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In Mongolia, human brucellosis became an issue in the 1960s. During the 1980s, thanks to livestock vaccination, human incidence was reduced to less than 1 case per 100,000 populations. In the 1990s, human brucellosis re-emerged due to the breakdown of government run disease surveillance and control programs and the lack of resources in the veterinary and medical sectors. Since 2000, the government of Mongolia has been implementing a mass brucellosis vaccination which extended until 2021. The brucellosis mass vaccination was not able to interrupt transmission from livestock to humans. This requires trace-back investigation of brucellosis using molecular epidemiological methods for medical and veterinary sectors in Mongolia. There is still a lack of understanding of the most important livestock-human brucellosis transmission, and no molecular epidemiological data is available for analysis of the current situation.

The principal objective of this PhD thesis was to provide the national brucellosis control program with evidence-based decision making to enhance its effectiveness. The evidences were provided through employing different research methods implemented in the selected areas of the study.

A simultaneous assessment of humans and livestock was conducted to help better understand the disease situation and understand access of the rural people to brucellosis diagnosis and treatment. Another study was carried out sampling of infected livestock and brucellosis patients from a hospital to identify the main *Brucella* spp. using bacteriological and molecular methods. *Brucella melitensis* turns out to be the main strain dominantly circulating in the country. The main reservoir host for *B. melitensis* is the sheep from which the strains spill over to goats and humans. A vaccine cool chain assessment of the national livestock vaccination checked the quality of brucellosis vaccines. Overall these assessments strive to improve the quality of the national brucellosis control program.

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Zolzaya Baljinnyam Molecular epidemiology of animal and human brucellosis in Mongolia

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List of Abbreviations

AVD	Aimag (Provincial) Veterinary Department
CFU	Colony Forming Unit
ELISA	Enzyme-linked immunosorbent assay
LPM	Livestock Project Mongolia
LPS	Lipopolysaccharide
MoFALI	Ministry of Food, Agriculture and Light Industry
MoH	Ministry of Health
NCCD	National Centre for Communicable Diseases
IVM	Institute of Veterinary Medicine
PIU	Project Implementation Unit of the LPM
RF	Russian Federation
SCVL	State Central Veterinary Laboratory
SH	Soum hospital
SVDBTCL	State Veterinary Drug, Biological Testing and Confirmation Laboratory
DVS	Department of Veterinary Services
RBT	Rose Bengal Test
OIE	World Animal Health Organization
FAO	Food Agriculture Organization
WHO	World Health Organization

Summary

Brucellosis is one of the most common zoonotic diseases worldwide with around half million human cases reported annually but up to 10 times as many cases are not reported. Humans contract brucellosis by direct contact with infected livestock and through consumption of raw dairy products. The disease not only debilitating in humans, but also has major economic consequences in loss of productivity due to illness and loss of animal production and reduced survival of newborns. In Mongolia, human brucellosis became an issue in the 1960s. During the 1980s, thanks to the mass vaccination of livestock, human incidence was reduced to less than 1case per 100,000 populations. In the 1990s, human brucellosis re-emerged due to the break down of the government-run disease surveillance and control program and the lack of resources in the livestock and medical sectors.

Brucellosis causes complex issues in public health, and economic, social and environmental problems in Mongolia. The country exports meat mainly to the Russian Federation (RF), but government of Russia restricts import of meat from brucellosis vaccinated animals so the current brucellosis control program caused obstacles for Mongolia exporting meat.

Animal numbers had greatly increased in Mongolia by the mid 1990s because the government had no longer controlled the numbers. The sheep, goat, cattle and horse demographic model predicts that livestock numbers will continue to grow exponentially in the future. The increase of livestock numbers and the usage of pasture lands should be considered when planning disease controls and meat export to other countries.

Mongolia has implemented several brucellosis mass vaccinations of livestock since 1975. The first ten year vaccination campaign was a successful vaccination program during the 1980's because of the strict top-down socialist regime approach of government, but the second 10 year vaccination was not implemented successfully due to dramatic increase of livestock numbers and lack of monitoring and quality control. The government is continuing to implement this mass vaccination until 2020.

The aim of the thesis is to contribute to Brucellosis control in Mongolia by
1) providing data on the current disease status in human and livestock to assist in the selection of an appriopriate control strategy;
2) results of molecular epidemiological study to give a better understanding of the circulating strains of *Brucella* spp. between different livestock species and livestock-to- humans;

3) vaccine cold chain evaluation and quality control of vaccines will provide overall assessment of the quality of brucellosis vaccines used for mass livestock vaccination

4) assessment of human brucellosis the diagnosis and treatment situation will provide a scientific evidence improving human brucellosis diagnosis and treatment standard in country. The thesis results will also give a more complete picture for brucellosis epidemiology and will deepen our understanding of disease risks as well as provide an important knowledge base for policy development, control activities and will improve the brucellosis control in the country.

1 Introduction

This dissertation provides a review of the brucellosis situation in Mongolia regarding the current control policy implementing in the country. A “One health” approach to brucellosis prevention and control not only fosters collaboration between the medical and veterinary sectors but also brings additional benefits through the sharing of limited resources, the exchange of disease information and the enhancement of the national control program. The simultaneous assessment of exposure among herders, rural people and their livestock from randomized cross-sectional multistage cluster proportional to size provides the current brucellosis status in humans and livestock. This gives us a more complete picture of the epidemiology, which deepens our understanding of the disease in Mongolia.

Even though livestock and human brucellosis is endemic in the country, almost no strains have been typed using molecular methods and no molecular epidemiological analysis has been conducted previously.
Human and livestock isolates were collected from the selected eastern region for the molecular epidemiological investigation and this enabled us to identify the main circulating *Brucella* spp, determine the main host species and path of transmission from livestock to human in Mongolia. This molecular epidemiological result helped to increase our understanding of the brucellosis epidemiology, specifically *B. melitensis*. This is the main strain dominantly circulating in the country, with sheep being the main livestock host transmitting *B. melitensis* to humans. The isolated strains provided molecular epidemiological evidence for the national control program, which has enhanced the current disease surveillance of the medical and veterinary sector.

The cold chain assessment showed that the almost no legal and practical documents existed in the country. The cold chain concept was not a part of the government vaccination program. There is no clearly defined role that shows who is responsible for this critical issue in the governmental veterinary administrative body.

1.1 Brucellosis current status

Brucellosis is one of the major zoonotic diseases in the world. Transmission to humans occurs through contact with secretions and excretions of infected animals. Routes of entry include skin abrasions, inhalation and ingestion. Raw milk, unpasteurized dairy products, uncooked or partially cooked liver and blood of infected livestock are main sources of infection for humans. Human-to-human transmission of the infection does not occur [1].
It was estimated that half a million human cases are reported annually worldwide, but underreporting suggests a figure 10 times higher owing to unspecific symptoms in humans [2]. Brucello-

sis has been successfully controlled by effective and well managed vaccination and test-slaughter strategies in Australia, the USA, South American and European countries. Brucellosis is endemic in livestock and causes human diseases in Africa, Central America, Central Asia, the Mediterranean region and the Near East. Human brucellosis is re-emerging as a major epidemic in countries of the former Soviet Union and Mongolia [1].

In most countries the importance of brucellosis in terms of burden of disease and societal cost is not known. Brucellosis can however have a considerable impact on both human and animal health, as well as wide socioeconomic impact in countries that rely on rural income from livestock breeding, fibers and dairy product. Human brucellosis can, only be eliminated by its control in livestock [1].

1.2 *Brucella* spp bacteriology

Brucellosis is a bacterial zoonotic disease caused by *Brucella* species. In 1887, Bruce reported the isolation of *Micrococcus melitensis* from a human case in Malta. Bang described *Bacterium abortus* isolated from a cow in 1897. Evans presented the close relationship between these two bacteria in 1918 [1, 3]. Based on Evans evidence, Meyer and Shaw were proposed the class: Alphaproteobacteria, order: Rhizobiales, family: Brucellaceae, and the genus *Brucella* in 1920. *Brucella* species are small, non-motile, coccobacillary or short rods of 0.5-0.7 μm by 0.6-1.5 μm most often arranged individually, without capsule and Gramm-negative bacteria.

Brucella species are aerobic, capnophillic, and catalase-positive [1-4]. *B. suis* (1929) was isolated from pig, *B. ovis* (1956) from sheep, *B. neatomea* (1957) from woodrat, *B. canis* (1968) from dogs and these are added to the genus as novel species.

B. ceti (1994) was isolated from bottlenose dolphins and *B. pinnepedialis* (1997) from seals. *B.microti* (2008) isolated from the common vole and *B. inopinata* (2010) from human [3-4]. Recently, a potential novel *Brucella* species was isolated from African bullfrogs. This isolates is motile and equipped with single laterally attached flagellum, as described for Ochrobacterium anthropi [5]. At the present time the pathogenic potential of these new novel species for humans is still unknown and the natural cycle of transmission and maintenance are not well understood [4].

1.2.1 *Brucella* spp structural and antigenic characteristics

Brucella follows a Gram-negative architecture: a cytoplasm encased in a cell envelope made of an inner membrane, a periplasm and an outher membrane (OM). The OM contains free lipids, proteins (Omp) and a lipopolysaccharide (LPS). The LPS is the dominant OM molecule and is critical in *Brucella*'s virulence and as an antigen.

While, *B.ovis* and *B.canis* have a rough type LPS (R-LPS) made of a lipid A (containing two types of aminoglycose) linked to an oligosaccharide, other *Brucella* spp. have a smooth (S) type LPS with an o-polysaccharide linked to the oligosaccharide [1,6].This is manifested in the surface of the colonies: R in *B. ovis* and *B. canis* and S in other *Brucella*. The S *brucella* can dissociate to yield mixtures of S and R colonies and cells as a result of mutation affecting the O-polysaccharide. Dissociation hampers species identification and its control is essential in vaccine and antigen production [1, 7].

Brucella O-polysaccharides create three basic epitopes: A (A=Abortus »5 contiguous sugars in α 1-2 linkage); C (or A=M; common to all S-brucella); and M=Melitensis. They are distributed in various proportions among S species and biovars so that neither A nor M is characteristic of *B. abortus* and *B. melitensis*, respectively.

In addition to the S-LPS, S brucella produces a free polysaccharide called native hapten [NH] [1, 8]. Bacteria cross-reacting with S brucella include *Stenotrophomonas maltophilia*, group N (0:30), *Salmonella* spp, *Vibrio cholerae*, *E.coli* 0:157, some *Escherichia hermanii* strains and *Yersinia enterocolitica* 0:9. The soluble fraction proteins common to all except the S-LPS cross-reacting bacteria, which make them useful for discriminating *Brucella* spp. infections from false positive serological reactions caused by latter [1].

1.2.2 Culture and selective media

Farrell's selective medium that was originally developed to test milk for *B.abortus,* is widely used, but inhibits *B.melitensis* strains. The modified Thayer–Martin's medium is less selective and gives better results with other species. It is recommended that both media are used in order to optimize culturing unknown field isolates [9]. Plates are incubated at 37°C in 5% to 10% CO_2 for up to 10 days. Although, CO_2 is a specific requirement for individual species, the majority of the *brucella* are capnophilic[6, 10]. On the selective media, *Brucella* colonies are visible after a two days incubation period. *Brucella* colonies are around 1-2 mm in diameter with smooth margins after 4 days incubation. The colonies are translucent and a pale honey colour when plates are viewed in daylight through a transparent medium. When viewed from above, colonies appear convex and pearly white. Colonies became larger and slightly darker several days later. Smooth (S) *brucella* cultures have a tendency to undergo variation during growth, especially with subcultures, and to dissociate to rough (R) forms. Checking for dissociation is easily tested by crystal violet staining: rough colonies stain red and smooth colonies stain pale yellow[7].

1.2.3 Identification and typing

Smears of specimens, particularly cotyledons, foetal abomasal contents and uterine discharges stain positive in Stamps modification of the Ziehl-Neelsen method (MZH). The smear shows MZH positive coccobacilli or short rods of 0.5-0.7 μm by 0.6-1.5 μm.

Brucella species are differentiated by colonial appearance, biochemical tests, and specific cultural requirements [10]. All *Brucella* species are catalase positive, all but *B.ovis* and *B.neotomae* are oxidase positive. Since *Brucella* cause false positive serological reactions, they are easily differentiated by bacteriological tests. Slide agglutination with anti-S sera helps to distinguish from S brucella to R forms. Species identification requires experience, specific anti-A and anti-M sera, phage and inhibition by dyes [1]. Biovar level typing is laborious and difficult to perform and reproduce. Molecular methods developed substitute the classical methods for species, biovar and vaccine typing.

Most molecular methods applied on colonies on isolation plates or on DNA help to avoid dangerous manipulation. One of the first PCR assays to differentiate among *Brucella* species was called Abortus-Melitensis-Ovis-Suis (AMOS). This PCR uses a single reverse primer, targeting the *Brucella* specific insertion element IS711 and four different forward primers, each specific for a given species as estimated by testing representative isolates. Table 1 shows different species identification. Species are differentiated on the basis of different PCR fragment sizes. The disadvantage was *B.canis* and *B.neotomae* were not identified and that some biovars within a given species gave negative results [1, 4].

A new conventional multiplex PCR (Bruce-ladder), using eight primer pairs in a single reaction covers all species and biovars. It rapidly replaced the AMOS-PCR as a diagnostic tool and is still in use. Bruce-ladder allows accurate species delineation of all existing species with differentiation at the biovar level [4]. In recent years the availability of microbial genome sequences data has revolutionized the development of in-depth molecular analyses and the subsequent of novel typing tools [4]. The DNA fingerprinting facilitated the development of multilocus sequence-based typing approaches such as multilocus sequence typing (MLST) and multilocus-variable number of tandem repeats (MLVA) [11]. The MLVA assays take advantage of array-length variations in tandem repeats. The first MLVA assay, named hypervariable octametric oligonucleotide fingerprints (HOOF-Prints), that was developed using *Brucella* genome, contains tandem repeats sharing the repeat unit “AGGGGAGT”. Eight highly variable such loci, present in most *Brucella* species, were used in the HOOF-Print assay. However, this assay could not use for identification purposes at the species level; additional selections of tandem repeats were subsequently required [4, 12]. Draft whole genome sequencing is being increasingly used to replace MLST and larger-scale SNP typing, because it is an unbiased approach and provides incomparable wealth of data. The cost of the

sequencing is now coming closer to the previously described assays. This will provide much higher resolution than MLSA [4].

Table 1 The current molecular methods used for *Brucella* spp identification and typing *

Level of identification	Test	Description
Species	AMOS-PCR	A multiplex PCR assay based on IS711 related polymorphism that differentiates *B.abortus* (biovars 1, 2 and 4), B.melitensis (biovars 1, 2, and 3) B.ovis, B.suis(biovar 1), plus vaccines B.abortus S19 and RBT 51
	Bruce-ladder-PCR	A single -step multiplex PCR assay that identifies B.abortus (biovars 3,5,6,7,9), B.melitensis, B.ovis, B.suis biovar2, 3, 4 B.canis, B.neotemae, B.pinnipedialis and B.ceti as well as the vaccine strains B.abortus S19 and RBT 51.
	MLSA-SNP	MLSA-SNP identifies the six classical Brucella species plus the marine strains as a group
Species and strain	MLVA-16	Two panels of primers were used, one comprising eight minisatellite markers (panel 1) which are used for species identification and a second group of eight microsatellite markers showing in a higher discriminatory power which are split into two groups; panel 2A and 2B. Panel 2B contained the most highly variable markers.
Strain	HOOF	Method based on multilocus hypervariable octameric oligonucleotide fingerprints (HOOF) analysis

*This table adopted from Brucellosis chapter Palmers book and modified.

1.3 Molecular epidemiology of Brucellosis

Molecular epidemiology is a science that seeks to answer questions about the aetiology, distribution and prevention of disease occurrence using molecular biological techniques. The identification of factors determines temporal and spatial disease distribution, transmission and makes progress toward possibility of intervention and prevention is a subject to epidemiological studies. A molecular technique helps providing more sensitive and specific measurements facilitates epidemiologic activities. This includes characterizing host-pathogen interactions, identifying transmission patterns and providing better understanding of disease pathogenesis at the molecular level.

Accurate diagnosis and typing procedures are essential for epidemiological studies. Study results aim to control and eradicate brucellosis. The standard bacteriological methods are not straightforward for identification of biovars. Extensive research in recent years has put forward genome se-

quencing which opens new molecular resources for typing different brucella biovars. Main objective is to improve the typing of biovars lead to propose using different PCR-based assays. One of the most widely used method to distinguish brucella biovars are the restriction fragment length polymorphism (RFLP) analysis of genes omp2a and omp2b, which differentiates between reference biovars 1, 2 and 3, 10 of the gene omp25c and of gene omp31. RFLP is able to differentiate biovars 1 and 3 from biovar 2[29]. More recently, another PCR based molecular typing approach for multiple-locus variable number tandem-repeat analysis (MLVA) has been developed [11, 12]. The homologous brucella genome contains a high percentage of DNA repeats that are either clustered in a specific genomic area or dispersed throughout the genome. MLVA is based on typing and indexing variations of these tandemly repeated DNA sequences. In a MLVA assay, a number of well-selected and characterised loci are amplified by PCR and separated by electrophoresis so that the size of each locus can be measured. From this size, the number of repeat units at each locus can be deduced. The resulting information is a code which can be easily compared to reference databases. Most of the early work on tandem repeats for identifying bacterial strains was conducted in the background of bioterror-related microorganisms for bio warfare. Single nucleotide polymorphism (SNPs) is another source of genetic variation. It represents polymorphisms at single base positions in the genome and which are distinguished from rare variations by a requirement for the least abundant allele to have a frequency of 1% or more. Recently, adapted mass spectrometry has been used increasingly for the rapid identification of bacteria and other pathogens.

1.4 Phylogeny

Molecular phylogeny is based on tandem repeat loci of brucella demonstrates distinct genetic differences between the different *Brucella* spp, in particular between *B. melitensis* and *B. abortus*. Such distinctions allow tracing back chains of transmission within different livestock species between livestock species and livestock to humans.

