые, рожд. до 2006)* — *поросята, рожд. в 2006*

[3.2.6.] ❒ Ит? *(2006 чейин туулган)* — *2006 туулган* күчүк
Dogs? *(adults, born before 2006)* _________ *(juveniles, born in 2006)* _______
Собаки? *(взрослые, рожд. до 2006)* — *щенки, рожд. в 2006*

[3.2.7.] ❒ Төө? *(2006 чейин туулган)* — *2006 туулган тайлак*
Camels? *(adults, born before 2006)* ________ *(juveniles, born in 2006)* ______
Верблюды? *(взр., рожд. до 2006)* — *верблюжата, рожд. в 2006*

[3.2.8.] ❒ Топоз? *(2006 чейин туулган)* — *2006 туулган мамалак*
Yaks? *(adults, born before 2006)* _________ *(juveniles, born in 2006)* ______
Яки? *(взрослые., рожд. до 2006)* — *ячата, рожд. в 2006*

3.3.1. **Бодо малдын продуктуулук параметри**
Productivity parameters of cattle
Параметры продуктивности КРС
Төл (Бир жылда ар тубар уйдан алынган төлдүн саны)
Fecundity _________ (Number of newborns per adult female per year)

Приплод (*Количество новорожденных на каждое маточное поголовье в год*) ___

0-1 жаштагы малдын өлүмдүүлүгү (бир жашка чейин өлгөн музоолордун саны мисалы 2 баш 10-дон)
Mortality of 0-1 year old animals ___ (Number of dead per all newborn in the first year of life (e.g. 2 of 10)
смертность 0-1-летних животных (Количество павших новорожденных в первый год жизни (например 2 из 10) ___

1-2 жаштагы малдын өлүмдүүлүгү (1-2 жаштагы малдын бир жыл ичинде өлгөн саны, мисалы 2 баш 10-дон)
Mortality of 1-2 year old animals ___ (Number of dead animals of all animals in this age class per year)
Смертность 1-2-летних животных (Количество павших животных из всего поголовья в этом возрасте в год) ___

2 жаштан жогорку малдын өлүмдүүлүгү 2 жаштан жогору малдын бир жыл ичинде өлгөн саны, мисалы 2 баш 10-дон
Mortality of >2 year old animals ___ (Number of dead animals of all adult animals per year)
Смертность> 2-летние животные (Количество павших животных из всего взрослого поголовья в год)
___

аборт жыл ичиндеги баардык малдардагы болгон аборттун саны (Мисалы 10 баш 50 дөн)
Abortion ___ (Number of abortions per of all pregnant animals per year (e.g. 10 of 50)
аборт *(Количество абортов от всех животных за год (например: 10 из 50).* ___

жыл ичиндеги сүт (лактация) (Бир жылдагы 1 уйдун берген сүтү)
Lactation per year ___(Liters of milk produced in one year per cow)
Лактация в год (Литр молока, произведенного в 1 год на корову)
___

3.3.2. Койдун продуктуулук параметри
Productivity parameters of sheep
Параметры продуктивности овец

Төл (Бир жылда ар тубар койдон алынган төлдүн саны)
Fecundity ___ (Number of newborns per adult female per year)
Приплод (Количество новорожденных на каждое маточное поголовье в год)
___

0-1 жаштагы малдын өлүмдүүлүгү (бир жашка чейин өлгөн козулардын саны мисалы 2 баш 10-дон)
Mortality of 0-1 year old animals ___ (Number of dead per all newborn in the first year of life (e.g. 2 of 10)
смертность 0-1-летних животных (Количество павших новорожденных в первый год жизни (например 2 из 10) ___

1-2 жаштагы малдын өлүмдүүлүгү (1-2 жаштагы койлордун бир жыл ичинде өлгөн саны, мисалы 2 баш 10-дон)
Mortality of 1-2 year old animals ___ (Number of dead animals of all animals in this age class per year)
Смертность 1-2-летних животных (Количество павших из всего поголовья в этом возрасте в год)
___

2 жаштан жогорку малдын өлүмдүүлүгү 2 жаштан жогору койлордун бир жыл ичинде өлгөн саны, мисалы 2 баш 10-дон

Mortality of >2 year old animals ________ (Number of dead animals of all adult animals per year)
Смертность> 2-летние животные *(Количество павших животных из всего* взрослого поголовья в год)

Аборт жыл ичиндеги баардык койлордун болгон аборттун саны(Мисалы 10 баш 50 дөн)
Abortion _______ (Number of abortions per of all pregnant animals per year(e.g. 10 of 50)
Аборт (Количество абортов от всех животных за год (напр.: 10 из 50.

3.3.3. **Эчкинин продуктуулук параметри**
Productivity parameters of goats
Параметры продуктивности коз

Төл (Бир жылда ар тубар эчкиден алынган төлдүн саны)
Fecundity __________ (Number of newborns per adult female per year)
Приплод (Количество новорожденных на каждое маточное поголовье в год)

0-1 жаштагы малдын өлүмдүүлүгү (бир жашка чейин өлгөн улактардын саны мисалы 2 баш 10-дон)
Mortality of 0-1 year old animals ________ (Number of dead per all newborn in the first year of life (e.g. 2 of 10)
Смертность 0-1-летних животных (Количество павших новорожденных в первый год жизни (например 2 из 10)

1-2 жаштагы малдын өлүмдүүлүгү (1-2 жаштагы эчкилердин бир жыл ичинде өлгөн саны, мисалы 2 баш 10-дон)
Mortality of 1-2 year old animals ____________ (Number of dead animals of all animals in this age class per year)
Смертность 1-2-летних животных (Количество павших животных из всего поголовья в этом возрасте в год)

2 жаштан жогорку малдын өлүмдүүлүгү 2 жаштан жогору эчкилердин бир жыл ичинде өлгөн саны, мисалы 2 баш 10-дон
Mortality of >2 year old animals __________ (Number of dead animals of all adult animals per year)
Смертность> 2-летние животные *Количество павших животных из всего* взрослого поголовья в год)

Аборт жыл ичиндеги баардык эчкилердин болгон аборттун саны (Мисалы 10 баш 50 дөн)
Abortion __________ (Number of abortions per of all pregnant animals per year (e.g. 10 of 50)
Аборт (Количество абортов от всех животных за год (например: 10 из 50)