1.5 Animal brucellosis

The most Brucella has atropism for both female and male reproductive organs in sexually mature animals. Each Brucella species tends to infect a particular animal species but cross species transmission can occur [7]. Animal brucellosis is characterized by epidemic late stage abortions. Infected animals serve as reservoirs of infection which is often persist and indefinitely. *Brucella* spp are shed in large numbers in urine, milk, placental and other fluids of infected animals prolonged period representing a major risk for public health. The main clinical feature of brucellosis is late abortion in sheep, goat and cattle. One study showed that among seropositive animals, it was estimated that 10-50% have aborted and 20% of them remain sterile. Aborted female animals are often not milked and all milk yields are lost during the lactation period [1].

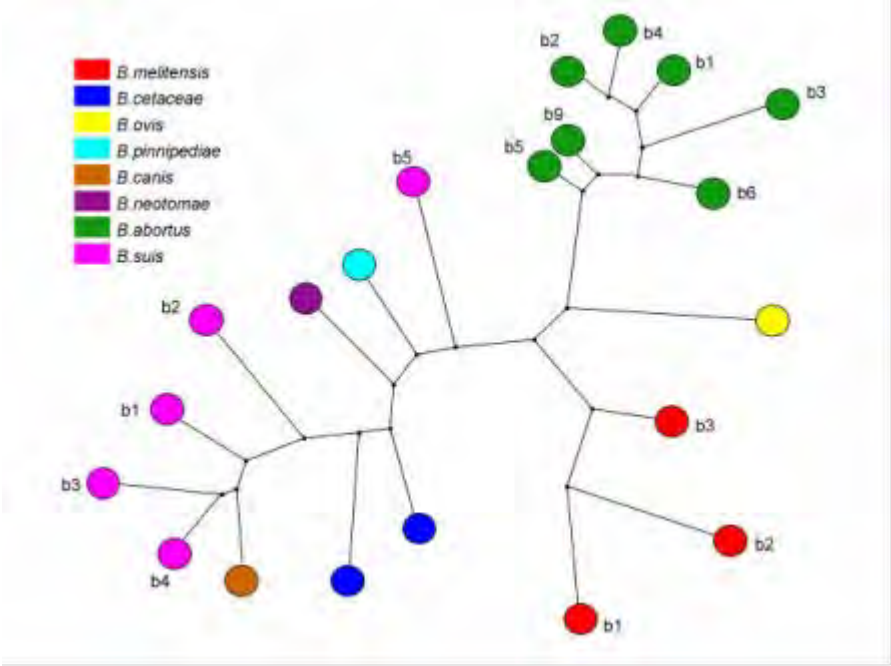

Figure 1 Phylogenetic tree of the genus Brucella spp from Le Fleche et al. 2006

1.5.1 Animal brucellosis bacteriology diagnosis

Brucellosis lacks pathognomonic clinical symptoms. Abortion, infertility and other manifestation are not specific which requires laboratory testing. Although Stamp's staining for smears of vaginal swabs, placentas and aborted foetus are useful to affirm the presence of the bacteria similar to *Brucella* spp. Therefore, *Brucella spp* isolation is the only diagnostic method. Culture should be always attempt to confirm the disease and to determine which *Brucella* species involved [1, 7]. However, culture is slow, expensive, cumbersome, and sensitivity depends on the type and number of samples. Also, culture depends on amount of bacteria shed in the collected samples, sample conservation and transported to the laboratory. An aborted foetus is taken from the field usually heavily contaminated and gives poor diagnostic results. Milk and vaginal swabs taken after the abortion are appropriate samples that frequently isolate *B. abortus* and B. *melitensis.* The spleen, iliac, mammary, cranial and prefemoral lymph nodes collected during the necropsy used for culturing [1, 13]. Culture samples on the selective media are described in the 1.4 section.

1.5.2 Animal brucellosis serological tests

Brucella spp triggers both humoral and cell-mediated responses. However, these responses may not be detected at early stages of infection an old animal, and a young heifer born from infected cow will not develop antibodies until first pregnancy. Moreover, an immune-response proves exposure with *Brucella* spp. (or cross reacting bacteria) but not necessarily infection. Setting up adequate standardization protocols to avoid conflicting situations are important because of the implications for the livestock trade [1,7].

1.5.2.1 Classical serological tests

The Rose Bengal Test (RBT) and the Complement Fixation Test (CFT) have been standardized for the diagnosis of cattle brucellosis [7]. RBT is a rapid and low cost plate agglutination test which is stained with 1 ml of 1% (w/v) Rose bengal dye (CI No. 45 440) and killed *B.abortus* S99 or S1119-3 suspension at pH 3.6-3.7[7]. It is dependent on the route of administration and age at vaccination which causes RBT to give a positive result in vaccinated animals [1].

CFT is a widely used and accepted confirmatory test although it is complex to perform, requiring good laboratory facilities and trained staff. Several methods have been proposed for the CFT using different concentrations of fresh or preserved sheep red blood cells (SRBCs) (2.5% or 3% suspension) is recommended but the test is most optimally carried out in a microtitre format. It is possible to use warm or cold fixation for the incubation of serum, antigen and complement: either 37°C for 30 minutes or 4°C for 14-18 hours. A sera giving a titre equivalent to 20 international CFT unit/ ml or more is considered to be positive [7]. But, CFT has several drawbacks. The classical tests are not optimal for the sero diagnosis in small ruminants. RBT shows lower sensitivity than in cattle, even though the problem is greatly reduced by increasing the proportion of serum: antigen to 3:1. It requires standardization and reassessment in the Mongolia [1, 7]. The Milk ring test (MRT) is used for screening pooled milk at the dairy farms. MRT is highly sensitive and easy to perform but specific for cattle [7].

1.5.2.2 The enzyme-linked immunosorbent assay (ELISA)

Many variations of the indirect ELISA have been described employing different antigen preparations, antiglobulin-enzyme conjugates and substrate /chromogens. Several commercial indirect ELISA are available that have been validated in the extensive field trials and are in wide use. In the interest of international harmonisation, the three OIE ELISA standard sera should be used by national reference laboratories to check or calibrate the particular test method in question [7]. The indirect ELISA shows sensitivity equal to or higher than the RBT, and higher than the CFT which is suitable for cattle, sheep and goats.

However, the specificity is influenced by antibody resulting from S19 or Rev.1 vaccination or other cross-reacting bacteria. To improve the specificity of S-LPS tests, competitive ELISA was developed in the context of vaccination with S19 and Rev.1. Moreover, specificity in the vaccination context is improved regarding to indirect ELISA but competitive ELISA does not eliminate the problem created by cross-reacting bacteria. The competitive ELISA has conflicting results on the sensitivity in cattle and sheep. In sheep, it does not outperform CFT and has lower sensitivity than indirect ELISA or the RBT [1].

1.5.2.3 The fluorescence polarization assay (FPA)

The FPA is a simple technique for measuring antigen and antibody interaction and may be performed in a laboratory setting or in the field. It is a homogeneous assay in which analyse are not separated and it is rapid test [1, 7]. The FPA uses *B.abortus* polysaccharide obtained from S-LPS labelled with fluorescein and measurements can be performed in a few minutes. The mechanism assay is based on random rotation of molecules in solution. Molecular size is the main factor influencing the rate of rotation, which is inversely related. Thus a small molecule rotates faster than a large molecule [7]. Its performance is similar to indirect ELISA, and RBT or tests in the absence of vaccination. In sheep and goats, FPA does not outperform CFT which requires further study [1].

1.5.2.4 Brucellin skin test

An alternative immunological test is the brucellin skin test, which can be used for screening unvaccinated herds, provided that a purified (free sLPS) and standardised antigen preparation (brucellin INRA) is used. The brucellin skin test has a very high specificity, such that serologically negative unvaccinated animals that are positive reactors to the brucellin test should be regarded as infected animals [7]. Also, the results of this test may aid the interpretation of serological reactions thought to be FPSR due to infection with cross-reacting bacteria, especially in brucellosis-free areas. Moreover, not all infected animals react. For this reason test alone are not recommended for diagnostic test purposes for international trade [7].

1.6 Human brucellosis and clinical manifestation

Human brucellosis clinical manifestation is dependent on which *Brucella* species are affecting humans. *B. melitensis* causes the more severe disease, followed by *B. suis and* then *B. abortus*. *B.melitensis* is the most pathogenic species producing the most intense symptoms, the greatest tissue damage, and the most frequent incidence of localisation in body organs, systems and tissue [14]. Human brucellosis is an acute or sub-acute febrile illness usually marked by an intermittent or remittent fever accompanied by sweat, malaise, headache, and anorexia, arthralgias, myalgias, back-ache and weight loss [1, 2, 14]. It can produce serious complication affecting cardiovascular, central nervous systems and other parts of the body. Endocarditis is the main causes of mortality. Some human cases showed the presense of lyphadenophaty, splenomegaly, and hepatomegaly, accompanied with spondylitis, sacroilitis, osteomyelitis, meningitis and orchitis [1, 14].

1.6.1 Human brucellosis diagnosis

A human brucellosis clinical manifestation is unspecific and differs between individials. It is critical to obtain epidemiological information on the occupation of the patient, whether there has been contact with an infected animal, or consumption of dairy products, or any recent travels before reaching a suspect for brucellosis [2]. Clinically suspect cases have to be confirmed using laboratory diagnostic tests. It is recommended to isolate cultures whenever possible in the pyretic phase, and is critical to ask if the patient has taken any antibiotic treatment previously. It is recommended blood samples, cerebrospinal and other fluid, or some tissues in focal forms be cultured in broth blood or Ruiz-Castaneda's biphasic system. Non-agglutinating and blocking antibodies are common in brucellosis, become agglutinating at pH « 5, and can be detected by Brucellacapt and Rose bengal test (RBT)[1, 14].

RBT (pH= 3.6-3.7) detects IgM, IgG and IgA. RBT is considered a qualitative test which is not effective in discriminating exposure from active infection in endemic areas. This problem can be overcome with test serum dilutions that allows for a diagnostic titre to be established. RBT titres increase with the time of evolution. RBT is most suitable in rural settings and small hospitals due to its simplicity and easy to perform. However, antibodies to this antigen persist in recovered patients for a long time [1, 15].

1.6.2 Human brucellosis treatment and relapses

Adult patients with acute brucellosis should be treated as outpatients with doxycycline-streptomycin or doxycycline-gentamicin combination. In focal forms, the same treatment but duration of the treatment must be decided in each individual case. Surgery should be considered for patients with endocarditis, cerebral, epidural, spleen, hepatic and other abscesses not resolving with antibiotic treatment [1, 2]. Pregnancy poses a special problem as tetracycline and streptomycin must be avoided and a rifampin monotheraphy is considered for the regime of choice. Trimethoprim-sulfamethoxazole (cotrimoxazole) plus rifampin is an alternative regimen but it can be teratogenicity if used in pregnancy week 13 and may induce kernicterus after week 36.

Children have fewer and milder symptoms. Since tetracycline is generally contraindicated for children less than 8 years old, rifampin-cotrimoxazole is recommended. Some studies showed that having treatment for longer than 6 months of cotrimoxazole provides good results. Depending on treatment regime followed, relapses occur in 5-30% of patients, usually 1 to 6 months after treatment but which tends to be milder than the original attack. The bacteria isolate from a relapsed patient maintains the same antibiotic-susceptibility. Hence, nearly all relapses respond to a repeated course of antimicrobial therapy [1, 2].

1.7 Burden of disease estimation

Brucellosis is one of zoonotic diseases cause major public health impact on human health, animal productivity and economic significance in worldwide. Human brucellosis incidence varies significantly from country to country even within countries [16].

Brucellosis endemic countries do not have strong health information system and official data accumulated the by passive surveillance which is more likely to underestimate the true burden of diseases [1]. Roth et al considered that brucellosis associates with class II (0.2) disability weight, as the disease is perceived as very painful and affecting occupational ability even during remission [1,17].

If the patient does not receive an appropriate treatment it may limits movement of the knee and elbow joints of Mongolian patients who suffered over 20- 30 years. (Personal communication with Dr. Enkhtuya, Sukhbaatar province).There is still estimate of the burden of Brucellosis not available up to present.

1.8 Brucellosis vaccines

1.8.1 Small ruminant vaccines

Rev.1 vaccine is a live, attenuated *B. melitensis* strain derived from a virulent strain. *B. melitensis* isolate became dependent on streptomycin for its growth. Rev.1 vaccine is considered currently as the best vaccine available for immunisation of small ruminants [1, 18, 19]. It stimulates protection against infection with *B.melitensis* in sheep and goats and also protects ram against infection with *B.ovis* [18, 19]. The standard dose (1-2 x 10^9viable bacteria-colony forming units (CFU) /animal) can be as 80-100% against challenges (1.5 x 10^9 virulent bacteria) while infecting 100% unvaccinated control animals. Rev.1 has been successfully used several countries in the world [1]. However, Rev. 1 has negative effects. Rev.1 vaccine strain can be pathogenic to humans' and precaution should be taken when handling and vaccinating livestock. If Rev.1 infection suspected and treatment should not include streptomycin due to it became dependent for its growth [18]. Subcutaneous vaccination with live Rev.1 vaccine strain causes interference with brucellosis serological diagnosis. Standard dose administration through subcutaneously which induces a protracted serological response that interferes in serological tests. The serological interference is often observed vaccinated animals older than 4 months or adult. However, same dose administered by conjunctival route stimulates similar immunity but markedly reduces the serological response, particularly in young animals. The conjunctival route of vaccine administration is recommended for brucellosis elimination program [1, 19]. Rev.1 has no negative effect in young ram, billy goats and lactating female animals. However, it causes high numbers of abortion administrating to pregnant female animals and vaccine strain shed in milk [1].

1.8.2 Cattle vaccines

Brucella abortus S19 is used to vaccinate cattle in high prevalence countries. S19 was able to induce protective immunity in cattle. In heifers vaccinated subcutaneously with the standard dose ($10x10^9$ CFU/ animal) the vaccine induces an infection that clears within a few months but does not last longer than a year [1, 7]. S19 is not dangerous as Rev.1 is, however, it can infect humans and for this reason biosafety precautions are required during vaccination and handling.

The rate of abortion in pregnant adult cows was decreased when a reduced dose was applied by a subcutaneous route in a study involving 10,000 cattle during the 7-8^{th} month of pregnancy. This route also induced udder infection and vaccine shedding in milk but in a very low proportion of animals. Moreover, both problems significantly decreased when reduced doses were applied by the conjunctival route [1].

Cattle may become infected with *B. melitensis* upon contact with infected sheep and goats. This situation leads into research but also taking account of the fact that *B. melitensis* infection in cattle is controlled with S19 vaccine [7]. *B.abortus* strain 45/20, a rough organism with little or no ability to induce O-chain antibodies could induce significant protection against infection with *B. abortus* and indicates that a rough organism can be used to induce protective immune responses while avoiding the diagnostic problems. The recommended dose ($1\text{-}3.4 \times 10^{10}$) is administered subcutaneously [18].

B.abortus strain 45/20 is called RB51. It is an R mutant obtained by repeated passage on media with rifampin and penicillin to develop a vaccine that would not interfere in the S-LPS serological tests [1].

1.9 Control and elimination

There are several points needed to be considered before implementing a brucellosis control and elimination program. The main points adopted from the Brucellosis chapter in Palmers infectious disease book [1].

1. Identification of all flocks and herds, and the proficiency of the veterinary services to vaccinate the whole livestock population in a short period of time
2. Sufficient public resources to cover intervention costs and compensation for culled animals at market value if the goal is elimination
3. Active involvement and cooperation of the farming community through an awareness building campaign
4. Well known disease status from randomized cross-sectional cluster surveys proportional and stratified by geographical regions
5. To have a basic understanding of circulating different *Brucella* spp. strains among livestock, and humans.

If herd or flock prevalence is higher than 10%. It is recommended to implement mass vaccination. The goal is to control the disease and to reduce prevalence of disease in the country or region, to prevent humans getting infected and also to decrease economic losses. In a herd or flock with prevalence between 5-10% a combined strategy of vaccination of young replacements, testing and slaughter of adults can eliminate the disease in the medium to long term. Herd or flock prevalence of less than 1-2% is a test-slaughter program with no brucellosis vaccination to eliminate the disease short term.

1.10 Mongolia

Mongolia has an area of 1.566.500 km ^{2} and stretches 1.250 km north to south and 2.400 km west to east. It is on average 1,500 m above sea level, with mountains over 4,000 m in the western and central part and in the lowest eastern part is about 800 m.

The 2,9 million population is mainly Khalkh, Kazakh, Buriat, Oirad, and Torguud ethnic groups but there are also Turkish, Russian and Chinese minorities. More than 1.5 million inhabitants live in the three major cities, of which about 1 million live in the capital city. Administratively the country is divided into 22 provinces (21 provinces plus Ulaanbaatar the capital city), 335 soums (districts) and 1,800 bags (the smallest administrative unit which equals a village). Fifteen percentage of the GDP is produced by agriculture sector of which 87% comes from livestock. Thirty five % of the population works in the agricultural sector, mainly as livestock keepers and herders. The total livestock-population is 45.1 million head which consists of 52.5% of small ruminants and the rest of cattle, yaks, horse and camels. The average size of a herd in one herder family is 244 animals consisting of sheep 109, goats 105, cattle/ yak 14, horses 14 and camels 2. The livestock population has been increasing since 1990, but approximately 75% of the total population in 2013 was due to the lost of marketing opportunities, rather than to an increase in productivity [20]. Traditional seminomadic livestock plays an important role in the national economy, main sources of food and rural employment. Mongolia has a pastoralist population herding mixed herds by following the natural breeding cycle and searching for water supply and adequate grazing areas for their livestock. Over 70 % of the land is degraded and 31% of the land is severely degraded plus the environment challenges caused by the increased sedimentation of the seminomadic pastoralist last decade [21]. However, the urbanization of the population meant fast-growing demands for meat, milk and dairy products in urban centres leading to an intensification of livestock production systems. Intensive farms have increased from 410 farms in 2003 to1706 farms by 2012 [20, 22].

1.10.1 Mongolian brucellosis situation

Before XIX century, the country was underdeveloped and no trained medical doctors and veterinarians were available to treat the people and sick animals. However the traditional practitioners used to treat human and livestock diseases with herbal medicine or acupuncture. The population was illiterate and no medical care was provided from the autonomic government. Mongolia became the second communist country in the world in 1921. This political change was positively influenced the establishment and development of a modern medical, veterinary medicine and education system in the country. The Department of Veterinary Services was established in 1923, and developed by Russian professionals from Union of Soviet Socialist Republic (USSR). The first veterinary diagnostics producing unit was established at Songino in 1924 [23].

The National University of Mongolia (NUM) was established with medical, veterinary, pedagogical and foreign language faculties in 1942. The NUM provided the first national professionals to work in the medical and veterinary sectors in the country.

Dr. Lebedyansky, a Russian veterinary specialist reported no mass abortion was noticed when he was working in Mongolia from 1925 to 1926. This might be the first report availabe that there was no brucellosis in Mongolia [24].
By 1930s, small private herds were brought together from different regions and established the government owned collective farms which provided water, infrastructure, and transport that ensured the mobility, free veterinary care, emergency podder, with the livestock numbers and movement of animals controlled by the state [21]. The government was solely responsible for providing free health services to the public, hospitalization of patients, and the implementation of preventative measures in the nation.

The first livestock brucellosis case was reported in cattle from Selenge province in 1932.The several small ruminant farms reported mass abortions and following laboratory investigations confirmed that brucellosis outbreak in 1940. Furthermore, the first human brucellosis case was officially registered in 1949. By 1950s, the prevalence of brucellosis in livestock was 17% in cattle, 3.5% in sheep, and 2% in goats in the country [24].
During the 1950s, the government of Mongolia had taken immediate action to control the human brucellosis, providing treatment for patients, and vaccinating the high risk groups using dried live *B. abortus* vaccine called “19-BA” which was produced in the former Soviet Union.

The Ministry of Agriculture made an effort to control livestock brucellosis through capacity building of veterinary laboratories, training the laboratory staff, and screening dairy herds by testing milk, and dairy products. A brucellosis serological test has been required for importing live animals to Mongolia since 1957. Moreover, the national control strategy included implementing appropriate herd management practices and improving hygienic approaches. The government of Mongolia launched national brucellosis health education campaign in the late 1950s [24].

Mongolia became a member state of the United Nations in 1962; the following year the country joined the World Health Organization (WHO) which opened opportunities for receiving international assistance. WHO started “Mongolia-001”a long term project focused on strengthening the health service and implementing an epidemiological survey which included the following;

(i) Estimated prevalence of main diseases followed by studying aetiology and distribution,

(ii) Development of effective preventive measurements and defined control strategies

WHO experts conducted large-scale epidemiological survey among livestock;

a) assessed brucellosis prevalence and distribution among different livestock species,
b) identified the main factors influencing the transmission of brucellosis from livestock to humans
c) Rev.1 vaccine trial among small ruminants plus cattle was conducted.

The WHO brucellosis survey was implemented in randomly selected 680 herds consisting of 413 small ruminant flock, 92 cattle herds, 89 camel herds, and 86 horse herds from Tuv, Bulgan, Dornod, Umnugobi, and Zavkhan provinces. The herd prevalence was 43% in cattle, 16.2% in sheep, 13.4% in goats, 4.9% in camels and 30.9% in horses [24, 25].

However, the survey result also provided evidence that urgent action was needed to control the brucellosis in the country. The government of Mongolia did not wait for the final conclusion and recommendations from the international experts of WHO. The government of Mongolia sought independent financial assistance from communist countries. The government implemented the first large scale test-slaughter strategy supported and financed by the Council for Mutual Economic Assistance (COMECON) former financial assistance organization of Soviet Union. The COMECON's supported team of veterinarians proposed the following action

i) examined adult animals for brucellosis, tuberculosis and glanders
ii) separated the reactors from herd based on serology test results
iii) developed brucellosis control plan for each province.

The COMECON veterinary team purpose was to eliminate brucellosis, tuberculosis and glanders mass testing and slaughter campaign. The veterinary team have had well-equipped mobile laboratories used for conducting serological tests from 1966 to1968. The mass testing and slaughter campaign was successfully implemented in the country as the government owned all livestock.
In total, there were 37.5 million livestock plus dogs tested with the allergic skin test, the complement fixation test (CFT) and the serum aglutunation test (SAT) during the mass testing and slaughter campaign. The identified positive reactors by serological and the allergic skin tests were immediately separated from the herd and slaughtered within a short period of time [26, 27].

The COMECON team and local veterinarians worked hard to reduce sero-positive reactors by 54.4% in sheep and 31.2% in goats. There is no serological test is appropriate in all epidemiologi-

cal situation. Consideration should be given to all factors that impact on the relevance of the test method and test results to a specific diagnostic interpretation or application [26].
The BST, SAT, and CFT serological tests had limited reliability as they were not able to detect the infected animals in the latent phase. Moreover, Mongolian pastoralists were situated over vast territory with large flocks, with continuous movement of livestock population, and high prevalence among different species of livestock. It was not possible to find replacement of healthy livestock in the country. However, the mass testing and slaughter campaign was not practical and less effective in controlling brucellosis in the Mongolian pastoralist conditions.

The government of Mongolia realized that the prevention of human brucellosis was directly linked to eradication of livestock brucellosis. Mongolia accepted the vaccination strategy suggested by WHO. In this frame work, the government of Mongolia conducted the Rev.1 and S 19 vaccine trials. The trail result showed that the Rev.1 vaccine was more effective against *Brucella melitensis* and *Brucella abortus* infection than the S19 vaccine which was less effective against *B. melitensis* from 1968 to 1969 [26]. The vaccine field trial provided the evidence that the vaccination of Rev.1 was effective for Mongolian local breeds in their pastoralist situation.

Ministry of Agriculture decided to implement a nationwide mass vaccination of cattle and small ruminants with financial and technical assistance from WHO and UNDP over the next five years. These included i) built the vaccine production factory called Biocombinat, and ii) started producing the Rev.1 and S19 vaccines.

In 1974, the Biocombinate vaccine production unit received *Brucella melitensis* 1 reference strain 16M (ATCC №.23456) from Central veterinary laboratory in Weybridge, United Kingdom. In 1976, the Biocombinate received *Brucella melitensis* 1 reference strain 16M second time from Department of bacteriology from the University of California in Berkerley, USA. The Biocombinate started producing the homemade vaccine by the Mongolian laboratory staff trained at the Hungarian biological laboratory in Budapest. The vaccine validation was conducted by the independent Mongolian State veterinary drug testing and confirmation laboratory. Also, the international independent vaccine quality control was conducted by WHO Brucellosis Reference Laboratory in Moscow, USSR and Central Veterinary Laboratory in Weybridge, UK. The locally produced Rev.1 vaccine dose was $1x10^9$ or $2\text{-}3x10^9$ live *Brucella* organisms according to the OIE (World Organization for Animal Health) and World Health Organization guidelines [26].
The locally produced vaccines were stored at below zero degrees and transported by airplane to the provincial center. The distilled water used as a vaccine diluent was transported by vehicles to the province center. The vaccination scheme was to vaccinate all female animals in the first year and following year by vaccinating the new born animals for the next 5 to 6 years until the flock was

replaced by the new animals. The mass vaccination was implemented in the country from 1974 to 1985.

The Mongolian livestock mass vaccination campaign provided practical information regarding the effective application of Rev.1 vaccine which became the important foundation for future campaigns worldwide [26]. In contrast, the Mongolian practice of using Rev.1 vaccine in cattle showed vaccine strains localized in the genital tract of breeding bulls providing evidence that this was not practical and which was not recommended to other countries [28]. The mass brucellosis vaccination continued until end of the international assistance which brought down livestock prevalence up to 0.01% in sheep and goats in 1985. During the 1980s, the human incidence was reduced to less than 1 case per 100,000 thanks to livestock vaccination. However, the government program did not lead to brucellosis elimination because of lack of funding and major political changes by end of 1980s [27, 29].

1.10.2 Livestock production in Mongolia

The Mongolian political system changed from communist regime to the democratic system in 1991. During the post-communist transition period human brucellosis was re-emerging in the country due to the breakdown of the government-run disease surveillance program and control of medical and veterinary sectors [30]. During the communist regime the country exported large number of livestock to the USSR and in this way it maintained a more or less stable livestock population (Figure 2.).

Figure 2 Frozen carcasses in Ulaanbaatar abattoir and transporting to the Soviet Union in the 1960's (Photo courtesy of Jan Kolar)

By the end of the communist regime exports of meat decreased dramatically because of a temporary loss of purchasing power. In addition, the Russian Federation (RF) allowed only the import of meat from non-vaccinated livestock. This requirement caused major obstacles for the country exporting meat because the current brucellosis control program was based on mass livestock vaccination. Over 10 million sheep and goats were ready for export to Russia or any other country but trade requirements limited the export.

Therefore, herders only had limited access to national markets and the stocking density was nearly twice the estimated capacity. The political changes allowed the herders for the first time to own their livestock and the state no longer the controlled the number of herds which resulted in a continuous increase in the Mongolian livestock population since 1991.

Mongolian seminomadic herders are subject to environmental changes and harsh winter conditions which can have dramatic consequences when there is a heavy snow fall and very low temperatures. Such snow storm disasters called “Dzud” occur periodically and prevent animals from feeding. In the last decade, two “Dzud” disasters occurred: In the years 2000-2001, there were consecutive snow storm disasters causing the loss of over 4 million animals and over 10.2 million animals lost during the harsh winter of 2010.

During a five month period 23.2% of total number of livestock was lost because of a lack of fodder and extreme low temperatures in 2010. Over 40% of herders who had lost their animals no longer had sufficient income so they sought employment and work by immigrating to urban areas. Herders end up unemployed or start low paid manual jobs in the urban areas.

Official data on livestock numbers reflects the increase of livestock numbers from 1930 onwards and the massive loss of small ruminants and cattle in the years 2000-2001 and 2009-2010 (Figure 3).

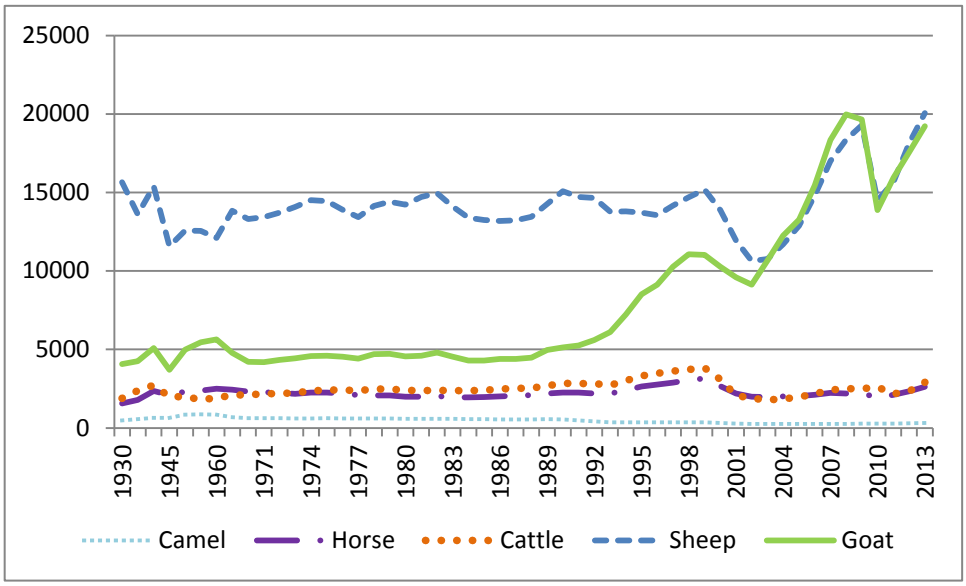

Figure 3 Dynamics growth of goats, sheep, cattle, horse and camels from 1930 to 2013 (Source: Mongolian National Statistical Office, 2013).

Pasture land management specialists found that Mongolian pastures could only support 25-30 million animals. Currently, 45.1 million heads of livestock are in Mongolia of which 52.5% are small ruminants. The total amount of pasture was 122.7 million km^2 in 1964 and pasture land assessment reported that 70% of pasure land was degraded and 31% is severely degraded in Mongolia.in the 2012s.The main causes are the doubling of the stock rates since 1990, seminomadic herders not having access to transportation provided by the government, the number of engineered wells has dropped from 35.000 to 20.000, access to the market and social reasons like education and health further inhibited by the movement of the herders.

For this reason, seminomadic herders reduced their mobility, increased sedentarization, and concentrated on their income-producing livestock-especially cashmere-goats that caused numbers have doubled since 1990. Mongolian seminomadic herders remain need of income from their livestock; they need adequate pastire land for their livestock, and skills to cope with natural variables, like dzud, drought, and heat, the intensification of the livestock production system, access to emergency fodder for winter, and the variables of mining, and government regulations. The livestock numbers have increased 75% in the last decade, but without the ablility to export all animal. It has caused over grazing which is one of the contributing factors to land degradation in the country [21].
Therefore, there is an urgent need to work rapidly towards brucellosis control and the control of other highly contagious disease like Foot and Mouth Disease (FMD) to gain open access to export to international markets, decrease the pressure on the grazing land and increase income for herders.

2 Research gap, aim and specific objectives

2.1 Research gap

In Mongolia, human brucellosis became an issue in the 1960s. During the 1980s, thanks to livestock vaccination, the human incidence was reduced to less than 1 case per 100,000 of the population. In the 1990s, human brucellosis re-emerged due to the breakdown of government run disease surveillance and control programs and the lack of resources in the livestock and medical sectors. Mongolia has one of the highest human brucellosis incidences in the world based on inactive surveillance data.

The recent systematic review of the global burden of human brucellosis showed that there was no quality epidemiological study available on human and livestock brucellosis prevalence for Mongolia. The government of Mongolia had implemented a mass vaccination for brucellosis from 2000 to 2010 and which is planned to continue until 2020.

Molecular epidemiological tools are not yet available for the medical and veterinary sector in Mongolia for a brucellosis trace-back investigation. Only a few strains had been typed in 2010 using molecular methods at the Himalaya Institute, Russian Federation. Unfortunately, those results are not accessible to the Mongolian medical doctors and veterinarians. There is still a lack of understanding of the most important livestock-human brucellosis transmission, and no molecular epidemiological data is available for analysis of the current situation.

This research study aims to provide scientific evidence of the human and livestock prevalence and to identify the main *Brucella* spp in livestock transmitting to humans.
No monitoring of the vaccine cool chain central, aimag (=province), soum (=village) has been done since 1975. Up to 2010, there was no official protocol or written document available in the veterinary sector on vaccine cool chain operatation and monitoring it. During the literature review process could not find any document on vaccination coverage. There was no document available 10 year vaccination program assessment or any evidence that livestock vaccination stopped the transmission of brucellosis to humans.

2.2 Aim

This dissertation aims to assess human and livestock brucellosis prevalence simultaneous serological surveys and the culture of the pathogenic agents. In this way a more complete picture of the disease epidemiology and helps to deepen our understanding of the disease risk and provide an important knowledge base for policy development and implementation.

2.3 Objectives

1. To assess the human and livestock seroprevalence and to estimate the proportion of underreporting of clinical illness in humans.
2. Identify the main Brucella spp circulating between humans and livestock.
3. To assess the brucellosis cold chain situation after the production, distribution, storage of vaccine at the province and soum level in Zavkhan province.
4. Contribute to the understanding of livestock demographics in Mongolia

2.4 Research questions and specific objectives

The specific objectives and main research questions are as following:

1. Understand the distribution and transmission of brucellosis in Mongolia;

    1.1. Simultaneous assessment of seroprevelance in human, sheep, goats, cattle, yaks, camels and dogs brucellosis in the country;

    1.2. What the association is between human and livestock seropositivity;

    1.3. What the human incidence of brucellosis is in the countryside;

2. What are the main human brucellosis symptoms, diagnosis and treatment scheme in Mongolia;

    2.1. What the main symptoms of human brucellosis are;

    2.2. How rural populations have access to the diagnosis and treatment of brucellosis in local areas;

    2.3. What kind of serological tests are used for diagnosis of human brucellosis and are these tests available in rural areas;

    2.4. What is the treatment regime offered for brucellosis patients at the soum and provincial hospitals;

    2.5. How many times human brucellosis is under reported;

    2.6. What is estimated to be the proportion of clinical illness among the seropositive;

3. What are the main strains circulating among livestock and humans;

    3.1. What is the main *Brucella* spp circulating among sheep, goats and cattle in Mongolia;

    3.2. What is the main host species that transmits *Brucella* spp to humans;

    3.3. What are the main *Brucella* species spilling over to humans;

    3.4. What are the main characteristic of the *Brucella* spp circulating in Mongolia.

    3.5. What conclusions can be made on brucellosis animal to human transmission based on the characterization of isolated strains;

4. How effective is the current brucellosis control program;

    4.1. What is situation of the cold chain of the brucellosis vaccine;

    4.2. How vaccine coverage can be evaluated in the rural area

3 Study design, data and ethical consideration

3.1 Study design

Zavkhan and Sukhbaatar provinces selected by the Ministry of Food and Agriculture for this study. A multistage cluster sampling was determined proportional to the soum size, bag, hotail (nomadic camp), people and invididual animal species per province. Livestock samples collected for isolation of *Brucella* spp from Dornod, Khentii and Sukhbaatar provinces.These province have not scheduled for livestock brucellosis vaccination from 2010 to 2013. This dissertation used several study designs and different empirical research methods. The subsequent section gives an overview of the data collection and data analysis. Detailed descriptions are included in the respective chapters.