[3.4.] Үй ээсинин билим деңгээли? Which is the highest level of education completed of the household head? *Уровень образования главы семьи?*
[3.4.1.] ❒ Билимсиз? No school attendance? *Без образования?*
[3.4.2.] ❒ Орто билимдүү. School. *Среднее образование.*
[3.4.3.] ❒ Ортокесиптик,техникалык билимдүү.College*. Среднее проф. тех. образование.*
[3.4.4.] ❒ Жогорку билимдүү. University. *Высшее образование.*

[3.5.] Тамак-аш камдоо: Procurement of nutrition: *Приобретение пищи*:
[3.5.1.] ❒ Сиздин чарба төмөнкүлөрдүн кайсынысын өндүрөт? Do your household produce his? *Что из следующего производит ваше хозяйство?*

[3.5.1.1.] ❒ Жашылча? Own vegetable? *Овощи?*
[3.5.1.2.] ❒ Сүт? Own milk products? *Молочная продукция?*
[3.5.1.3.] ❒ Эт? Own meat? *Мясная продукция?*

[3.5.2.] ❒ Сиздер үйгө негизги азык-түлүктү каяктан аласыздар. Do your household procure his food mainly from. *Ваше домашнее хозяйство обеспечивается основными продуктами главным образом от:*
[3.5.2.1.] ❒ Базарданбы же дүкөндөнбү? Market or store? *Рынка или магазина?*
[3.5.2.2.] ❒ Өзүңөр өндүрөсүңөрбү? Own production? *Собственное производство?*

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4. Epidemiology of human brucellosis. Эпидемиология.

[4.1.] Сиздердин үй-бүлөөдөн кимдир бирөөнө бруцеллез диагнозу коюлду беле? Have any members of your household been diagnosed with Brucellosis? *Имеют ли члены вашего семейства поставленный диагноз на бруцеллез?*
[4.1.1.] ❒ Жок No *Нет*
[4.1.2.] ❒ Ооба Yes Да (Кан алуу үчүн врачка маалыматтаңыз *For blood taking inform the doctor Сообщите врачу для взятия крови*)

[4.2] Сиз өзүңүздүн малдардын ичинен бруцеллез менен ылаңдаганын кездештирдиңизби? Have you noticed Brucellosis cases in your herd? *Вы заметили случаи заболевания бруцеллеза в вашем стаде?*
[4.2.1.] ❒ Жок No *Нет*
[4.2.2.] ❒ Ооба Yes *Да*
Эгер кездештирсеңиз If yes *Если да,*
[4.2.3.] Качан, When, *Когда*? Жыл Year *Год*: ________
Айы Month *Месяц*: __________
Кайсы мал? On which animal? *Какое животное?*
[4.2.4.] ❒ Бодо малданбы? Cattle? *Крупный рогатый скот?*
[4.2.4.1.] Кандай белгилер болгон? Symptoms you recognized *Какие были симптомы*?:
____
[4.2.4.2.] Сиз ылаңды кандайча тастыктадыңыз? How did you confirm the disease? *Как Вы подтверждали болезнь*? ________________
[4.2.5.] ❒ Жылкыданбы? Horses? *Лошади*?
[4.2.5.1.] Кандай белгилер болгон? Symptoms you recognized? *Какие были симптомы*?:______________________
[4.2.5.2.] Сиз ылаңды кандайча тастыктадыңыз? How did you confirm the disease? *Как Вы подтверждали болезнь*? ________________
[4.2.6.] ❒ Эчкилер? Goats? *Козы*?
[4.2.6.1.] Кандай белгилер болгон? Symptoms you recognized? *Какие были симптомы?*:____
[4.2.6.2.] Сиз ылаңды кандайча тастыктадыңыз? How did you confirm the disease? *Как Вы подтверждали болезнь?* ________________
[4.2.7.] ❒ Койлор? Sheep? *Овцы*?
[4.2.7.1.] Кандай белгилер болгон? Symptoms you recognized *Какие были симптомы?*:____
[4.2.7.2.] Сиз ылаңды кандайча тастыктадыңыз? How did you confirm the disease? *Как Вы подтверждали болезнь*? ________________
[4.2.8.] ❒ Чочколор? Pigs? *Свиньи?*
[4.2.8.1.] Кандай белгилер болгон? Symptoms you recognized *Какие были симптомы?*:____
[4.2.8.2.] Сиз ылаңды кандайча тастыктадыңыз? How did you confirm the disease? *Как Вы подтверждали болезнь?* ________________
[4.2.9.] ❒ Иттер? Dogs? *Собаки*?
[4.2.9.1.] Кандай белгилер болгон? Symptoms you recognized? *Какие были симптомы*?:____
[4.2.9.2.] Сиз ылаңды кандайча тастыктадыңыз? How did you confirm the disease? *Как Вы подтверждали болезнь?* ________________
[4.2.10.] ❒ Төөлөр? Camel? *Верблюды*?
[4.2.10.1.] Кандай белгилер болгон? Symptoms you recognized? *Какие были симптомы*?:____
[4.2.10.2.] Сиз ылаңды кандайча тастыктадыңыз? How did you confirm the disease? *Как Вы подтверждали болезнь*? ________________

4.3. Сиз аборт болгонун байкадыңызбы? Do you observe abortion? *Вы наблюдали аборт*?
4.3.1. ❐ Ооба Yes *Да*
4.3.2. ❐ Жок No *Нет*
Эгер байкасаңыз кайсы малдан? If yes, in whit species: *Если да, то у каких животных, это было*:
[4.3..3.] ❐ Бододонбу? Cattle? *Крупный рогатый скот*?
[4.3.4.] ❐ Койдонбу? Sheep ? *Овцы*?
[4.3.5.] ❐ Эчкиденби? Goat? *Козы*?

[4.4.] Сиздин малыңыз бруцеллезго текшерилген беле? Have your animals ever been tested about Brucellosis? *Ваши животные когда-либо проверялись на бруцеллез*?