3.2 Data sources

A cross-sectional survey was conducted among randomly selected 169 hotail (2-3 herder families live as a nomadic camp). A total of 574 people and 8.054 livestock species, plus dogs were sampled during the field study in Zavkhan and Sukhbaatar provinces in 2010. All study participants were interviewed using a questionnaire to obtain their demographics and risk factors. A livestock questionnaire on animal health status was also answered by one family member.The laboratory test results from human and livestock samples were also incorporated with data.

A cross sectional survey was conducted among randomly selected rural population from four soums and two bags from Arkhangai, Khuvsgul, Selenge, Uvs, Umnugobi, and Govi-Altai provinces. There are 2,282 rural people participated in the study from Nov 2011 to Jan 2012.

Herders also participated in a cross-sectional study included in the follow up study while collecting livestock samples for isolating *Brucella* spp from different livestock species of Dornod, Khentii and Sukhbaatar provinces from April 2011 to April 2013.

3.3 Data analysis

All data entered in duplicated into Access 2003 database (Microsoft, USA), in order to detect data entry errors, and compared using the data compare function of Epi Info 3.5.3 (Centers for Disease Control Prevention, USA). All statistical analyses were performed in Stata 10.1 (StataCorp LP, USA).

The seroprevalences of people and livestock species (also when stratified to age classes) were calculated considering clustering within hot ails (a logistic regression model with the outcome alone and with a random effect (RE) at the hot ail level in Stata 10.1.

A generalised linear latent and mixed model was used to assess an association between human and livestock seropositivity. This multilevel model allowed for inclusion of the denominator (number of people sampled per camp) in the analysis.

Risk factors of human seropositivity, herd and individual livestock seropositivity analysis of associations, have been summarised in categories such as for profession and reported symptoms. Factors possibly associated with seropositivity in humans and livestock (explanatory variables with $p \leq 0.2$ in univariable analysis) were evaluated with multivariate logistic regression models (with RE at the hot ail level) using backward stepwise selection and a removal level for covariates at $P = 0.10$ based on the likelihood-ratio test (LRT).

Sequencher ® version 5.0 sequence analysis software: MLVA locus band sizes were estimated using Sequencher ® version 5.0 sequence analysis software (Gene Codes Corporation, Michigan, USA).

SAS TM (Statistical Analysis System Inc. Cary, USA) program was used for MLVA analysis. VNTR data was used for the cluster analysis using SAS TM proc cluster using the unweight pair-group method with arithmetic averages, (UPGMA).

Phylogenetic trees were drawn using SAS TM proc tree. Hunter- Gaston diversity index (HGDI) was calculated for every locus using online tool V-DICE available at the HPA website (http://www.hp-bioinformatics.org.uk/cgi-bin/DICI/DICI.pl)

3.4 Ethics Statement

This study was approved by the Ethics Committee of the Ministry of Health of Mongolia (ref. No 03/2010), the Ethics Commission of Health Science University, Mongolia (ref. No 15 / 1A / 2011) and the Scientific Committee of Veterinary Research Institute (ref N 256/2010). In Switzerland, approval was given by Ethics Commission of the Cantons of Basel-Stad and Basel- Land (Ethikkommission beider Basel) (ref.169/10) and Research Commission of the Swiss Tropical and Public Health Institute of Basel. Informed written consent was obtained from all participants. In the case of children informed consent was obtained from their parents or guardians. Herders gave oral consent to test livestock samples and aborted foetuses.The collection of livestock samples were always supervised by the veterinarian.

4 Collaboration

4.1 Animal Health Project, Swiss Agency for Development and Cooperation in Mongolia

This research study was embedded in the frame-work of the Animal Health Project (7F-06 231.02.05) and the Livestock Project (7F-06231.01.31). The reseach work was funded by Swiss Agency for Development and Cooperation (SDC) in Mongolia.

The Animal Health project aims to improve Mongolian animal health system which includes control of zoonoses focusing on brucellosis. The thesis findings will provide as evidence and recommendations for the Ministry of Health and Ministry of Food, Agriculture after the thesis defence. It will assist improvement of the current livestock brucellosis control. The main findings will be published in the form of a booklet which will be distributed to the Mongolian medical and veterinary authorities.

4.2 Swiss Tropical and Public Health Institute

This thesis work has been conducted with the close supervision of Prof. Dr. Jakob Zinsstag and Dr. Esther Schelling who supervised all project activities within the Swiss Tropical and Public Health Institute for the period of May, 2010 to Dec, 2015.

Dr. Felix Roth also collaborated with the project and exchanged his opinions and suggestions. Prof. Sebastian Gagneux, Dr. Sonya Borrell, and Ms. Julia Feldmann collaborated with the laboratory work and Dr. Maria Balliff helped to teach the first step of molecular typing tests at the TB research laboratory at the Swiss TPH Institute.

4.3 Institute of Bacteriology, University of Bern

Dr. Paola Pilo supervised all laboratory work and gave practical support and coordination of day–to-day work for molecular typing of human and livestock samples Dec 2012-Feb 2013 and Oct 2013-Jan 2014.

4.4 Mongolian medical and veterinary collaborators

Dr.Tugsdelger Sovd who was Head of the Public Health and Policy Department, MoH and Dr. Tsolmon Bandi was an officer in charge of zoonotic disease from The Department of Veterinary Medicine, MoFA supported the field work.

The Sukhbaatar and Zavkhan provinces veterinary department provided the data on animals and households which has been used for the design of the multistage cluster sampling. The Dariganga, Khalsan, Sukhbaatar, Tuvshinshiree, Aldarkhaan, Ider, Otgon and Ulaistai soum governors provided hot ail lists per bag.

Awareness building material produced in collaboration with National Center for Communicable Diseases. The brucellosis awareness building material such a handout and small booklets were given to the participant herders as well as soum physicians and local veterinarians. The Aldarkhaan, Ider, Otgon, Ulaistai, Dariganga, Khalsan, Sukhbaatar and Tuvshinshiree soum veterinarians, medical doctors and nurses assisted in conducting of the survey. Aformentioned soum hospitals were responsible for treatment of human by RBT positive patients following treatment regime with antibiotics provided by the project. The Sukhbaatar and Zavkhan province health department Dr. Gundegmaa and Dr.Dondog closely monitored the treatment process at the Soum hospital level. Dr. Selenge, Dr. Narangarav, Dr. Enkhtuya, Dr. Bujinlkham, nurse Mrs.Nergui collobrated from NCCD.

Dr. Batbaatar, Dr. Erdenebaatar, veterinarian B. Enkhtuul, O.Khurtsbaatar. G.Ulziisaikhan, B.Aruintuya supported the field work for collection of livestock samples, and the laboratory work was done in the Laboratory of Infectious Disease and Immunology at the IVM in Ulaanbaatar, Mongolia. Dr. Gantsetseg collaborated from the SCVL tested the livestock sera with the RBT and two competitive ELISA.

D.Uuganbayar, Kh.Bayarnyam, G.Oyunbadam, S. Khishigt, and Dr. Bayarsaikhan collaborated and independent quality control of the brucellosis vaccines at the State Central Veterinary Drug and Biological Testing and Confirmation Laboratory.

5 Representative Seroprevalences of Human and Livestock Brucellosis in Two Mongolian Provinces

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5.1 Abstract

Mongolia implemented a brucellosis livestock mass vaccination campaign from 2000 to 2009. However, the number of human cases did not decline since 2004 and the current epidemiological situation in Mongolia was uncertain. The objective of this study was to estimate the representative seroprevalences of humans and livestock in two provinces in view of their comparison with officially reported data. A representative cross-sectional study using cluster sampling proportional to size in humans, sheep, goats, cattle, yak, horses, camels and dogs was undertaken to assess the apparent seroprevalence in humans and animals. A total of 8054 livestock and dog sera and 574 human sera were collected in Sukhbaatar and Zavkhan provinces. Human and animal sera were tested with Rose Bengal and ELISA tests. The overall apparent seroprevalence of brucellosis was 27.3 % in humans (95% CI 23.7-31.2), 6.2 % (95% CI 5.5-7.1) in sheep, 5.2% (95% CI 4.4-5.9) in goats, 16.0% (95% CI 13.7-18.7) in cattle, and 2.5% (95% CI 0.8-7.6) in camels, 8,3% (95% CI 6.0-11.6) in horses and 36.4% (95% CI 26.3-48.0). More women than men were seropositive (OR=1.7; $P < 0.0014$). Human seroprevalence was not associated with small ruminant and cattle seroprevalence at the nomadic camp (*hotail*) level. Annual incidence of clinical brucellosis, inferred from the seroprevalence using a catalytic model, was by a factor of 4.6 (1307/280) in Sukhbaatar and by a factor of 59 higher (1188/20) in Zavkhan. This represents a 15-fold underreporting of human brucellosis in Mongolia. The lack of access to brucellosis diagnostic testing at the village level hinders rural people receiving treatment. In conclusion, this study confirms the high seroprevalence of brucellosis in Mongolia. Stringent monitoring and surveillance and quality control of operational management of a nationwide mass vaccination of small and large ruminants is warranted to assure its effectiveness. More research is needed to understand the complex animal-human interface of brucellosis transmission at different scales from farm to provincial level.

Keywords: apparent seroprevalence, incidence, brucellosis, Mongolia, human, livestock

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5.2 Introduction

Brucellosis is the most common zoonotic diseases worldwide. Brucellosis is endemic in livestock and causes human disease in Africa, Central America, Central Asia, the Mediterranean region and the Near East. (Zinsstag et al. 2011, Bonfoh et al. 2012, Dean et al. 2012b). Brucellosis is caused by members of the *Brucella* genus. Infection is caused by *B. melitensis* in small ruminants and *B. abortus* in cattle and yaks. Brucellosis is mainly transmitted to humans through direct contact with infected livestock, placenta material and vaginal discharge or through the consumption of contaminated unpasteurised milk and dairy products. Human brucellosis causes varied clinical manifestations including intermittent fever, sweating, joint and low back pain, headaches, fatigue, weight loss and general weakness persisting for a long time (Dean et al. 2012a).

Clinical signs of human brucellosis are often unspecific which makes it difficult to differentiate from other febrile conditions (Baba et al. 2001). Laboratory tests are essential for diagnosis and only the isolation of bacteria provides a proof of infection. However, bacteriological diagnosis is expensive, difficult and dangerous. Serological tests are easier to implement in limited resource settings. In the absence of specific antibiotic treatment, brucellosis in humans may persist and progress to neurological or other severe complications (Diaz et al. 2011).

In Mongolia, traditional nomadic livestock systems are maintained where sheep, goats, cattle, yaks, camels and horses are kept in flocks of varying sizes. Herd move continuously depending on the availality of pasture during the summer and autumn, with limited mobility during the cold season. Herder families keep guard dogs to protect their livestock from wild predators. These dogs consume infected placenta/foetal materials and carcasses from the herds. Herders utilise traditional practices of home slaughtering animals for household consumption, milking mares to produce a traditional fermented drink called “airag” and milking female camels consume milk during the summer and autumn. Fresh cow’s milk is also used to feed weak newborn animals, which are brought into their traditional mobile houses during cold season (Madkour 2001).
In Mongolia, animal brucellosis was first documented in 1935 and human brucellosis was officially registered for the first time in 1949. The national livestock surveys result showed that apparent brucellosis seroprevalence was 17% in cattle, 3.5% in sheep and 2% in goats in the 1950s. The government of Mongolia received assistance from World Health Organization to implement brucellosis control in the 1960s. A total of 4,816 human sera were analysed during the national survey, using the serum agglutination test (SAT) and complement fixation test (CFT) to show that 1.7% of

urban and 4.4% of rural populations were seropositive. Participants were classified according to occupational exposure risk as meat factory workers, sheep herders, dairy farmers and students from the Agricultural University. The national survey results provided evidence that human brucellosis had emerged endemic in the country (Jezek et al. 1972).

The government of Mongolia was alarmed that human brucellosis had emerged in the country and urgently sought assistance from the Council for Mutual Economic Assistance (COMECON), the former economic assistance organisation of the Soviet Union. Subsequently, the country launched a national brucellosis control programme using a test-slaughter strategy from1965 to 1968 (Enkhbaatar et al. 2004). In total, 37.5 million livestock and dogs were tested with an allergic skin test, CFT and SAT during programme. The animals with positive serological test results were immediately separated from the herd and slaughtered within a short period of time (Kolar J. 1984). Mongolia was able to implement the test-and-slaugther strategy because the government owned all livestock. Therefore, it was mot necessary to compensate herders when animals were slaughtered.

However, the government realised that the test-and-slaughter strategy was not practical and was less effective to control brucellosis under pastoralist conditions (Kolar 1987). An epidemiological team commissioned by WHO, conducted a Rev.1 vaccine trial for small ruminants showing that the vaccine was highly effective for the local breeds of livestock in pastoralist conditions (Kolar 1982, Enkhbaatar at al. 2004). The Ministry of Agriculture approved a mass vaccination strategy using Rev.1 for small ruminants and S19 vaccine cattle and yaks which was impelemented from 1975 to 1986. More than 33 million small ruminants and 9.5 million cattle/yaks were vaccinated during the mass vaccination campaign, making it the most successful nationwide vaccination programmes in the world. (Kolar J. 1987). The annual incidence of human brucellosis decreased from 25 per 100,000 in 1975 to 0.23 per 100,000 in 1986. However, the coverage achieved by mass vaccination campaigns was not assessed in a formal way (Kolar 1977, Selenge et al. 2011).

After 1990, Mongolia no longer dependent on the former Soviet Union, and it went through democratic reform and privatisation of all sectors. The health care and veterinary services were weakened due to lack of resources during the post-socialist transition period. This influenced the breakdown of government-operated disease surveillance and control activities from 1990 onwards. Brucellosis re-emerged as a preventable human disease in most countries which were part of the former Soviet Union and Mongolia. Government reported human brucellosis annual incidence rose from 4.9 per 100,000 in 1990 to 67 per 100,000 in 1999 (Selenge et al. 2011). In a mathematical transmission model, a simultaneous fit of sheep-human and cattle-human contact rates indicated that >90% of the human brucellosis cases were related to small ruminant transmission (Zinsstag et al. 2005). However it was not known which proportion of the nomadic herders had access to bru-

cellosis diagnosis and treatment in rural areas. It is possible that the true incidence was underestimated (Ebright et al. 2003). International experts recommended to the WHO that Mongolia needed to implement livestock brucellosis mass vaccination. In the year 2000, the Ministry of Food and Agriculture (MoFA) budgeted the equivalent to 10.5 million USD for a 10-year livestock mass vaccination campaign following WHO recommendations. A cross-sector economic analysis showed the mass vaccination would be largely profitable to the Mongolian society (with a benefit-cost ratio of 3.1). If costs were shared between the public health sector and the agricultural sectors, cost-effectiveness could be at 20 USD per averted Disability-Adjusted Life Years (DALY) (Roth et al. 2003). However the mass livestock vaccination campaign which started in 2000 seemed to lose traction as reported cases of human brucellosis did not decline further after 2004 (Selenge et al. 2011). The epidemiology of brucellosis in Mongolia became uncertain and needed to be reassessed. The objective of this study was to assess the seroprevalence of brucellosis in humans, in different livestock species and dogs from two selected province (*aimag*). Additionally, the study aimed to evaluate the association between human and livestock seropositivity (Bonfoh et al. 2012). We hypothesised that human brucellosis seroprevalence could be statistically associated to brucellosis seroprevalence in small ruminants. The study results are compared with government reports to inform Mongolian policy makers from the medical and veterinary sectors to define the new strategy for the National Brucellosis Elimination program.

5.3 Material and Methods

Study design

This study took place from June to October, 2010. Human and livestock aggregated data was available at the provincial and national level from the Mongolian National Statistical Office. Household census information and official livestock annual census data from year 2009 was available from the district (soum) governors' offices in all selected districts.

A cross sectional study design, similar to Bonfoh et al.(2012), utilised cluster sampling propotional to size for all species (Bennett 1991). An intraclass correlation coefficient (rho) of 0.1 between clusters was assumed, which is appropriate for highly contagious diseases like brucellosis (Otte and Gumm 1997).The sample size calculation was optimised to assure that the lower 95% confidence limit was higher than zero, and for larger prevalences, the largest standard error was 2.5%. The sample size was further optimised to assure the feasibility of sampling herds within available budget. We assumed a brucellosis sero-prevalence for livestock based on reported annual serological test results of Sukhbaatar and Zavkhan province from 1990 to 2008. Brucellosis seroprevalence were 0.5% for small ruminants, 2% for cattle and 3% for camels. Human brucellosis seroprevalence among rural population was assumed to be 20% according to the Mongolian National Centre for Communicable Diseases (Dagvadorj et al, 2003).

Sukhbaatar, Zavkhan provinces were selected by MoFA (Map 1). Zavkhan province was represented the western region and Sukhbaatar province represented the eastern region of the country. Sukhbaatar province (Map 2) reported high human brucellosis incidence in the country annually and high livestock prevalence, as well as high cross-province livestock movements. Human brucellosis incidence was not known and only sporadically a few cases were reported by Zavkhan province's health department. There was low-cross province livestock movement in Zavkhan province (Map 3). Four districts were randomly selected proportional to size for livestock from each province. Similarly, the third level of sampling, 8 villages (*bags*) and the fourth stage, 10 nomadic camps (*hot ail*), were also randomly selected proportional to size. Nomadic camps were composed of two or three nomadic herder families camping together. Their herds of all different livestock species mix and shared the same pasture and water points. The selection of nomadic camps was done using the official household lists of livestock annual census data from the governor's office in eight villages (Bennet 1991). The nomadic camps were selected by drawing random numbers.

Simultaneous Sampling of Human and Livestock

The study was conducted in summer of 2010 by two field teams composed of three veterinarians, a medical doctor and a nurse. The field teams visited 86 nomadic camps from 4 districts of Sukhbaatar and 83 nomadic camps from 4 soums Zavkhan province. The field team was able to find selected nomadic camps by assistance of a local veterinarian. Approval was first sought from the chief of a nomadic camp and members were then gathered together to explain the aims and methods of study. Children under 16 years old were sampled if their parent or guardian provided written consent to participate in the study. Participants were selected from those were willing by randomly drawing names from a hat. The planned sample size was 3-4 people per nomadic camp, thus totalling 60-80 people per district. A medical doctor collected blood sample from the participants. Basic data relating to demography (age, sex, and birthdate), risk factors of exposure to infected livestock , consumption of dairy and animal source products and the presence of specific symptoms during the previous month (fever, headache, sweats, sleeping difficulties, fatigue, weight loss, joint and muscle pain and back pain) was collected through through structured interviews.