[4.4.1.] ❐ Жок No *Нет*
[4.4.2.] ❐ Ооба Yes *Да*
Эгер текшерилсе: If yes: *Если да*:
[4.4.2.1.] Жылы Year *Год*: ____________ Айы Month *Месяц*: __________
Эгер бруцеллез менен ылаңдаган мал чыкса аны сойгонсузбу? If yes, have the animals with the result Brucellosis positive been slaugtered? *Если да, вы забивали животных с положительным результатом на бруцеллез?*
[4.4.2.2] ❐ Ооба Yes *Да* Качан? When? *Когда?*
Жылы Year Год: ________ Айы Month *Месяц*: _________
[4.4.2.3] ❐ Жок No *Нет*
[4.4.3.] ❐ Билбейм I don't know *Я не знаю*

[4.5.] Сиздин малыңыз бруцеллезго каршы эмделгенби? Have your animals ever been vaccinated against Brucellosis? *Ваши животные когда-либо прививались против бруцеллеза*?
[4.5.1.] ❐ Жок No *Нет*
[4.5.2.] ❐ Ооба Yes *Да*
Эгерде ооба болсо If yes Если да:
[4.5.2.1.] Жылы Year Год: ________ Айы Month *Месяц*: _________
[4.5.3.] ❐ Билбейм I don't know *Я не знаю*

[4.6.] Сиз малды чарбаңызда соесузбу? Do you slaughter animals in your household? *Вы забиваете животных в вашем домашнем хозяйстве*?
[4.6.1.] ❐ Ооба Yes *Да*
[4.6.2.] ❐ Жок No *Нет*

[4.7.] Сиз малдардын терисин чарбаңызда иштетесизби? Do you process the skin of any animal in your household? *Вы обрабатываете кожу любого животного в вашем домашнем хозяйстве*?
[4.7.1.] ❐ Ооба Yes *Да*
[4.7.2.] ❐ Жок No Нет

[4.8.] Сиздин чарбаңыздан алынган пробалардын саны: Numbers of samples taken in the household: *Количество взятых проб на исследование в вашем хозяйстве*:
[4.8.1.] Койдон Sheep *Овцы*………………..
[4.8.2.] Эчкиден Goats *Козы*………………….
[4.8.3.] Бододон Cattle *Крупный рогатый скот*…………………

Интервью буткөн убакыт:
Time of ending of interview:
Время окончания интервью:

15.2 *Questionnaire for farmer survey (2012)*

Анкета для исследования хозяйств **** Чарбаларды изилдөө үчүн суроолор

(Livestock owners & herders * владельцы животных и животноводы мал ээлери жана малчылар)**

Final version from 25/ 06/ 2012

1. General information on interview (общая информация об интервью) интервью боюнча жалпы маалымат

[1.1.] ***N° interview (интервью №):*** ________________________

[1.2.] Initials interviewer имя анкерирующего) маалымат алуучунун аты: __________ ____

[1.3.] Date of interview (дата): Year (год): ***2012*** Month (месяц): ________ Day (число):

[1.4.] Time of beginning and end of interview (время начала и конца интервью) башталышы жана бүтүү убактысы: _________________________________

[1.5.] Location of interview and origin of animal (место и происхождение животных) малдын турган жана келген жери: _______________________________________

2. General information on farmer (общая информация о фермере) фермер жөнүндө жалпы маалымат

[2.1.] Initials interviewed (имя) аты жөнү ________________________________

[2.2.] Age of the (возраст) жашы: _____________ years old (лет) жаш

[2.3.] Sex (пол) жынысы:

[2.3.1.] ❒ male (М) Э

[2.3.2.] ❒ female (Ж) А

3. Household characteristics and measures for stratification of households (характеристика и измерение стратификации домохозяйств) чарбаны стратификациялоо чарасы жана мүнөздөмө

[3.1.] Main occupation of head of household (Основное занятие главы семьи) Үй ээсинин негизги кызматы:

[3.1.1.] ❒ Government employee-Public servant (Гос.служащий) Мам.кызматкер

[3.1.2.] ❒ Private entrepreneur (Частный предприниматель) Жекече ишмер?

[3.1.3.] ❒ Livestock herder (Животновод) Малчы?

[3.1.4.] ❒ Other self-employed (Работающие не по найму) Башка кызматта? _________

[3.2.] Which is the highest level of education completed of the household head (Уровень образования главы семьи) Үй ээсинин билим дэңгээли?

[3.2.1.] ❒ No school attendance (Без образования) билимсиз?

[3.2.2.] ❒ School (среднее образование) Орто билимдүү

[3.2.3.] ❒ College (средне специальноеобразование) Орто-кесиптик билим

[3.2.4.] ❒ University (Высшее образование) Жогорку билимдүү

4. ***Heard composition*** (Состав стада) Короонун (малдын) түзүлүшү? Which number of animals do you have in your household? (какое количество животных есть в вашем стаде) Сиздин короодгу малдын саны? *(Please cross and fill in a number if there are any * Пожалуйста заполните если есть таковое * Төмөндө суроолорго жоон бериниз:)*

	Age (возраст) жашы	Female самки Ургаачы	Male самцы эркек		Age возраст жашы	Female самки Ургаачы	Male самцы эркек		Age возраст жашы	Female самки Ургаачы	Male самцы эркек
Cattle КРС бодо	0-1			Sheep овцы кой	0-1			Goat козы Эчки	0-1		
	1-2				1-2				1-2		
	> 2				> 2				> 2		
	Total Всего Баардыгы				Total Всего Баардыгы				Total всего Баардыгы		

(0-1) young (*under 1 year*) (телята, ягнята, козлята до 1 года) 1 жашка чейинки музоо, козу, улак
(1-2) juveniles *(1-2 years)* (телки, ярки-бычки от 1-2 лет) 1-2 жашка чейинки торпок, токту-борук чебич
(>2) adult *(up to 2 years)* (взрослые коровы старше 2 лет) 2 жаштан жогору уй-бука-өгүз, кой-кочкор-ирик, эчки-теке

5. ***Slaughtering monthly at the slaughterhouse (помесячный убой в бойне) Атайын мал сойуучу жайдагы ай сайын мал союу***

	Малдын жашы	Jan Январь	Feb Февраль	Mar Март	Apr Апрель	May Май	Jun Июнь	Jul Июль	Aug Август	Sep Сент	Oct октябрь	Nov Ноябрь	Dec Декабрь
Female Самки Ургаачы бодо	0-1												
	1-2												
	> 2												
Total slaughtered всего забито союлган бодо													
Male Самцы Бука-өгүз	0-1												
	1-2												
	> 2												
Total slaughtered всего забито Баардык букалар													
Total cattle всего КРС жалпы бодо													

Female Самки Кой (ургаачы)	0-1												
	1-2												
	> 2												
союлган кой													
Male Самцы кочкор	0-1												
	1-2												
	> 2												
Total slaughtered всего забито кочкор													
Total sheep Всего овец жалпы кой-кочкор													
Female Самки Эчки (ургаачы)	0-1												
	1-2												
	> 2												
Total slaughtered всего забито баардыгы													
Male Самцы Теке	0-1												
	1-2												
	> 2												
Total slaughtered всего забито баардыгы													
Total goat всего коз Жалпы эчки-теке													

5.4. Do you slaughter animals in your household ? (Вы забиваете животных дома) Сиздер малды үйдөн соёсуздарбы?

[5.4.1.] ❒ no (нет) жок?