Head of nomadic camps were interviewed using structured questionnaires on livestock health status, number of aborted animals during last lambing season and brucellosis vaccination status of the herd for last 3 years.

In each hotail 20 sheep, 20 goats, 5 cattle/yaks, 5 camels, 4 horses and a dog if present during the field visit. Animals under 9 months of age and sick animals excluded. Small ruminants were gathered in the sheep pen. A team member pointed at a first animal. From there every tenth small ruminant was selected until a total of 20 goats and 20 sheep were sampled (Otte and Gumm 1997).

The selection of five cattle was made focus on cows. The team member pointed at the first horse from there every second animal was selected until four horses were sampled. Any camel present at the time of visit was sampled. Any dog which the owner was willing to to hold was sampled.

Serological Testing (RBT and ELISA)

Human and livestock blood samples were centrifuged the same day. Human serum samples were transported frozen in cool boxes, according to national biosafety standards, from the field site to the National Centre for Communicable Diseases (NCCD) in Ulaanbaatar. Livestock serum samples were transported in cool boxes frozen from the field site to the State Central Veterinary Laboratory (SCVL) in Ulaanbaatar. All human sera were tested by standard Rose Bengal Test (RBT; Tulip Diagnostics Ltd, Bambolim, India) mixing 25 µl antigen with 25 µl serum, which was read after 4 min. Positive sera were serially diluted with saline to obtain dilutions from ½ to 1/32. They were retested by the modified Rose Bengal Test protocol of Diaz et al protocol (2011). Additionally, all sera were tested by indirect IgG ELISA (Diagnostic Automation INC, California, USA). Twenty were positive by the standard RBT and modified RBT but negative by indirect IgG ELISA. Those sera were retested by IgM ELISA (NovaLisa™, NovaTec Immundiagnostica GMBH, Dietzenbach, Germany).

Study participants were asked about the following human risk factors: dictrict where the family was staying at the time of study, age class, sex, occupation, consumption of raw milk and raw milk products, consumption of dried meat and raw or half-cooked liver. Husbandry practices recorded were: keeping weak new born animals in the family dwelling place, direct contact with livestock placenta/ aborted foetuses and the disposal of aborted foetus/placentas (feeding dogs or discarding in the field). The observations of abortions among livestock species were also recorded.

Livestock sera were tested following the standard protocol of RBT (Biokombinate State Owned Enterprise, Ulaanbaatar, Mongolia and Brucellosis National Reference Center, Santa Fe, Spain). Cattle and yak sera were tested using 25 µl antigen and 25 µl serum ratio and small ruminant sera were tested with 25 µl antigen and 75 µl serum, which was read after 4 minutes according to the World Organization for Animal Health (OIE). Camel, horse and dog sera were tested using 25 µl antigen and 25 µl serum according to national standards. Additionally, livestock sera were tested by two competitive ruminant IgG ELISAs (COMPELISA of VLA®, UK and SVANOVIR ®, Sweden).

Data Analysis

Human and livestock data were double entered into Access 2003 database (Microsoft, USA). Data bases were validated using the “Data compare” routine in Epi Info 3.5.1 (Centers for Disease Control and Prevention, USA). Human and small ruminants modified RBT results were compared with indirect ELISA and competitive ELISA. We report here apparent seroprevalence because the sensitivity (Se) and specificity (Sp) of RBT test for camels, yaks, horses and dogs were not established. True prevalences were estimated for some species using the Rogan–Gladen estimator TP = (AP+Sp - 1) / (Se + Sp - 1), where TP is the true prevalence, AP is the apparent prevalence, Se is the sensi-

tivity and Sp is the specificity (Gladen and Rogan 1978). We considered that Se=91.7% and Sp=91.2% for human RBT produced by Tulip Diagnostic Ltd (Ruiz-Mesa et al. 2005). For small ruminants, we considered Se=94% and Sp=100% for cattle, we used Se=99.7 and Sp=99% (Mainer-Jaime et al. 2005). The apparent seroprevalences was calculated considering clustering within nomadic camps.

The apparent human seroprevalence of the RBT was used to estimate the incidence of brucellosis exposure by catalytic two way model under equilibrium conditions (Muench 1959)

$$\frac{dS}{dt} = -aS + bI \quad (1)$$

$$\frac{dI}{dt} = aS - bI, \quad (2)$$

S is susceptible population and *I* is seropositive population. The 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* (3).

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

We estimated the seroconversion rate *a* and loss of *b* simultaneously from the data using Microsoft Excel Software (Microsoft, USA).

The assumed average duration of brucellosis seropositivity (1/*b*) was 10.9 years (Bonfoh et al. 2012). Serological test results were converted into binary outcomes (0= seronegative, 1= seropositive), depending on the cut-off value of each test. Logistic regression, modelling for the outcome of seropositive humans and livestock, included random effects (RE) on the district and nomadic camp level. For the human seroprevalence calculation, the clustering of individuals within nomadic camps was used as cluster unit.

Univariable analyses of explanatory variables (biologically plausibly associated with brucellosis seropositivity) were evaluated by a logistic regression model with a RE at nomadic camp level.

Seroprevalences of people and livestock species (also stratified to age classes) were calculated considering clustering within nomadic camps using in Stata 10.5 (StataCorp LP, Texas, USA). A generalised linear latent and mixed model was used to assess the association between human and livestock seropositivity. This multilevel model allowed for inclusion of the denominator (number of people sampled per nomadic camp) in the analysis. Risk factors of human seropositivity, herd and individual livestock seropositivity analysis of associations, have been summarised in categories such as for profession and reported symptoms. Factors possibly associated with seropositivity in humans and livestock (explanatory variables with $P \leq 0.2$ in univariable analysis) were evaluated

with multivariate logistic regression models (with RE at the nomadic camp level) using backward stepwise selection and a removal level for covariates at P = 0.10 based on the likelihood-ratio test (LRT).

The study was approved by Ethics Committee of the Ministry of Health of Mongolia (ref.№ 03/2010) and Scientific Committee of Veterinary Research Institute (ref № 256/2010). In Switzerland, approval was given by Ethics Commission of the Cantons of Basel-Stad and Basel- Land (Ethikkommission beider Basel) (ref.169/10).

Clinical brucellosis patients with a positive serological test result and clinical symptoms were registered and received doxycycline (100 mg) orally twice a day for 45 days and gentamicin (5 mg/kg) intravenously daily for 2 weeks.

Informed written consent was obtained from all participants. In the case of children informed consent was obtained from their parents or guardians.

5.4 Results

Seroprevalence in Humans and Animals

A total of 8054 different livestock and dog sera and 574 human sera were tested with at least one of serological test (Table 1). A total of 4,123 livestock sera were collected from Sukhbaatar and 3931 livestock sera from Zavkhan province. The apparent human seroprevalence was 28.5% (95% CI 24.0-33.6) in Sukhbaatar and 25.9% (95% CI 20.2-32.6) in Zavkhan.

In Sukhbaatar province, 318 herders and their family members (161 male and 157 female) participated from 83 nomadic camps. Participants were classified by their occupations as herders (*n* = 260), students or pupils (*n* = 45), children staying home (*n* =6), government employees (*n* = 4) and veterinarians (*n* = 3). Participants younger than 10 years had a seroprevalence of 16%. The seroprevalence was highest (>50%) among participants older than 45 years of age (Table 2).

In Zavkhan province, 256 herders and their family members (123 males and 133 females) participated from 69 nomadic camps, but only 250 blood samples were tested for serology. Participants were classified by their occupations as herders (*n* = 218), students or pupil (*n* = 16), government employees (*n* = 10), veterinarians (*n* = 7), children staying home (*n* = 6) and disabled/unemployed (*n* = 5). Only 11 children were younger than 15 years were sampled, but their seroprevalence was similarly high when compared with adults (Table 2).

Table 1. Total sample size by species and number of samples examined with different diagnostic tests

Species	Total	RBT (India) [1]	RBT (Mongolia) [2]	RBT (Spanish) [3]	ELISA IgG (human) [4]	ELISA IgM (human)[5]	ELISA (ruminant) [6]	ELISA (ruminant) [7]
Human	568	568			374	31		
Sheep	3338		1691	1647			242	131
Goats	3321		1988	1633			203	140
Cattle	817		366	451			130	55
Yaks	14			14				1
Camel	118		118				111	11
Horses	373		112	145			12	2
Dogs	72		72				46	13

[1] Rose Bengal Test produced by Tulip Diagnostic, India.

[2] Rose Bengal Test produced by Biocombinate, Mongolia.

[3] Rose Bengal Test produced by Brucellosis National Reference Centre, Spain.

[4] Indirect enzyme-linked immonosorbent assay detecting IgG in humans Diagnostic Automachine, USA

[5] Indirect enzyme-linked immonosorbent assay detecting IgM in humans Nova Tech Immunodiagnostic GmbH

[6] Competetive enzyme-linked immonosorbent assay detecting IgG in ruminants produced by VLA, UK

[7] Competetive enzyme-linked immonosorbent assay detecting IgG in ruminants produced by Svanovir, Sweden

Table 2. Human apparent seroprevalences by age class Sukhbaatar and Zavkhan province

Age class	n	n pos[1]	% [95% CI for binary outcome]	Seroprevalence[b]	95% CI[2]
Sukhbaatar					
<10 yrs	19	3	15.7 [3.4 – 39.6]	17.8	7.2 – 37.6
10 - < 15 yrs	17	1	5.9 [0.1 – 28.7]	6.1	0.8 – 32.7
15 - <20 yrs	25	2	8.0 [0.1 – 26.0]	8.1	2.0 – 27.2
20 - <45 yrs	184	48	26.1 [19.9 – 33.1]	26.1	20.3 – 32.8
≥ 45 yrs	73	37	50.7 [38.7 – 62.6]	50.9	38.6 – 63.1
Zavkhan					
<10 yrs	1	0	0	0	0
10 - < 15 yrs	10	3	30 [6.7-65]	na	na
15 - <20 yrs	6	0	0	0	0
20 - <45 yrs	142	40	28.1 [20.9 – 36.3]	29.0	21.1 – 38.4
≥ 45 yrs	91	21	23.1 [14.9-33.1]	23.5	15.7 – 33.6

[1] Positive with 1:1 RBT.

[2] Calculated with a random effect on the level of hot ail to consider potential clustering within nomadic camps.

Livestock seroprevalence were relatively homogenous between 5 and 8% in sheep, goats and cattle in Sukhbaatar province. In Zavkhan province, 2% of sheep and goats compared 15% of cattle were seropositive. Seroprevalences were 3.4% in camels, 1% in horses and 41.3 % in dogs in Sukhbaatar. In Zavkan province, 11% of tested horses were seropositive (Table 3). In Zavkan province, only 14 yaks and 9 dogs were sampled.

The overall apparent seroprevalence of brucellosis was 27.3 % in humans (95% of 23.7-31.2), 6.2 % (95% of CI 5.5-7.1) in sheep, 5.2% (95% of CI 4.4-5.9) in goats, 16.0% (95% of CI 13.7-18.7) in cattle, 2.5% (95% CI 0.8-7.6) in camels and 8.3% (95% CI 6.0-11.6) in horses and 36.4% (95% CI 26.3-48.0) in dogs. The overall apparent seroprevalence in yaks not calculated because only 14 yaks were sampled during the study.

In Table 4, true prevalences of human, sheep, goats and cattle using the Rogan-Gladen formula show minor changes for humans and livestock.

Table 3. Apparent seroprevalence estimates of brucellosis in Sukhbaatar and Zavkhan province for humans and different livestock species in 2010

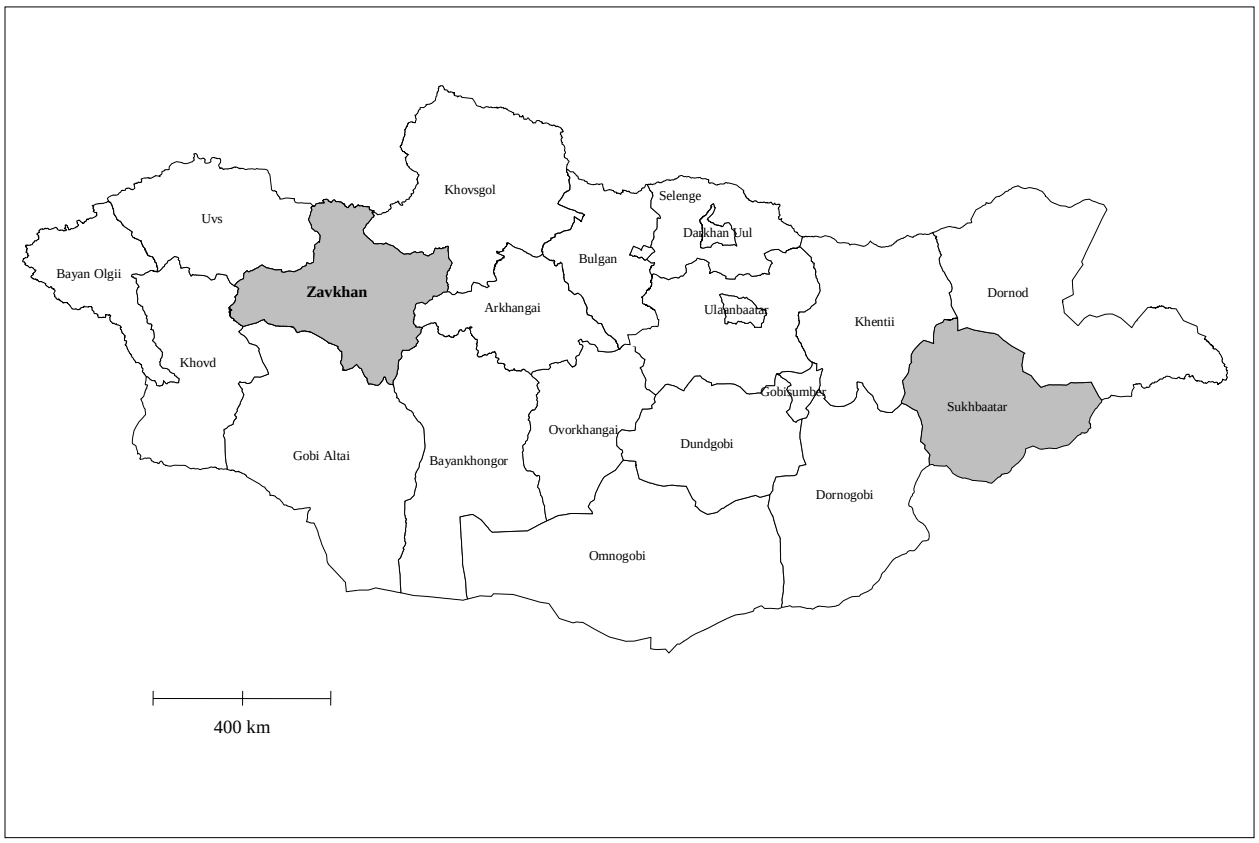
Species	n	Seroprevalence % [1]	95% CI
Sukhbaatar province			
Human	318	28.5	24.0-33.6
Sheep	1682	7.1	4.8-10.4
Goat	1671	5.1	3.0-8.6
Cattle	359	7.8	4.9-14.5
Camel	118	3.4	2.6-4.4
Horses	228	0.9	0.02-3.4
Dogs	65	41.3	29.38-54.36
Zavkhan province			
Human	250	25.9	20.2 -32.6
Sheep	1656	1.9	0.9-3.6
Goat	1650	1.7	0.9-3.2
Cattle	458	15.3	9.9-22.7
Yak	14	0	
Horses	145	10.9	4.3-25.4
Dogs	9	0	

[1] Seroprevalence and 95% CI calculated with xtgee model specifying nomadic camps as a random effect.

Correlation Between Human and Livestock Seropositivity

Generalised linear latent and mixed models were run first in each species alone, then for three ruminant species all combined, and finally, regrouped as small ruminants and large animals (cattle, camels and horses). No association between the serostatus of the livestock and status was found (data not shown).

Observations of livestock abortions were not associated with human brucellosis seroprevalence. The presence of a livestock species in a nomadic camp was not significantly associated with human seropositivity of any species. No livestock variable was significantly associated in the multivariable analysis, and thus was not considered in the risk factor analysis of human seropositivity (data not shown). Over 49% of participants (n = 556) reported that they fed aborted foetuses and placenta to the dogs.



Map 1 Mongolian map: selected Sukhbaatar and Zavkhan provinces are in *grey shade*

Reported Symptoms and Incidence of Apparent Brucellosis Seropositivity in Humans

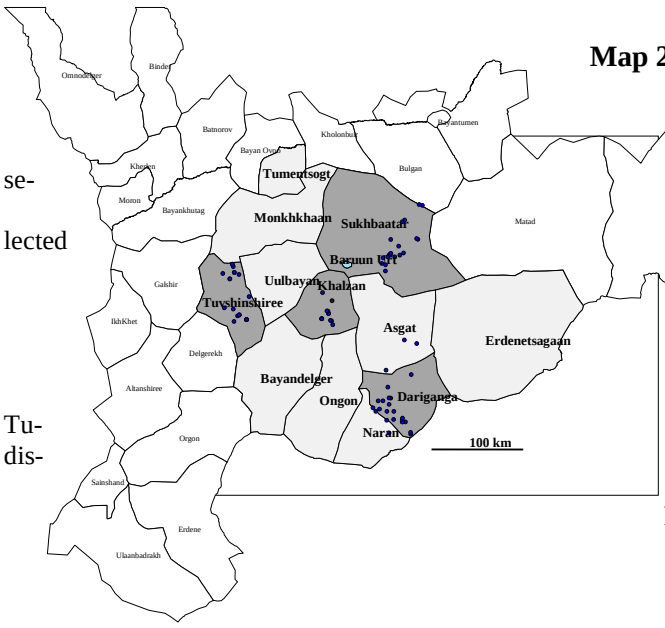
Reported disease events experienced during the previous month were compared to the sero-status of participants. Joint, muscle and back pain, weakness, night sweat, sleep disturbance and neuralgia were significantly associated with seropositivity (Table 5). Night sweat and neuralgia remained in

the stepwise backward multivariable model (Table 19). In Sukhbaatar province, a total of 91 participants (n =318) were seropositive, of which 62 (68%) reported at least one symptom and 54 (59%) at least two symptoms. Overall, there were 17% (95% CI 13-22) of participants who had two cardinal symptoms of brucellosis at time of interview. In Zavkhan province, among 64 seropositives, 41 (64%) have reported at least one symptom or a self diagnosis and 34 (53%) reported two symptoms. Overall, there were 13.6% (95% CI 9.6-18.4) of participants who were seropositive who had two cardinal symptoms of brucellosis at time of interview.

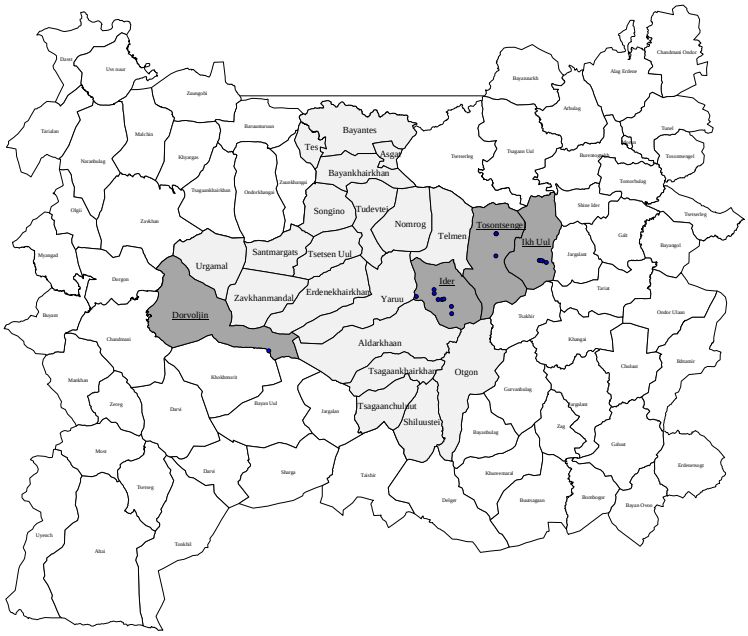
Among all seropositives in Sukhbaatar and Zavkhan, 66% had at least one symptom, whereas among the seronegative 47% had a least one symptom ($P < 0.001$; Table 5). Human incidence of apparent sero-conversion was estimated at 2.6% (95% CI 2.2-3.1) per year in Sukhbaatar province and at 2.3% (95%CI 1.9-3.0) in Zavkhan province by Rose Bengal Test.

Risk Factors of Human Brucellosis Seropositivity

In Sukhbaatar, the univariable analysis showed significant associations with age classes (in comparison to adults 20-45 years, 10-20 year old participants were at lower risk, whereas the elders were at higher risk). Being a female was a risk factor, and students had increased risk when compared to herders. For behaviour related risk factors, the consumption of half-cooked liver alone was associated as a risk factor with seropositivity. The observations of abortions were not associated with brucellosis seroprevalence (Table 6). For Zavkhan, the same univariable analysis was carried out. Similar results were found for the association with age classes (Table 7). Older people had a higher risk and only the consumption of half-cooked liver was associated with being seropositive (Table 6).



Map 2 Location of participating nomadic camps from selected Tudistricts in *grey shade* from Sukhbaatar province Dariganga, Khalzan, Sukhbaatar and vshinshiree



Map 3 Location of participating nomadic camps from selected Durvuljin, Ider, Ikh-Uul, and Tosontsengel in *grey shade* from Zavkhan province (only 17 of the 83 nomadic camps have the coordinates recorded)

History of Human Brucellosis Diagnosis and Treatment

In Sukhbaatar province, 18% of participants (*n*=318) reported that they were diagnosed for brucellosis in the past. The median time of past testing was 3.5 years (Q1 = 1.5 and Q3 = 20 years). Out of 14 participants diagnosed with brucellosis, 12 participants received treatment soon after the diagnosis was made at the province center.

Seventy-nine nomadic camps reported that the nearest health centre was at the district centre and only seven nomadic camps were reported that the provincial hospital was the nearest. The median distance to the health centre was 20 km. The most frequent use of transport to reach the health centre was by motorcycle (84%), by car (27%), by horse or camel ride (12%), by walking (2%) and picked up by an ambulance of the health centre 1%.

In Zavkhan province, 15% of the participants (*n*=254) reported that they had been diagnosed with brucellosis in the past. The median time of past brucellosis testing was 23 years (Q1 = 6.6 years and Q3 = 30.6 years). Among 21 participants (n=38) diagnosed with brucellosis, 30% received brucellosis treatment. Among nine patients who received treatment, one patient was at a tertiary hospital, two were at a province hospital, four patients were at the district hospital, one person at the bag feldsher unit and one elsewhere.

Table 4. Comparison of apparent and true seroprevalences in Sukhbaatar

	Apparent prevalence	Se	Sp	True prevalence
Humans	28.6	0.99	0.99	28.2
Sheep	1.9	0.94	1	2.0
Goats	0.7	0.94	1	0.74
Cattle	1.6	0.997	0.99	0.61

Underreporting of Human Brucellosis

The study showed an apparent seroprevalences of humans of 26.0% in Zavkhan and 28.5% in Sukhbaatar provinces. In Sukhbaatar, 17 new human cases per 10,000 were reported in 2008 and 28 new cases per 10,000 in 2010 (Selenge et al. 2011).

In Zavkhan province, there was no new human case reported in 2008, and two new cases reported per 10,000 in 2010.

An extrapolation of the results from Zavkhan to the whole country would mean that Mongolia had on average 6650 (95% CI 5180 – 8370) new brucellosis cases in 2010. This would reprsent an incidence of newly reported cases of 237 per 10,000 (95% CI 185 – 299) in 2010. Againts the incidence of 15 humans per 10,000 populations in 2010, this represents a 15 fold underreporting. By using the data from Zavkhan rather than Sukhbaatar province, a more conservative estimate is calculated. Assuming that 50% of the seropositive have clinical symptoms (which was the case in our study) the incidence of clinical brucellosis in Sukhbaatar would be 131 (95% CI 110-154) per 10,000 and 119 (95% CI 93-150) per 10,000 population in Zavkhan. These results would indicate an underreporting of the annual incidence of clinical brucellosis by a factor of 4.6 (1307/280) in Sukhbaatar and by a factor of 59 (1188/20) in Zavkhan.

Table 5. Number of reported symptoms and human serostatus from Sukhbaatar and Zavkhan provinces

N reported	*Seropositives*		*Seronegatives*	
symptoms	*n*	*%*	*n*	*%*
Sukhbaatar				
0	29	32	101	44
1	14	15	41	18
2	6	7	22	10
3	8	9	13	6
>3	34	37	50	22
Zavkhan				
0	23	36	41	22
1	7	11	37	20
2	6	9	19	10
3	7	11	19	10
>3	21	33	70	38

5.5 Discussion

This is the first study of simultaneous assessment of brucellosis seroprevalence in humans and livestock. It shows a more complete epidemiological picture and deepens the understanding of infection patterns at the animal-human interface. The findings of this study are relevant for the country towards the development of a national brucellosis control programme by the medical and veterinary sector. Our study provides evidence that brucellosis is a major public health problem among the rural population of Mongolia. All livestock species were seropositive by serological test. This means brucellosis may cause economic impact through a reduction of livestock productivity (Roth et al.2003).

In the absence of a perfect test, brucellosis serology is difficult to interpret. All serological tests have limitations when used for screening (OIE 2009). In this study, the apparent seroprevalence has been adjusted for the performance of the Rose Bengal Test. However, it remains only an approximation of the true disease seroprevalence in the population. RBT and ELISA are suitable tests for screening at the national and local level according to the OIE guidelines. Two competitive ruminant ELISAs were used for livestock diagnosis and two different ELISAs were used for human diagnosis. More research is needed to identify the appropriate cut-off values for diagnostic tests in endemic settings. There are no specific serology tests for diagnosis in the horses, yaks, camels and dogs. We included horses, yaks and camles in the study because of the consumption of milk and milk products in Mongolia. Dog RBT results showed that dogs are likely to be infected. More research is needed to find appropriate serological tests and cut-off values for brucellosis diagnosis in yaks, camels, horses and dogs.

Traditional and cultural practice may influence zoonotic disease risk. Human seroprevalence was higher among women than men. Woman play important role taking care of newborn animals and milking all animals during the lactation period. Almost half of the participants reported that they fed dogs with aborted foetuses and placentas. Public education campaigns are needed among rural people informing them about the appropriate disposal of abortion by-products and other hygienic practices for preventing brucellosis.

Access to Human Diagnosis and Treatment

According to the current national standard of human brucellosis diagnostics and treatments (MNS 5348-39:2003) any person with a positive result by standard RBT and history of exposure to infected livestock and consumption of diary products should be considered as a suspected brucellosis case with a need to be confirmed by ELISA, PCR or bacteriology. Confirmed human cases receive brucellosis specific treatment requiring a 10 day hospitalisation, which is covered by national insurance scheme. Outpatient treatments must be paid out of pocket. Unfortunately, most of the district health centres do not have any brucellosis diagnostic tests available and district medical doctors prescribe medical treatment based on clinical symptoms. Suspected brucellosis cases have to travel to the provincial hospital to receive a primary diagnosis by RBT. Provincial hospitals do not have ELISA test or bacteriological methods to confirm the diagnosis. Therefore, suspected patients need to travel to the capital city to confirm a diagnosis. Brucellosis patients who are not diagnosed by a confirmatory test are not included in the official data registry, which may explain the high level of underreporting. The limited annual budget of district level health centres does not allow covering 45 days of treatment cost for all patients.

Based on these findings, we recommended to the Ministry of Health (MoH) that standard RBT can be used as a primary test and modified RBT can be used as a confirmatory test at the district level. RBT is a cheap and simple test at the district level under Mongolian conditions. Patients without complications can receive the 45 days of treatment regime as recommended by WHO without hospitalization. These recommendations were accepted by the MoH, who modified the diagnostic method and accepted treatment regime. Currently, MoH and NCCD are revising the national standards for brucellosis diagnosis and treatment procedures to our study results.

Table 6. Univariable and multivariable analysis of risk factors of human seropositivity in Sukhbaatar province

	Univariable					LRT
	n	**pos**	**%**	**OR**	**p-value[a]**	***P*(LRX2)**
Districts						
Dariganga	89	26	28.6	1		0.24
Sukhbaatar	79	23	25.3	1	0.99	
Tuvshinshiree	77	16	17.6	0.6	0.21	
Khalzan	73	26	28.6	1.3	0.37	
Age (years)						
<10 yrs	19	3	15.8	0.5	0.34	<0.001
10 - < 15 yrs	17	1	5.9	0.2	0.09	
15 - <20 yrs	25	2	8.0	0.2	0.06	
20 - <45 yrs	184	48	26.1	1	--	
≥ 45 yrs	73	37	50.7	3	0.001	
Sex						
Male	161	36	22.4	1	--	0.012
Female	157	55	35.0	1.9	0.013	
Occupation						
Herder	260	85	32.7	1	--	0.001
Student	45	3	6.7	0.15	0.002	
At home	6	1	16.7	0.4	0.42	
Other	7	2	28.6	0.8	0.82	
Raw milk consumption						
No	284	84	29.6	1		--
Yes	34	7	20.6	0.6	0.27	
Raw milk product consumption						
No	202	65	32.2	1		--
Yes	116	26	22.4	0.6	0.065	

Dried meat consumption						
No	205	60	29.3	1		--
Yes	113	31	27.4	0.9	0.73	
Raw liver consumption						
No	292	81	27.7	1		--
Yes	26	10	38.5	1.6	0.25	
Half-cooked liver consumption						
No	179	42	23.5	1		--
Yes	138	48	34.8	1.7	0.027	
Newborn						
No	122	36	29.5	1		--
Yes	195	55	28.2	0.9	0.80	
Direct contact to aborted fetus/retained placenta						
No	168	43	25.6	1		--
Yes	150	48	32	1.4	0.21	
Feeding dog abortions/placenta						
No	193	57	29.5	1		--
Yes	125	34	27.2	0.9	0.65	
Dumping of abortions/placenta						
No	283	80	28.3	1		
Yes	35	11	31.4	1.2	0.7	
Sheep abortions						
No	98	26	26.5	1		--
Yes	139	39	28.1	1.1	0.79	
Goat abortions						
No	93	21	22.6	1		--
Yes	145	45	31	1.5	0.16	
Cattle abortions						

No	217	62	28.6	1		--
Yes	20	3	15	0.4	0.20	
Horse abortions						
No	225	62	27.6	1		--
Yes	12	3	25	0.9	0.85	

Human Risk Factors

The higher risks for students compared to herders was difficult to interpret. The higher risk of women may be explained by their direct contact with weak new borns, feeding them at home and milking livestock during the lactation period. In contrast to a study in Kyrgyzstan (Bonfoh et al. 2012), we could not demonstrate a significant relationship between human and animal seroprevalence at the nomadic camp level. This may reflect complex contact networks which blur the detection of human-animal transmission at the nomadic camp level. In Zavkhan province, small ruminants had a higher seroprevalence than in Sukhbaatar province. This may be explained by differences in husbandry systems and local tradition. More research is needed using molecular typing of circulating strains to ascertain the main transmission pathways in livestock and to humans.

The national brucellosis mass vaccination programme was officially implemented from 2000 to 2009. The government of Mongolia did not consider the livestock number when the mass vaccination budget was planned in 1999. The livestock numbers doubled during the intervention period but the number of vaccine doses was never adjusted for the livestock number since 2000 (Shabb et al.2013). Based on structured questionnaires on livestock health information, 163 out of 169 nomadic camps reported that brucellosis vaccine was never offered to their herds by the local veterinarian last 3 years. Only one nomadic camp from Zavkhan province was included in the brucellosis vaccination programme in 2009. There was not enough vaccine, and there was a lack of monitoring of the vaccination coverage and quality control of the implementation of national vaccination programme at the village level. The vaccination coverage rate (35%-53%) did not reach the critical immunization rate during the mass vaccination programme period from 2000 to 2003 year (Roth et al.2003).The quality of the two brucellosis vaccines, in terms of viable colony forming units, and cold chain were never assessed during the mass vaccination period.The poor performance of the past brucellosis mass vaccination campaign is certainly one of the reason for high seroprevalence of human brucellosis found in the study. Based on known vaccination coverage and basic reproductive number, it can be effectively interrupted (Zinsstag et al. 2005). If the coverage was found to be too low in some areas, then revaccination of those areas could be considered. Future mass vaccination campaigns must include an assessment of vaccination coverage for each animal species. The quality of the brucellosis vaccine and the cold chain should be followed up continuously. Independent vaccination coverage surveys help to monitor effectiveness of the mass vaccination. Pro-

vincial level of veterinarians and medical doctors has been trained on basic epidemiological concepts and methods implementing such coverage surveys for the ongoing mass vaccination campaign since 2011.

Table 7. Invariable analysis of risk factors of human seropositivity in Zavkhan province

	Univariable				
	n	**pos**	**%**	**OR**	**p-value[a]**
Soum					
Durvuljin	96	18	19.6	1	
Ider	34	11	32.3	2.1	0.22
Ikh-Uul	88	24	27.2	1.6	0.30
Tosontsengel	38	11	30.5	2.0	0.22
Age (years)					
<10 yrs	1	0	0	-	-
10 - < 15 yrs	10	3	30	1.5	0.6
15 - <20 yrs	6	0	0	-	-
20 - <45 yrs	142	40	28.2	1	
≥ 45 yrs	91	21	23.1	0.8	0.41
Sex					
Male	120	36	30	1	
Female	130	28	22	0.6	0.18
Occupation					
Herder	218	55	25.4	1	-
Student	16	3	18.7	0.8	0.76
Other	22	6	27.3	1.1	0.94
Raw milk consumption					
No	234	61	26.0	1	
Yes	14	2	14.3	0.53	0.45
Raw milk product consumption					
No	118	30	25.4	1	

Yes	130	33	25.3	1.0	0.99
Dried meat consumption					
No	242	62	25.6	1	
Yes	5	0	0	1.4	1
Raw liver consumption					
No	246	62	25.8		
Yes	8	0	0		--
Half-cooked liver consumption					
No	124	32	25.8	1	
Yes	124	31	25.0	0.97	0.93
Keep new-borns livestock in dwelling place					
No	199	51	25.6	1	
Yes	47	12	25.5	1.1	0.77
Direct contact to aborted foetus/retained placenta					
No	102	25	24.5	1	
Yes	146	37	25.3	0.9	0.79
Feeding dog abortions/placenta					
No	146	36	24.6	1	
Yes	104	28	26.9	1.0	0.96
Dumping of abortions/placenta					
No	208	56	26.9	1	
Yes	42	8	19.0	0.55	0.251
Sheep abortions					
No	114	30	26.3	1	
Yes	109	28	25.6	1.1	0.79
Goat abortions					
No	155	40	25.8	1	
Yes	68	18	26.4	1.1	0.83
Cattle abortions					
No	178	48	26.9	1	

Yes	45	10	22.2	0.38	0.65
Mare abortions					
No	211	55	26.0	1	
Yes	12	3	25.0	0.88	0.87

5.6 Study Limitations

There are several limitations to this study. The first immune-response proves exposure to *Brucella* (or cross reacting bacteria) but not necessary infection.