[5.4.2.] ❒ yes (да) ооба?

If yes how many animals per year (Если да то сколько животных в год) Эгерде ооба болсо, анда жылына канча?

	Age (возраст) жашы	Female самки	Male самцы		Age возраст жашы	Female самки	Male самцы		Age возраст жашы	Female самки	Male самцы

		Ургаачы	эркек			Ургаачы	эркек			Ургаачы	эркек
Cattle КРС бодо	0-1			Sheep овцы кой	0-1			Goat козы Эчки	0-1		
	1-2				1-2				1-2		
	> 2				> 2				> 2		
	Total Всего Баардыгы				Total Всего Баардыгы				Total всего Баардыгы		

[5.4.3.] What purpose of slaughtering animals at home (Какова цель забоя животных дома) Малды үйдөн сойгондун максаты кандай?
[5.4.3.1.] Home use (for food) (использовать дома для пищи) Үйдө тамак-ашка пайдалануу?
[5.4.3.2.] Sale (продажа) сатуу
5.5. Do you process the skin of any animal in your household ? (Вы обрабатываете дома шкуру) Сиз үйдөн тери иштетесизби?
[5.5.1.] ❐ no (нет) жок
[5.5.2.] ❐ yes (да) Ооба

6. *Sales of animals* (Продажа животных) малдарды сатуу
Please fill the table of monthly sale parameters

	Малдын жашы	Jan Январь	Feb Февраль	Mar Март	Apr Апрель	May Май	Jun Июнь	Jul Июль	Aug Август	Sep Сент	Oct октябрь	Nov Ноябрь	Dec Декабрь
Female Самки Ургаачы бодо	0-1												
	1-2												
	> 2												
Total sold всего продано сатылган бодо													
Male Самцы Бука-өгүз	0-1												
	1-2												
	> 2												
Total sold всего продано баардык сатылган букалар													
Total sold cattle всего КРС продано жалпы													

сатылган бодо													
Female Самки Кой (ургаачы)	0-1												
	1-2												
	> 2												
союлган кой													
Male Самцы кочкор	0-1												
	1-2												
	> 2												
Total sold всего продано сатылган кочкор													
Total sheep Всего овец жалпы кой-кочкор													
Female Самки Эчки (ургаачы)	0-1												
	1-2												
	> 2												
Total sold всего продано баардыгы сатылды													
Male Самцы Теке	0-1												
	1-2												
	> 2												
Total sold всего продано баардыгы													
Total goat всего коз Жалпы эчки-теке													

7. *Productivity parameters of household* (Параметры продуктивности домохозяйств) чарбанын продуктивдүүлүк(өндүрүгүч) параметри

7.1. Fecundity - Did you had newborns in the last year? (Плодородие – сколько новорожденных за этот год?) Тукумдуулук – ушул жылда сиздин кородо канча мал төлдөдү?

(Number of newborns per adult female per year) (количество приплода на взрослое поголовье на год) (жылына мал башына алынган төлдүн саны)

[7.1.1.] ❒ cattle (КРС) Бодо _______________

[7.1.2.] ❒ sheep (овцы) кой _______________

[7.1.3.] ❒ goat (козы) эчки ________________

7.2. Of 10 reproductive female animals in your herd, how many newborns? Количество полученного приплода на 10 репродуктивных животных в вашем стаде? Сиздин короодогу ар 10 тубар малдын башына алынган төлдүн саны?

[7.2.1.] ❒ cattle (КРС) Бодо ____________?

[7.2.2.] ❒ sheep (овцы) кой ____________?

[7.2.3.] ❒ goat (козы) эчки _____________?

7.3. Of 10 newborns animals, how many have died before the end of the first year of life? Сколько из 10 голов приплода текущего года пало в течение первого года жизни? Быйылкы ар 10 баш төлдүн канчасы канчасы жыл ичинде өлдү?

[7.3.1.] ❒ cattle (КРС) Бодо __________?

[7.3.2.] ❒ sheep (овцы) кой __________?

[7.3.3.] ❒ goat (козы) эчки __________?

7.4. Abortion (Number of abortions (lost) per of all pregnant animals per year (e.g. 10 of 50); Аборты (Ежегодное число абортов (потеря) из всех беременных животных (например 10 из 50); Аборт (Ар бооз малдын качасы музоо (козу, улак) салды? (мисалы 50 дөн 10 баш)

[7.4.1.] ❒ cattle (КРС) Бодо __________?

[7.4.2.] ❒ sheep (овцы) кой __________?

[7.4.3.] ❒ goat (козы) эчки __________?

7.5. For cattle only what on the average lactation of you cattle? Только для КРС, средний удой ваших коров за лактацию? Бодо мал үчүн, саан уйдан алынган сүт

[7.5.1.] ❒ less 2000 litre or how many litre (меньше 2000 литров или сколько литров) 2 миң литрден кем жээ ______________

[7.5.2.] ❒ more 2000 litre or how many litre (больше 2000 л или сколько) 2 миң литрден ашык же ______________

[7.5.3.] ❒ I do not know (не знаю) билбейм?

---

8. Procurement of nutrition of animals: (Закупка корма для животных) Малга тоют камдоо:

8.1. Do your household produce his (Ваше домашнее хозяйство производит) Сиздин чарба төмөндөгүлөрдөн чыгарабы:

[8.1.1.] ❒ own hay (собственное сено) Өздүк чөп?

[8.1.2.] ❒ own grain (cereal) (собственное зерно) өздүк эгин?

[8.1.3.] ❒ own silage (собственный силос) өздүк силос?

8.2. Do your household procure his food mainly from (Ваше домашнее хозяйство обеспечивает кормами главным образом от) Сиздин чарба негизинен тоютту

[8.2.1.] ❒ market or store (рынка или от магазина) базардан же дүкөндөн?