Brucellosis serology is difficult to interpret due to the absence of perfect tests. The OIE guidelines for brucellosis state that RBT and ELISA are suitable screening tests at the local and national level. The RBT and ELISA should be validated in the country level using culture positive livestock sera and negative livestock sera from the national collection of reference sera. RBT and ELISA are currently not validated in Mongolia because of the lack of culture positive and negative sera. There may also have been temporal variantions in pathogen exposure and clinical symptoms that were not captured by the cross-sectional study design.

5.7 Conclusion

Our study reports a high seroprevalence of human brucellosis in Mongolia and indicates that the annually reported cases are significantly under-reported. Herders have limited access to brucellosis diagnosis at the district level which prevents them from receiving an adequate treatment. Human brucellosis can only be effectively controlled if livestock mass vaccination is implemented at high coverage. This is in compliance with the guideline of World Organization for Animal Health (OIE). The Mongolian government must implement effective monitoring of vaccination coverage and quality control of the implementation of mass vaccination programmes at the district and village level. Mass vaccination should be accompanied by educational and communication programmes.

The study results already influenced the public health policy of the MoH regarding the case definition and diagnosis of human brucellosis at the primary and secondary health care levels in the country. The MoH is currently revising the national standards for human brucellosis diagnosis and treatment procedures. The human and livestock linkage of brucellosis transmission was investigated by molecular typing of *Brucella* spp. collected and is reported separately. The MoFA started implementing a vaccination coverage survey after the mass vaccination in 2012.

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5.9 Disclaimer

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6 Seroprevalence Survey of Brucellosis Among Rural Reople in Mongolia

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6.1 Abstract

Background: After the transition from socialism to a market economy in 1990, human brucellosis re-emerged in Mongolia. The aim of our study was to estimate a representative seroprevalence of *Brucella* spp. and to determine risk factors for brucellosis seropositivity among rural people.

Methods: A cross-sectional study with multistage random selection was conducted in eight provinces of Mongolia. Study participants were interviewed using a questionnaire to obtain their brucellosis history, current symptoms and likely risk factors. Blood samples were drawn to determine brucellosis seroprevalence using the Rose Bengal Test.

Results: A total of 2,856 randomly selected rural people aged four to 90 years were enrolled in the study. The seroprevalence of *Brucella* spp. was 11.1% (95% confidence interval [CI] 10.0-12.1), ranging between 2.3% and 22.6% in the eight provinces; 39.2% (n=*609*) of nomadic camps had at least one seropositive participant. Risk factors associated with brucellosis seropositivity were being older than 45 years old (adjusted odd ratio [AOR] =6.9, 95% CI 5.1-8.7) being a veterinarian (AOR = 6.9, 95% CI 1.5-5.0).

Conclusion: Our study confirms that human brucellosis seroprevalence among rural people in Mongolia is high. Human brucellosis can be effectively controlled if high-coverage livestock mass vaccination is implemented with coverage survey after the vaccinations to ensure completeness. This mass vaccination should be accompanied by public awareness and educational programme.

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6.2 Introduction

Brucellosis is a zoonosis, and the infection is almost invariably transmitted by direct or indirect contact with infected animals or their products. It is an important human disease in many parts of the world, especially in the Mediterranean countries of Europe, North and East Africa, the Middle East, South and Central Asia and Central and South America.[1]

Brucellosis us caused by members of the *Brucella* genus. Transmission of infection to humans occur through breaks in the skin, following direct contact with tissues, blood, urine, vaginal discharges, aborted fetusus or placentas. [2]
The most frequent symptoms of brucellosis are fever, chills or shaking, malaise, generalized aches and pains all over the body, joint and low back pain, headaches, anorexia, easy tiredness and general weakness.[3]

Mongolia has the second highest incidence of human brucellosis worldwide; another seven republics of the former Soviet Union included in the 25 countries with the highest incidence. According to data from the National Statistical Office of Mongolia, a rapid increase in notified cases observed between 1990 and 2000. This increase may have been the result of the evolution from a socialist state to a free market economy which led to the loss of rigorous livestock control [4]. During this period, changes to the health system precluded early recognition of the disease or interventions that considered the emerging trends in humans and animals. [5] In Mongolia, factors contributing to the incidence of brucellosis include traditional eating habits, standard hygiene measures, and methods for processing milk and its products and rapid movement of animals.[3]

In 2011, a national brucellosis survey was conducted that sampled 168,027 head of livestock from 11 528 nomadic camps (two or more than four herder families that share the same pasture and water source) of 337 districts of 21 provinces. [6] Twenty-one provinces, 57.3% of all districts and 8.0% of all nomadic camps had seropositive livestock including camels, cattle, sheep and goats. Livestock seroprevalence was found in 0.7% of camels, 1.8% of cattle, 0.7% of sheep and 0.5% of goats using parallel interpretations of Rose Bengal Tests (RBT), complement fixation tests and competitive enzyme-linked immunoabsorbent assay (ELISA).[6]

The aim of our study was to estimate the seroprevalence of *Brucella* spp. and to determine risk factors for brucellosis seropositivity among rural people.

6.3 Methods

6.3.1 Study design and population

Eight provinces were selected for the cross-sectional surveys. Between June and September 2010 surveys were conducted in Sukhbaatar and Zavkhan provinces, selected for convenience. [7] Between November 2011 and January 2012, the same surveys were conducted in a further six provinces: Arkhangai, Khuvsgul, Selenge, Uvs, Umnugovi and Govi-Altai (Figure 1). In each province, four districts were selected using randomization in Excel (the rand () command). Twenty nomadic camps and four to five individual participants were randomly selected based on the required sample size.

The cluster sample size calculation as described elsewhere [7] assumed a human brucellosis seroprevalence among rural people of 20%. [8] In addition, the number of cluster and number of individuals per cluster was optimized according to the feasibility and the available budget.

The study was approved by the Ethics Committee of the Health Science University of Mongolia and the Ethics Committee of the Canton of Basel of Switzerland. All participants were informed about the study and what they could expect regarding diagnosis, reporting and treatment; all signed a consent form. A child younger than 16 years of age was included in the study with signed consent from of his/her parents.

6.3.2 Data collection

Study questionnaire

All study participants were interviewed using a questionnaire which included demographics, risk factors and clinical symptoms for brucellosis. The questionnaire was pre-tested during the 2010 study in Sukhbaatar and Zavkhan[7] and revised for the extended study to improve understanding of questions and to eliminate overly-sensitive questions.

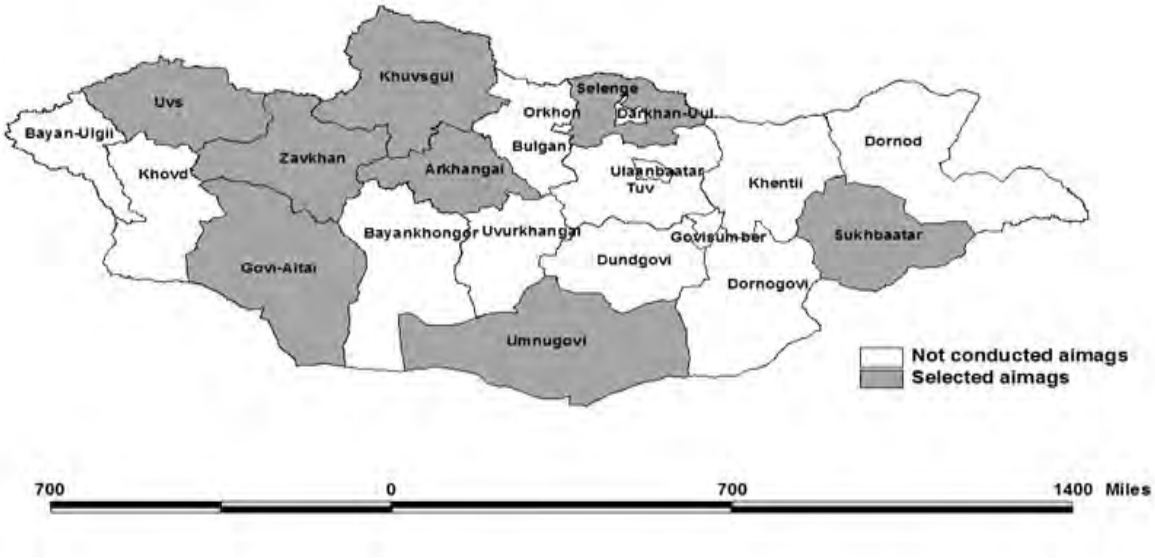
Blood sample collection and handling

Venous blood was taken with 5 ml Vacutainer® tubes. The blood samples were centrifuged in 3000 round/minute for five minutes. Separated 1.5 ml tubes of serum were kept in a cool box and transported to the provincial laboratories for storage and cooling before shipment to the serological laboratory of the National Centre for Communicable Diseases of Ulaanbaatar, where they were tested for brucellosis.

Serological test

Sera were tested with the RBT for detection of antibodies to Brucella abortus/melitensis from the Tulip Diagnostic Ltd (Bambolim, India). Positive sera were re-tested with the RBT using ½ to 1/32 dilutions, 9 and with enzyme immunoassay for the qualitative determination of IgG class antibodies against Brucella from NovaTec Immundiagnostica GMBH (Dietzenbach, Germany). ELISA test was performed according to manufacturer's instruction.

Figure 1. Map of Mongolia by province highliting provinces where study was conducted



Data entry and statistical analysis

All data were double-entered in Access-2007, compared in Epi Info ™ 3.5 to correct entry errors and analysed using Stata 10.1 (StataCorp LP, USA). Study particpants who tested positive by either ELISA or RBTS were considered seropositive by statistical analysis.

To assess the association between risk factorts and human brucellosis seropositivty we used Person χ^2 or Fisher's exact tests for explanatory variables such as demographics, behaviour related risk factors and reported clinical symptoms. We also conducted univariate logistic regression using a binary serological outcome with the xtgee command and random effect on the nomadic camp level. A miltivarate logistic regression model with (random effectr at the nomadic camp) using backward stepwise selection and a removal for covraiates at P=0.10 based on the likelihood ratio test was then constructed. Variables with *p* value less then 0.05 in the univariate analysis were included in the multivariate model.

To determine the proportion of the general population seroconverting each year due to brucellosis exposure, the seroprevalence data were divided by the duration of seropositivity, assumed to be 10.9 years.[10] Using a conservative estimate of 20% of seroconversions representing true clinical cases (note that among all seropositives detected, 58.5% had at least two symptoms at time of interview) these proportions were multiplied by 0.3 and converted to rates per 100,000 person years for the general population.

Table 1 Number of participants seropositive for *Brucella* spp. *by province and district, Mongolia 2010 to 2012

Province	Number of districts surveyed	Number of participants	Seropositives	% of positivity	Confidence Interval
Khuvsgul	4	400	46	11.5	8.72–14.2
Umnugovi	4	400	49	12.3	9.64–14.9
Govi-Altai	4	398	30	7.5	4.17–10.8
Selenge	4	391	60	15.3	12.9–17.6
Arkhangai	4	400	9	2.3	0.45–9.15
Uvs	3	293	17	5.8	1.27–10.3
Sukhbaatar	4	318	72	22.6	20.5–24.6
Zavkhan	4	256	33	12.9	9.7–16.1
Total	31	2856	316	11.1	10.0–12.1

*based on parallel interpretation of the RBT and ELISA test

6.4 Results

There were 2,856 participants from 609 nomadic camps from 31 soums in the eight selected provinces between 4 to 90 years of age (median 38 years). This included 2,260 (79.1%) herders, 142 (5.0%) students, 96 (3.4%) office workers, 70 (2.45%) workers, 37(1.3%) retired people, 20 (0.7%) veterinarians, 18 (0.63%) entrepreneurs, 16 (0.56%) unemployed adults, 13(0.5%) children under 6 years and 184 (6.4%) other residents.

Seroprevalences

The seroprevalence of *Brucella* spp. among participants was estimated at 11.1% (95% CI 10.0 – 12.1) ranging from 2.3% to 22.6% between the eight provinces (**Table 1**) and 4.1% to 43.8% in the 28 districts. Within nomadic camps, 39.2% (95% CI 38.2-41.0) had at least one to four seropositive members (Table 2).This equated to an annual incidence of seroconversion of 1145 per 100 000 and an overall annual incidence of 229 clinical cases per 100 000.

Seroprevalences was higher in females than in males (11.2% compared with 10.9%, P=0.46). By age group, the highest seroprevalence was found in those 45 years and above at 15.5% (95% CI 13.9-17.0), with the lowest in the four to 10 year age group at 2.6% (95% CI 1.5-20.4). All occupation categories included seropositive cases ranging between 2.8% and 30.0%.

Analysis of risk factors for brucellosis

Risk factors associated with being seropositive by univariate analysis included: being 45 years old and above (odds ratio [OR] = 6.6, P=0.046), being a veterinarian (OR = 3.5, P=0.016), contacted with aborted animal fetus and placentas (OR = 1.35, P=0.016) and comsumption of undercooked liver (OR=1.51, P=0.001) (**Table 3**).

In the multivariate analysis, only two variables remained associated with being seropositive: being 45 years old and abobe (adjusted odds ration [AOR]=6.9, 95% CI 5.1-8.7) and being a veterinarian (AOR=2.8, 95% CI 1.5-5.0). Among veterinarians who participated in the study, 72.7% assisted in livestock obstetric work, and 50% had direct contacted with aborted animal fetus and placentas. The risk factor for veterinarians was also much higher compated with other occupations (P<0.001).

Table 3. Univariate analysis of risk factors of brucellosis seropositivity* in Mongolia, 2010 to 2012

Characteristic	Number of participants	Number seropositive (%)	OR (95% CI)	*p* value
Age group (years)				
4–9	39	1 (2.6)	1.0	-
10–14	69	4 (5.8)	2.3 (1.2–4.1)	0.440
15–19	96	3 (3.1)	1.2 (0.6–2.7)	0.864
20–44	1769	171 (9.7)	3.9 (1.2–7.6)	0.151
≥45	883	137 (15.5)	6.6 (4.5–10.2)	0.046
Sex				
Males	1181	132 (11.2)	1.0	-
Females	1675	184 (10.9)	1.0 (0.9–1.2)	0.968
Occupation				
Herder	2260	263 (11.6)	1.3 (0.9–2.5)	0.087
Student	142	4 (3.0)	0.9 (0.3–2.5)	0.345
Office worker	96	7 (7.3)	0.7 (0.2–1.6)	0.267
Worker	70	7 (10)	0.9 (0.5–2.0)	0.733
Retired	37	7 (18.9)	2.0 (0.8–4.2)	0.112
Veterinarian	20	6 (30.0)	3.5 (1.6–7.9)	0.016
Entrepreneur	18	4 (22.2)	2.3 (1.0–4.6)	0.119
Unemployed	16	1 (6.3)	0.5 (0.3–1.3)	0.521
Children under six	13	1 (7.7)	0.7 (0.3–1.6)	0.708
Other	184	16 (8.7)	0.8 (0.4–1.7)	0.328
Risk factors				
Animal obstetric work	778	93 (11.9)	1.5 (0.9–2.5)	0.121
Contact with aborted animal fetuses and placentas	769	104 (13.5)	1.4 (1.0-2.1)	0.016
Consumption of raw milk	295	32 (10.8)	1.2 (0.9–1.8)	0.546
Consumption of raw liver	38	11 (28.9)	0.8 (0.5–1.2)	0.612
Consumption of undercooked liver	1067	146 (13.7)	1.5 (0.9-4.3)	0.001
Consumption of fresh animal blood	143	12 (8.4)	1.5 (1.0–1.7)	0.332

OR: odds ratio; CI: confidence interval * Based on parallel interpretation of RBT and ELISA

History of human brucellosis and clinical symptoms

Of the study participants, 2.7% (*n*=76) reported receiving treatment for human brucellosis in the past; median time since past brucellosis treatment was 14 years (Q1=3.3 and Q3=20 years). With the exception of testicular pain, there were significant differences between age groups in reporting clinical symptoms; the age group of 20 to 44 years and 45 years and above reported more clinical symptoms for human brucellosis. Females also reported more headaches; joint, back and muscle paon; weakness and sleeping disturbance than males **(Table 4)**.

Reported clinical symptoms at the time of the study were compared to the serostatus of participants. Overall, 165 of the 316 (52.2%) brucellosis seropositive participants and 1186 of the 2540 (46.7%) seronegative participants reported symptoms. Among all seropositives, 36.7% reported more than three symptoms (P<0.001). Headache; joint, back, and muscle pain; night sweats and sleeping disturbances were significantly associated with brucellosis seropositivity (**Table 5**).