[8.2.2.] ❒ own production (собственное производство) өздүк өндүрүш?

9. ***Disease control & access to service*** (***Контроль за болезнью & доступ к обслуживанию) Дартты контролдоо & тейлөөгө укук)***

9.1. Have you noticed Brucellosis cases in your herd (замечали ли Вы случаи Бруцеллеза в Вашем стаде) Сиздин короонузда бруцеллез кездешти беле?

❒ no (нет) жок?

❒ yes (да) ооба?

If yes, (если да, то) эгерде оба болсо, анда

[9.1.1.] when (когда) качан ? Year (год) жыл: ________ Month (месяц) ай: _________

on which animal (какие животные) кайсыл мал?

[9.1.2.] ❒ Cattle (КРС) Бодо?

[9.1.2.1.] Symptoms you recognised (симптомы распознаны) кандай симптомдор:

____

[9.1.2.2.] How did you confirm the disease (Как Вы подтвердили болезнь) Сиз дартты кантип тактадыныз? ____

[9.1.3.] ❒ Sheep (овцы) кой?

[9.1.3.1.] Symptoms you recognised (симптомы распознаны) кандай симптомдор:

____

[9.1.3.2.] How did you confirm the disease (Как Вы подтвердили болезнь) Сиз дартты кантип тактадыныз? ________________

[9.1.4.] ❒ Goat (козы) эчки?

[9.1.4.1.] Symptoms you recognised (симптомы распознаны) кандай симптомдор:

____

[9.1.4.2.] How did you confirm the disease (Как Вы подтвердили болезнь) Сиз дартты кантип тактадыныз? ____

❒ I don't know (не знаю) билбейм?

9.2. Have your animals ever been tested about Brucellosis (Ваши животные когда-либо проверялись на Бруцеллез) Сиздин мал Бруцеллезго текшерилди беле?

❒ no (нет) жок ?

❒ yes (да) ооба?

If yes (если да) эгерде ооба болсо:

[9.2.1.] Cattle (КРС) Бодо Year (год) жыл: _______ Month (месяц) ай: _____

[9.2.2.] Sheep (овцы) кой Year (год) жыл: _________ Month (месяц) ай: ________

[9.2.3.] Goat (козы) эчки Year (год) жыл: ________ Month (месяц) ай: ______

❒ I don't know (не знаю) билбейм?

9.2.4 If yes, have the animals with the result Brucellosis positive been slaughtered (Если да, то животные с положительным результатом на Бруцеллезом были забиты) Эгерде оба болсо, анда дарт табылган мал союлду беле?

❒ yes (да) ооба ?

[9.2.4.1] When (когда) качан ? Year: (год) жыл: ________ Month (месяц) ай: _______

❒ no (нет) жок?

❒I do not know (не знаю) билбейм?

9.3. Have your animals ever been vaccinated against Brucellosis (ваши животные когда-либо вакцинированы против Бруцеллеза) сиздин мал качандыр бруцеллезго каршы эмделди беле?

❒ no (нет) жок?

❒ yes (да) ооба?

[9.3.1.] If yes (если да) эгерде ооба болсо:
Year: (год) жыл: ________ Month (месяц) ай: _______

❒ I don't know (не знаю) билбейм?

9.4. Have your vaccinated against Brucellosis animals ear notching? (ваши животные вакцинированные против Бруцеллеза имеют выщипы (надрезы) на ушах) Сиздин эмделген малдын кулагына эн салынды беле?

❒ no (нет) жок?

❒ yes (да) ооба?

9.5. Have you noticed PPR cases in your herd (Вы когда либо замечали случаи Чумы МРС в Вашем стаде) Сиздин короодо кыргын болгонун байкадыныз беле?

❒ no (нет) жок?

❒ yes (да) ооба?

If yes(если да) эгерде ооба болсо,
[9.5.1.] when (когда) качан? Year (год) жыл: ________ Month (месяц) ай: _________
on which animal (какие виды животных) малдын касыл түрүндө?

[9.5.2.] ❒ Sheep (овцы) кой?

[9.5..2.1.] Symptoms you recognised (симптомы распознаны) кандай симптомдор:

____

[9.5.3.] ❒ Goat (козы)?

[9.5.3.1.] Symptoms you recognised (симптомы распознаны) кандай симптомдор:

____

10. ***Numbers of samples taken in the household and lab results количество проб, взятых в этом домашнем хозяйстве и результатах лабораторных исследований***

[10.1.] Cattle (КРС) Бодо…………… [10.1.1.] brucellosis....................
[10.2.] Sheep (овцы) кой…………… [10.2.1.] brucellosis………. [10.2.2.] PPR………………
[10.3.] Goat (козы) эчки…………..… [10.3.1.] brucellosis………. [10.3.2.] PPR........................

15.3 *Questionnaire for brucellosis patients*
(Impact of Brucellosis on households)

Final version from June 2013

Acute brucellosis ___ Chronic brucellosis____

1. General information on interview

[1.1.] N° interview : ___________________________

[1.2.] Initials interviewer : ___________________________

[1.3.] Date of interview : Year: ________ Month: ________ Day: ______

[1.4.] Time of beginning of interview : ___________________________

[1.5.] Location of interview : ___________________________

2. General information on farmer

[2.1.] Initials interviewed ____

[2.2.] Age of the: _______________ years old

[2.3.] Sex:

- [2.3.1.] ❐ male ?
- [2.3.2.] ❐ female ?

[2.4.] Adress (rayon, village)_____________

3. Household characteristics and measures for stratification of households

[3.1.] Main occupation of head of household:

- [3.1.1.] ❐ Civil servant ?
- [3.1.2.] ❐ Private employer ?
- [3.1.3.] ❐ Livestock herder ?
- [3.1.4.] ❐ Does not work
- [3.1.5.] ❐ Other self-employed ? ___________________

4. Perception and interaction with the health care system

[4.1.] How often did you address the health system from the beginning of your illness?

- [4.1.1.] ❐ never
- [4.1.2.] ❐ 1-2 times
- [4.1.3.] ❐ 3-5 times
- [4.1.4.] ❐ 6-9 times
- [4.1.5.] >=10

[4.2.] Where did you treat your brucellosis the last time :

[4.2.1.] ❐ Family medicine (plants etc.) ?
[4.2.2.] ❐ Traditional healer/doctor ?
[4.2.3.] ❐ Pharmacy ?
[4.2.4.] ❐ Private doctor ?
[4.2.5.] ❐ hospital ?

[4.3.] Why did you come to consult in this health centre/hospital ?

[4.3.1.] ❐ Only place to get the treatment ?
[4.3.2.] ❐ Cheaper than alternatives ?
[4.3.3.] ❐ I know someone at this centre/hospital ?
[4.3.4.] ❐ Better quality than alternatives ?
[4.3.5.] ❐ closest facility
[4.3.6.] Others ____________

5. Epidemiology of human brucellosis

[5.1.] Have any other members of your household been diagnosed with Brucellosis ?

[5.1.1.] ❐ no ?
[5.1.2.] ❐ yes ?
[5.1.2.1.] If yes, when ? Year: ______ Month: ______
[5.1.2.2.] If yes, what kind of treatment did he or she receive ?
[5.1.2.2.1.] ❐ no treatment ?
[5.1.2.2.2.] ❐ Drugs ?
[5.1.2.2.3.] ❐ Hospitalisation ?
[5.1.2.2.4.] ❐ Other __________

6. Costs incurred to household

[6.1.] How much do you spent for the current health care recourses for the disease (Brucellosis) ?
(please cross and fill number in:)

[6.1.1.] ❐ For transport costs: ________ Som
[6.1.2.] ❐ For drugs: ________ Som
[6.1.3.] ❐ For hospitalisation: ________ Som
[6.1.4.] ❐ For laboratory or X-rays: ________ Som
[6.1.5.] ❐ Fees for doctors or others: ________ Som
[6.1.6.] ❐ Food during hospitalisation ________ Som
[6.1.7.] ❐ Other costs: ________ Som

[6.2.] Up to now how many days have you been away from your household (inclusive hospitalisation) in 2013 because of the disease Brucellosis ?
(Please fill number of days in) __________ days

7. Loss of productivity

[7.1.] Are you still able to work as usual being ill from Brucellosis?

[7.1.1.] ❒ yes ?
[7.1.2.] ❒ no ?
[7.1.2.1.] If no, since when have you been unable to perform your routine work? Year: ______ Month: ________ Day: _______

8. Opportunity costs

[8.1.] Who replaces you for your routine work or part of your routine work while you are away from your household or while you are being treated ?
[8.1.1.] ❒ nobody ?
[8.1.2.] ❒ relatives living in the same household ?
[8.1.3.] ❒ other relatives not living in the same household ?
[8.1.4.] ❒ other than relatives ?

[8.2.] Has your income decreased since you are ill from Brucellosis in 2013?

[8.2.1.] ❒ no ?

[8.2.2.] ❒ yes ?
if yes, by how much?
<u>here two possibilities to answer:</u>
[8.2.2.1.] ❒ by how many percent?_______
<u>or:</u>
[8.2.2.2.] ❒ by __________ Som. *(Please insert the sum)*

9. Availability of cash for treatment of Brucellosis

[9.1.] Has it occurred in your household, that you had no cash to pay for health care (incl. drugs and transport) ?
[9.1.1.] ❒ no ? *(If no, then continue please with question [8.1.])*
[9.1.2.] ❒ yes ?
If yes, is it linked to the present disease?
[9.1.2.1.] ❒ yes ?
[9.1.2.2.] ❒ no ?

[9.2.] If it has occurred, that your household has had no cash to pay for health care (incl. drugs

and transport), did you stop the consumption of health care services ?

[9.2.1.] ❒ yes ? *(If yes, then continue please with question [8.1.])*
[9.2.2.] ❒ no ?

[9.3.] If it has occurred that your household has had no cash to pay for health care, did you receive health care without spending cash?
[9.3.1.] ❒ yes ?
If yes, did you:

[9.3.1.1.] ❒ avoid payment ?
[9.3.1.2.] ❒ seek for exemption ?

[9.3.2.] ❒ no ?
if no, did:

[9.3.2.1.] ❒ borrow money ?
[9.3.2.2.] ❒ delay payments or postpone consumption, investments, education?
[9.3.2.3.] ❒ Open up new income fields by for example engaging household members in extra work, begging or charity, selling assets as live stock or equipment ?

[9.4.] If it has occurred that your household has had no cash to pay for health care, did you reduce consumption in health care?
[9.4.1.] ❒ no ?
[9.4.2.] ❒ yes ?
If yes, did you:
[9.4.2.1.] ❒ delay consumption of health care ?
[9.4.2.2.] ❒ reduce attendance or length of stay ?
[9.4.2.3.] ❒ cut level of treatment ?
[9.4.2.4.] ❒ do not complete treatment regime ?

[9.5.] If it has occurred, that your household has had no cash to pay for health care, did you diversify consumption in health care?
[9.5.1.] ❒ no ?
[9.5.2.] ❒ yes ?
If yes, did you:
[9.5.2.1.] ❒ shift demand to other providers ?
[9.5.2.2.] ❒ not seek for treatment ?

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17. Curriculum vitae (CV)

Information

Name:	Joldoshbek KASYMBEKOV
Marital status:	Married
Children:	One son and two daughters
Date of Birth:	09/04/1967
Citizenship:	Kyrgyz Republic
Tel.:	+996 312 61 76 03 (KGZ) +996 700 83 29 32 (cell ph)
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Education

2010-2014	**PhD** in Epidemiology at the Swiss Tropical and Public Health Institute “Epidemiology, cost and surveillance of brucellosis in people and livestock of Kyrgyzstan” at the Faculty of Natural Sciences of the Basel University, Switzerland, supervised by J. Zinsstag, Professor, PhD, DVM
2000-2003	**Candidate of Veterinary Sciences (PhD)** in 16.00.03 at the Research Institute of Livestock, Veterinary and Pasture “Improvement of the Methods of Diagnostic of Animal Brucellosis” at the Kyrgyz Agrarian University, supervised by V.I. Kim, Professor, PhD, DVM
1997-1999	**Faculty of Veterinary Medicine** Kyrgyz Agrarian Academy
1991-1996	**Faculty of Zoo engineering (Animal Breeding)** Kyrgyz Agrarian Academy

Employment Record

23.11.2015-Present	**Animal Health Specialist at the APIU MoAH KR** **Responsibilities:** providing technical, information and advisory assistance in updating strategies, strategic plans and control implementation of the approved strategies, plans of actions aimed at improvement of animal health, veterinary and laboratory services in the Kyrgyz Republic. Working closely with respective central, local structures of MoAM, MoH, SIVPSS (State Veterinary Service), ARIS, KSRVI, KNAU, VSB, private veterinary services and their associations and other stakeholders; assist in timely preparation of procurement plans, work programs, budgets, progress reports and M&E reports required under World Bank/IFAD guidelines; jointly with the project implementing agencies – partners communicate and work with OIE towards achieving of the expected

	results in veterinary on a regular basis; provide support to the technical assistance and advice for the project activities associated with animal health, and integrate inputs and recommendations of the international specialists effectively; participate in designing and updating normative and legal acts, technical guidelines, training modules and other information and training materials in veterinary / animal health; prepare descriptive and analytical reports;
03.10.2014-23.11.2015	**Education and Continuous Professional Development Specialist** of the Veterinary Chamber of the Kyrgyz Republic **Responsibilities:** define educational, professional and qualification requirements, adoption of certification system for private veterinarians; elaboration of curriculum of continuous professional education, consult and provide systematic support for veterinarians on requirements needed for update their knowledge and experience, development of minimum requirement of skills, knowledge, abilities of the veterinarian based on required OIE standards, development and updating of training programs, development the technical manuals, training module, preparation of the trainers for training and etc.
03.10.2014-Present	**Head of Virology**, Institute of Biotechnology of the National Academy of Sciences of the Kyrgyz Republic. **Responsibilities**: providing training to laboratory research, led work on the development of new and improvement and their introduction into production, guide, planning, research, development of laboratory facilities and collaboration with international scientists, tutoring young scientists and etc.
07.2013-10.2014	**Senior scientist**, Institute of Biotechnology of the National Academy of Sciences of the Kyrgyz Republic **Responsibilities:** conducting research and development on the subject in accordance with approved procedures, participation in carrying out experiments, observations and formulating conclusions, participation in the implementation of the results of research and development, tutoring young scientists and etc.
09.2010-04.2014	**PhD candidate** of the Human and Animal Health unit of the Epidemiology and Public Health Department of the Swiss Tropical and Public Health Institute. Basel University **Responsibilities:** Study of programmes of Epidemiology and training courses in the field of Public Health. Data collection and providing Statistical (STATA-12, Epi Info^{tm}7) and Economical analysis.
26.02.2008-31.08.2010	**National Consultant** Contractor FAO UN project on Avian Influenza and other Transboundary Animal Diseases **Responsibilities:** Maintain liaison with the Chief Veterinary Officer (CVO) to ensure project activities are appropriate and consistent with national

	objectives; Planning, implementation of training, workshops in AI and TAD's surveillance and diagnosis and conduct of national training activities; Ensure the timely ordering and delivery of project equipment and supplies to maintain continuity of project activities; Action as focal point for HPAI activities by collecting related information and support FAO in its resources mobilization efforts including preparation, in close cooperation with TCEO and AGAH.
02.09.2008-30.03.2009	**Chief of Information and Communication** State Veterinary Department of the Kyrgyz Republic **Responsibilities:** Responsible for WAHID & WAHIS, Designing and planning preventive activities of diseases and coordination of their implantation (Brucellosis mass vaccination project; FMD, PPR, Anthrax, Sheep Pox mass vaccination); Action to develop National information System on animal disease registration, data base, training of Veterinarians for the Internet using; Cooperation with International Organizations and Donors on behalf of State Veterinary Department. Design projects and activities on surveillance of disease and ect.
18.01.2008-02.09.2008	**Deputy Chief of the Department for Analysis and Planning of Epidemiological Activities** State Veterinary Department of the Kyrgyz Republic **Responsibilities:** Responsible for WAHID & WAHIS, assist to designing and planning preventive activities of diseases listed A & B; Design projects and activities on surveillance of disease and ect.
2003-2008	**Head of Brucellosis and TB department** Research Institute of Livestock, Veterinary and Pasture **Responsibilities:** Planning, developing and managing scientific researches on Brucellosis and TB: vaccine and diagnostic study, analysis of data and reporting, scientific writing (publishing)
2000-2002	**Consultant, Veterinarian – biologist** Veterinary component of the Sheep Development Project of World Bank at the Ministry of Agriculture **Responsibilities:** Consultation activities for farmers, private veterinarians, data collection, analysing (mass vaccination of small ruminants), and preparing reports to the MoA, State Veterinary Department.
1998-2000	**Veterinary Specialist** Kyrgyz State Control Research Institute of Veterinary **Responsibilities:** Scientific research: developing vaccine and diagnostic methods, clinical trial, data collection, analysis of data and reporting
1996-1998	**Head of Scientific Department** Joint-Stock Company "Uyk" **Responsibilities:** Planning and developing scientific studies
1994-1996	**Veterinarian**

	State Veterinary Department
	Responsibilities: Clinical trial, data collection, analysing (working with laboratory animals)
1989-1994	**Veterinary specialist**
	Kyrgyz Research Institute of Veterinary
	Responsibilities: Trial-experiments, data collection (working with laboratory animals)

Trainings at the Swiss Tropical and Public Health Institute

1-7.07.2011	Inter Regional Training Course 1-7 July 2011 NCCR N-S, Kathmandu, Nepal
20-22.09.2011	poster on “BioValley Life Science Week 2011” Basel, 20-September 2011, Day: Infectious Disease of Global Impact.
5-7.03.2012	Oral presentation at seminar on Transboundary Ecosystem Health in Pamir’s, Dushanbe, Tajikistan,
17.08.2012	Oral presentation at Sanitary and Epidemiology and State Veterinary Department in Bishkek, Kyrgyzstan
1-10.09.2013	2nd International Graduate School North-South Summer School on Health and Environment, Abidjan, Coˆte d’Ivoire
04.11.2013	Oral presentation, Monday Seminar at Swiss TPH

Other trainings

2013	International Graduate School (IGS) North-South Summere school training on contribute to sustainable Development- and global change-related issues through research held in Abidjan, Côte d'Ivoire.
2011	Inter-Regional Training Course (IRTC) 2011 of the National Centre of Competence in Research (NCCR) North-South held in Kathmandu, Nepal.
2009	FAO - Training workshops on Communication strategy; Standard of Operation Procedures (SOPs); Socio-Economics of Animal Disease Prevention and Control with a Specific Focus on Highly Pathogenic Avian Influenza, Ankara, Turkey,
2009	FAO-OIE- Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe) GIS training course on epidemiology and control of emerging avian diseases - Padova (Italy), September 2009
2008	FAO – Crisis Management Centre, Animal health (CMC-AH) training on “Orientation for Rapid Response to Animal Health Emergencies”. 22-27 June 2008, Rome – Italy.
2008	Advanced training on World Animal Health (OIE) Information System and Database (WAHIS & WAHID), World Organization for Animal Health (OIE), Paris, France
2005-2008	EU Specific Support Action Project, Training and Mentoring early career scientists from candidate,

	associated and Mediterranean countries in a whole food chain approach to quality and safety
2007	"Laboratory Animal Care and Research and Techniques", United States Army Medical Component-Armed Forces Research Institute of Medical Sciences (USAMC-AFRIMS) USAFIRM Bangkok, Thailand
2007	"Veterinary Epidemiology" IFDC Kyrgyz Agro-Input Enterprise Development Project (KAED/IFDC) of USAID, Bishkek, Kyrgyzstan
2007	"Successful negotiations" International Scientific Technical Centre (ISTC), Bishkek, Kyrgyzstan
2002	Information and Communication Technology Malaysian Technical Cooperation Programme (MTCP, ICT-2002);
1998	Method of culture of vaccine and batch control, Join Stock company "Altyn-Tamyr (2 month) Bishkek, Kyrgyzstan
1998	Diagnostic techniques of and developing skills on routine serology tests, Republican Central State Veterinary Laboratory" (3 month), Bishkek, Kyrgyzstan

Additional Responsibilities

Since-2011	Expert of the World Organization for Animal Health (OIE), member of OIE ad hoc Group on Brucellosis (http://www.oie.int/doc/ged/D10866.PDF, p 5) http://www.oie.int/doc/ged/D12311.PDF, p5 http://www.baphiq.gov.tw/userfiles/A_SCAD_Aug-Sept2011.pdf, p 90.
2008-2011	Co-coordinator of the Swiss-Kyrgyz project on molecular epidemiology study of Brucellosis
2008-2010	FAO UN National consultant of the project of Central Asia Regional Network on Avian Influenza and other Transboundary Animal Diseases in Kyrgyzstan
2008-2009	Coordinator of the World Bank's project on Brucellosis prevention
2006-2007	Co -coordinator of the Kyrgyz-Swiss Project of joint animal and human health on Comprehensive Brucellosis study in Kyrgyzstan
2003-2008	Part-time trainer of the "Consulting and Training Center" (renamed "Training, advisory and Innovation Centre")

Languages

Kyrgyz (native language), Russian, English speaking, reading and writing good

Books and chapters

Guide to practical exercises zoonosis

Chapter 1	Diagnosis, prevention and control measures of zoonosis
Chapter 2.	Studies on zoonosis; "Guide to practical exercises zoonosis" ISBN 9967-10-192-X; UDC 619:616.9 (075); LBC (BBK) 48 P-85. 2005, Sham Publisher, 381 p. Recommended by the Ministry of Education of the Kyrgyz Republic as a textbook for students of higher educational institutions, specialty 560501 "Veterinary medicine" (in Russian)
Herders' Manual	
Part A	Pasture plants
Part B	Pasture management
Part C	Animal welfare, Animal health and disease prevention
Part D	Annexes
	Herders' Manual. – B.: University of Central Asia. – B.: ISBN 978-9967-26-502-8; 2011. - 124 p. Published: University of Central Asia, 138, Toktogul Str., Bishkek, 720001, Kyrgyz Republic; e-mail: info@ucentralasia.org, www.ucentralasia.org (in Kyrgyz, Russian and English version, http://msri.ucentralasia.org/events.asp?Nid=248)

Peer-reviewed articles

Kasymbekov J.B., Kim V.I	Ring test with salt. Invention patent. № 493. 31.12.2001.
Zinsstag J, Schelling E., Bonfoh B., Fooks A.R., Kasymbekov J., Waltner-Toews D. & Tanner M.:	Towards a 'One Health' research and application tool box. Veterinaria Italiana Volume 45 (1) 121-133 (2009) PM:20391395
Bonfoh B, Zinsstag J, Fokou G, Weibel D, Ould Tableb M, Ur-Rahim I, Maselli D, Kasymbekov J, Tanner M(2011)	Pastoralism at the crossroads: new avenues for sustainable livelihoods in semi-arid regions. In: Wiesmann U, Hurni H (eds), Research for sustainable development: foundations, experiences, and perspectives. Bern: Geographica Bernensia, 549-570.
Bonfoh, B, J Kasymbekov, S Dürr, N Toktobaev, M Doherr, T Schueth,J Zinsstag, and E Schelling. 2012.	"Representative Seroprevalences of Brucellosis in Humans and Livestock in Kyrgyzstan." Representative seroprevalences of brucellosis in humans and livestock in Kyrgyzstan. EcoHealth 9, 132-138. PM:22143553
Zinsstag J, Kasymbekov J, Schelling E, Bonfoh B., 2012	It's **time** to control brucellosis in Central Asia. (2) , 1-4.Magazine article
J. Kasymbekov, J. Imanseitov, M. Ballif, N. Schürch, S. Paniga, P. Pilo, M. Tonolla, C. Benagli, K. Akylbekova, Z. Jumakanova, E. Schelling, and J. Zinsstag,	Molecular Epidemiology and Antibiotic Susceptibility of Livestock Brucella melitensis Isolates from Naryn Oblast, Kyrgyzstan. PLoS Negl Trop Dis, vol. 7, no. 2, p. e2047, Feb. 2013
Dürr S, Bonfoh B, Schelling E, Kasymbekov J, Doherr MG, Toktobaev N, Schueth T, Zinsstag J (2014)	Bayesian estimate of seroprevalence of Brucellosis in humans and livestock in Kyrgyzstan. *Rev Sci Tech* (in press), Journal Article

Zinsstag, J. and Dean, A. and Baljinnyam, Z. and Roth, F. and Kasymbekov, J. and Schelling, E. (2015)	Brucellosis surveillance and control: a case for one health Brucellosis surveillance and control: a case for one health. In: One health: the theory and practice of integrated health approaches. Wallingford, S. 153-162.