Table 4. Reported clinical symptoms among study participants by age group and sex, Mongolia, 2010 to 2012 (*N* = 2856)

		Age group						Sex		
Symptoms	**n**	**0-9**	**10–14**	**15–19**	**20–44**	**≥ 45**	***p* value***	**Male**	**Female**	***p* value***
		%	%	%	%	%		%	%	
Fever	135	0.7	1.6	0.7	52.6	44.4	0.009	3.8	5.4	0.053
Headache	1268	0.3	0.7	2.0	57.9	39.1	<0.001	34.3	51.8	<0.001
Joint pain	1287	0.4	0.5	1.5	50.7	46.9	<0.001	38.7	49.5	<0.001
Back pain	1351	0.1	0.4	1.4	57.6	40.5	<0.001	43.6	49.8	<0.001
Muscle pain	590	0.5	1.0	1.0	46.4	51.1	<0.001	14.9	24.7	<0.001
Weakness	964	0.3	0.3	0.4	50.7	48.3	<0.001	26.9	38.6	<0.001
Night sweats	336	0.9	0.6	0.6	45.8	52.1	<0.001	11.4	12.0	0.812
Sleeping disturbance	530	0.2	-	0.4	42.3	57.1	<0.001	14.5	21.4	<0.001
Weight loss	233	1.3	1.3	1.3	40.7	55.4	<0.001	7.2	8.8	0.115
Miscarriage	31	-	-	-	90.3	9.7	0.015	-	100.0	<0.001
Testicular pain	10	-	-	-	50.0	50.0	0.749	100.0	-	<0.001

Table 5. Reported clinical symptoms by sero-status among study participants, Mongolia, 2010 to 2012 (*N*=2856)

Clinical symptoms	Category	Number of participants	Number sero-positive (%)	*p* value
Fever	No	2721	301 (11.0)	
	Yes	135	15 (11.1)	0.561
Headache	No	1588	167 (10.5)	
	Yes	1268	149 (11.7)	<0.001
Joint pain	No	1569	155 (9.9)	
	Yes	1287	161 (12.5)	0.014
Back pain	No	1505	151 (10.0)	
	Yes	1351	165 (12.2)	0.038
Muscle pain	No	2266	234 (10.3)	
	Yes	590	82 (13.9)	0.009
Weight loss	No	2623	287 (10.9)	
	Yes	233	29 (12.4)	0.379
Weakness	No	1892	194 (10.3)	
	Yes	964	122 (12.7)	0.058
Night sweats	No	2520	266 (10.6)	
	Yes	336	50 (14.9)	0.013
Sleeping disturbance	No	2326	242 (10.4)	
	Yes	530	74 (13.9)	0.010
Abortion	No	1644	182 (11.1)	
	Yes	31	2 (6.4)	0.713
Testicular pain	No	1171	131 (11.3)	
	Yes	10	1 (10.0)	0.620

6.5 Discussion

We report a seroprevalence of *Brucella* spp. among rural people of 11.1% (with a range between provinces from 2.3% to 22.6%) and an annual incidence of 229 per 100 000. This high incidence reflects an increase in human brucellosis after the transition in Mongolia from socialism to a market economy leading to livestock privatization and collapse of the veterinary sector.[4]

Although several earlier studies also estimated the seroprevalences of *Brucella* spp. in Mongolia among high-risk people including herders, veterinarians and raw animal processing technicians,[11-14] these differed from our study in time, study design and methodology and should not be compared. The result from our study was higher than the 0.1 to 10.1% reported among high-risk people in other countries, [10.15-21] which is not surprising as Mongolia is ranked second in the world for brucellosis incidence.[5]

We also estimated a much higher incidence compared with that reported from notification data, [22] despite the fact that we have taken conservative assumption that 20% of seropositive cases are clinical cases.

According to the multivariate analysis, adults aged 45 years and above and veterinarians had a higher risk for brucellosis. This age group plays important role in the livestock herding and birthing, and veterinarians have direct contact with animals and aborted materials when doing veterinary examinations. We also found seropositives in all age groups, including in young children (four to

nine years), which may indicate ongoing exposure and transmission of brucellosis in rural Mongolia. These groups should be targeted with material about protection against brucellosis infection.
This study will serve as a baseline of the seroprevalence of Brucella spp.in rural people in Mongolia before the implementation of a nationwide livestock vaccination campaign; it also will be used for ongoing brucellosis surveillance. A decrease of human incidence and repated sero-surviellance surveys in humans will indirectly assess the efficacy of the vaccination campaign in livestock.[23]

There are several limitations to the study. First, association between human and livestock seropositivty was not assessed in provinces (with exception of Zavkhan and Sukhbaatar [7]). There are also may have been temporal variantions in risk factors for childhood brucellosis, interpretation of reported clinical symptoms for brucellosis based on seropositivity and pathogen exposure that were not captured by the cross-sectional study design.

6.6 Conclusion

Our study confirms that human brucellosis seroprevalence among rural people in Mongolia is high and that the incidence is much higher than the notification data suggests. As recommended by the Food and Agriculture organization of the United Nations, the World Animal Health Organization (OIE) and World Health Organization, mass livestock vaccination is required in Mongolia in the nomadoc livestock production system.

Safety measures to avoid brucellosis include wearing protective clothes such as gloves, using metal hooks to collect aborted foetuses and placentas for burial or burning, washing hands after handling livestock and completely cooking liver from small runinants. This information should be included in educational materials to prevent as many as possible new cases, especially at the beginning of the mass vaccination campaign while strains still circulate. We have developed written and pictorial educational materials mainly for children. The literacy rate in Mongolia is extremely high and thus printed media are appropriate. In parallel, the surveillance, treatment and diagnostic capacities for human brucellosis must be increased in provinces and districts. Education and awareness programmes are should be implemented particularly before the livestock birthing season.

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6.8 Acknowledgements

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7 Investigation of Human and Livestock *Brucella*. spp Isolates in Mongolia using Multi Locus Variable number of tandem repeat Analysis (MLVA-16) Method

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7.1 Abstract

Brucellosis is one of the most common zoonotic diseases found worldwide with around 500.000 human cases reported annually. In Mongolia, human brucellosis became an issue during the 1960s and since1975, a vaccination strategy of small ruminants and cattle has successfully reduced human brucellosis from 48 cases to less than 0.2 cases per 100,000 at the end of the 1985. After 1990, human brucellosis re-emerged due to the severe decline in medical and veterinary services and lack of monetary resources during the post-communist transition period. By 2003, Mongolia was ranked the second highest in terms of human brucellosis cases worldwide. It is therefore surprising, that despite the high prevalence of brucellosis, there has been hardly any genetic characterization of brucellosis in the country. In this study we characterized 58 isolates of *B.melitensis* and *B.abortus* from humans and livestock within five provinces of Mongolia using a 16 Multi Locus Variable number tandem repeat Analysis (MLVA-16).

The 58 Mongolian strains were genetically more diverse when compared with Central Asian strains. Human strains were most closely associated to *B.melitensis* strains from sheep and goats. To the best of our knowledge, this is the first report on MLVA-16 characterized *Brucella* spp. strains from Mongolia. MLVA-16 has a high potential to improve brucellosis surveillance and trace back to outbreaks which occurred during the national control program from 2010 to 2021.

Key words: Brucella, Mongolia, human, livestock, genotyong, MLVA-16

Manuscript is ready to be submitted to the Journal of Clinical Microbiology

7.2 Introduction

Brucellosis is the most common zoonotic disease in the world. Brucellosis is endemic in livestock and a source of human diseases in Africa, Central America, Central Asia, the Mediterranean region and the Middle East [1-4].

Brucellosis is caused by bacteria of the genus *Brucella* which circumscribe ten recognized species. Ovine and caprine brucellosis are predominantly caused by *Brucella melitensis*, while bovine brucellosis caused by *Brucella abortus*. Cross species infections do occur between different breeds of domestic animals when kept in close contact [5, 6]. *B.melitensis* and *B.abortus* are highly pathogenic and are transmitted to humans through direct contact with infected animals, abortion material, blood, raw milk and unpasteurized dairy products of infected livestock [7]. *B. melitensis* causes the most important clinically apparent disease in humans [8]. It is estimated that half a million human cases are reported annually in the worldwide each year. However, it is estimated that at least a quarter of human cases go unreported due to unspecific and undiagnosed symptoms of the human disease [9]. The disease causes a debilitating and disabling illness in humans causing major economic consequences due to lost time at work due to illness, loss of animal production and reduced survival of new-born animals. The elimination of brucellosis is only possible by intervening in the target animal reservoirs [10].

In Mongolia, traditional extensive nomadic livestock systems are maintained with different species of domestic ruminants beingt kept in variably sized flocks are kept. These herds move continuously depending on the availability of pasture land according to the climate and breeding season. Mongolian herders have a particular habit to consume partially cooked liver, unpasteurized cheese and cream. Herders also keep traditional practices of slaughtering animals at home for household consumption, providing obstetric assistance during lambing and feeding of weak new-born lamb and kids in their ger (traditional mobile house) during cold weather [6, 7].

In Mongolia, the first animal brucellosis was documented in 1935 and the first cases of human brucellosis were officially reported in 1949. During the 1960ies, human brucellosis seroprevalence was 45% among herders and 16% among abattoir, wool, cashmere and hide factory workers [11]. As a result, the government of Mongolia implemented an “elimination” program based on a test-and-slaughter strategy during the late 1960s. This strategy was not practical for Mongolian pastorlists because it was impossible to restrict animal movement and the normal continuous sharing of the same pasture lands and water points [12].

Since 1975, a mass vaccination strategy has been implemented by the government of Mongolia using Rev.1 vaccine for small ruminants. Over 30 million small ruminants were vaccinated during the ten years vaccination campaign. As a result, human brucellosis declined from 48 cases (1974 year) to 0.23 cases per 100,000 population in1985.The small ruminant’s seroprevalence result was below 0.01% by 1985[12].

During the 1990s, Mongolia experienced a political and economic transformation via democratic reform and privatisation. During this transition, medical and veterinary services weakened due to lack of resources during the post-communist transition period and government-run livestock disease surveillance and control programs ceased to function [2]. Human brucellosis re-emerged reports detailed imcreases in human brucellosis from 4.9 cases to 67 cases per 100, 000 population in 1999 [13]. By 2003, Mongolia was ranked the second highest in terms of reported human brucellosis cases worldwide and national survey result showed that 20% among herders [4]. Human brucellosis cases are still significantly under reported due to lack of diagnostic facilities in the village health centres [14-16].

A recent study found an apparent seroprevalence of brucellosis in 27.3% among herders, 6.2% in sheep, 5.2% in goats, 16% in cattle, and 2.5% in camels in Sukhbaatar and Zavkhan provinces in 2010 [16]. In 2011, national brucellosis surveillance conducted sampling 168 000 head of livestock. National brucellosis surveillance result showed that seroprevalence was 0.53% in goats, 0.72% in sheep, 1.8% in cattle/yaks and 0.74% in camels [17].The overall apparent seroprevalence among rural Mongolian people was estimated at 11% ranging from 2.3% to 22.6% in eight provinces when calculated an annual incidence of seroconversion of 1145 per 100,000 and an overall annual brucellosis human incidence was 229 clinical cases per 100,000 population [18].

Since 2000, Mongolia expanded its natiowide mass vaccination of small ruminant to include cattle and the mass vaccination program has been extended until 2021 [19, 20]. To implement vaccination program the country has been divided into three sections based on number of livestock, the production capacity of required amount of S19 and Rev.1 vaccine doses, and personnel capacity. Within the first section, 14.2 million small and large ruminants were vaccinated in 2011 and 23.2 million livestock were vaccination the following year in the second section. In 2013, 6.6 million livestock were vaccinated third section which locates in Eastern part of Mongolia. All large and small ruminants of the three zones are to be vaccinated every second year until 2020[14, 17].

In Mongolia, most human brucellosis cases are caused by *B. melitensis* the dominant species, but also by *B.abortus* [21-22]. *B. melitensis* biovar 2 has been found in humans and *B. abortus* biovar 3 found in cattle. In 2008, this was confirmed by the international brucellosis reference laboratory in the United Kingdom [23-24]. However, routine identification of *Brucella* spp. and biotyping work has been restricted due to limited resources, laboratory facilities and lack of trained personnel [15]. Therefore, the main reservoir hosts and transmission dynamics within livestock species and from livestock to humans are not well understood in Mongolia.

Further characterization of *Brucella* genus field strains by Multi Locus Variable number of tandem repeat Analysis (MLVA-16) assay is important to better understand the epidemiology of the human and animal brucellosis and to trace the sources of infection. Such information will enhance the on-

going brucellosis elimination program [25, 26].The objective of the study was to characterize Mongolian strains using variable number tandem repeats (VNTR) and to compare results to genotypes from isolates of Central Asia and other regions. Full sequences of the isolated strains are published elsewhere.

7.3 Material and Methods

Ethics Statement

The study was approved by the Ethics Committee of Health Science University, Mongolia (ref. №15/1A/2011) and Scientific Committee of Veterinary Research Institute, Mongolia (ref. № 256/2010). In Switzerland, approval was given by the Ethics Commission of the Cantons of Basel-Stadt and Basel-Land (Ethikkommission beider Basel) (ref.169/10) and the Research Commission of the Swiss Tropical and Public Health Institute of Basel, Switzerland. Informed written consent was obtained from all human participants. Herders gave oral consent to take livestock samples and collect aborted fetuses.

Livestock sample collection

Livestock samples were collected from Khentii, Sukhbaatar and Dornod provinces of Mongolia from 2011 to 2013. Based on an earlier serological study [16], the field team again visited herder families who had positive serology test results between years 2011 to 2013. With the permission of the herders, the team collected aborted fetuses, vaginal discharge from female animals' recently aborted, collected blood and milk samples. The team dissected aborted fetuses and collected stomach contents in sterile tubes; spleen, liver, lung, and lymph nodes put into plastic bags at the provincial veterinary laboratory. Vaginal swabs were transported in the BD BBL™ Culture swab plus, Amies Medium without Charcoal (Becton Dickinson, France). Milk samples were collected in sterile tubes. All samples were kept in cool boxes during transportation to the Veterinary Research Institute (VRI) in Ulaanbaatar, Mongolia.

Human sample collection

Since 2011, medical doctors have explained the purpose and procedures of diagnosis, reporting and treatment to brucellosis patients admitted to the inpatient hospital of National Centre for Communicable Disease (NCCD), Ulaanbaatar, Mongolia. All study participants signed a written consent form. For children younger than 16 years old, parents or guardians gave the permission and signed the consent form. Study participants were interviewed using a structured questionnaire to obtain demographic and brucellosis exposure information. Blood samples were taken from participants according to the national standard. *Brucella* spp was cultured from six patients. In addition, six human isolates previously collected at the NCCD between years 2000 to 2009-were included in the study.

Culture

All samples were cultured in a biosafety level III laboratory at the Veterinary Research Institute. Human blood samples were cultured in biphasic method Castaneda which used both solid and liquid medium in the same container. Samples incubated at 37°C with 5-10% of CO_2 for 1-6 weeks. In addition, six human isolates previously collected at the NCCD between years 2000 to 2009-were included in the study.

Livestock samples such as milk, stomach contents, various internal organs and vaginal swabs were cultured on solid media. The modified Thayer-Martin's medium prepared using Blood Agar Base №2 (CM 0171, Oxoid AG, Basel, Switzerland) with 5-10% v/v inactivated horse serum (SR0035, Oxoid AG, Basel, Switzerland) and *Brucella* spp selective antibiotic supplement (SR0083 Oxoid AG, Basel, Switzerland).
Also used were, Farrell's medium using Brucella medium base (CM 0169, Oxoid AG, Basel, Switzerland) with 5-10% v/v inactivated horse serum (SR0035, Oxoid AG, Basel, Switzerland) and *Brucella* spp selective antibiotic supplement (SR0083 Oxoid AG, Basel, Switzerland). All samples were cultured on the modified Thayer-Martin and Farrell's medium in parallel at 37° C with 5-10% of CO_2 for 2-5 days. Seven *B.melitensis* field strains were isolated from sheep in 2009 from Khentii province were included in the study.

DNA extraction and genotyping

Extraction of DNA was performed with the QIAamp DNA Mini Kit (Qiagen, Germany) according to the manufacturer's instruction. Multiplex PCR (Bruce-ladder), using eight primer pairs in a single reaction was used determine *Brucella* spp according to the protocol [27].
Samples were genotyped using the Multiple Locus Variable number of tandem repeat Analysis (MLVA) 16 panels proposed by Le Fleche and modified with another tandem repeat locus bruce19 [26]. Protocols are available online on the MLVA-NET for *Brucella* (http://mlva.u-psud.fr). In brief, two panels of primers were used; one comprising eight minisatellite markers (panel 1) which were used for species identification and a second group of eight microsatellite markers showing a higher discriminatory power. The latter part comprised two groups; panel 2A and 2B. Panel 2B contained the most highly variable markers; bruce04, bruce07, bruce09, bruce16 and bruce30 [26].
The PCR was performed with total volume of 30 µl containing approximately 10 µl reaction buffer, 10 µL *Taq* DNA polymerase (Roche, Mannheim, Germany), 100 µm of each deoxynucleotide triphosphate and 3 µl of each flanking primers (Sigma Aldrich, St. Gallen, Switzerland). The amplification was run in a Biolabo thermocycler as described previously [29].
The amplicons were separated by electrophoresis on 2.5% agarose gels to analyse tandem repeats. A 100 base pair ladder (Promega, Mannheim, Germany) was used as a molecular size marker. DNA extracts of *B. melitensis* 16 M and *B. abortus* S544 were used as positive controls.

Sequencing

A subsample of the amplicons was sequenced for confirmation of the band sizes (Table S1). Direct sequencing of the PCR products was performed with an automated sequencer ABI Prism 3100 genetic analyser (Applied Biosystems, Foster City, CA, USA) with the BigDye Terminator cycle sequencing kit according to the manufacturer's instruction (Applied Biosystems, Foster City, CA, USA). The length of the sequenced locus was analysed using the Sequencher ® version 5.0 sequence analysis software (Gene Codes Corporation, Michigan, USA).Sequencing results are provided in the Supplementary data table S1.

Data analysis

Allelic diversity was calculated using the formula below;

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

where x_i is the relative frequency of the *i*th allele of the locus, *n* the number of isolates in the sample [n/ (n-1)] is a correction for small samples. VNTR data were used for the cluster analysis using SAS™ (Statistical Analysis System Inc. Cary, USA) proc cluster using the unweighted pair-group method with arithmetic averages (UPGMA) [26]. Hunter-Gaston diversity index (HGDI) was calculated for every locus using online tool V-DICE available at the HPA website (http://www.hpa-bioinformatics.org.uk/cgi-bin/DICI/DICI.pl) [30].

For assessment of the phylogenetic place of the Mongolian isolates, strains were selected from the online database by Maquart (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 biovar [31]. Phylogenetic trees were drawn using SAS™ proc tree.

7.4 Results

Forty eight livestock *Brucella* spp isolates collected from Dornod, Khentii, and Sukhbaatar provinces from different livestock species (2 cattle, 6 goats and 40 sheep). Seven sheep isolates were collected during an outbreak of brucellosis from Delgerkhaan district, Khentii province in 2009. Forty livestock strains were isolated from samples collected during the lambing season of 2011-2013. In addition, 11 human isolates were collected from brucellosis patients (Table 1). All human and livestock strains were confirmed as field strains (i.e. no vaccine strains).

A conventional PCR was used for species identification of *B. melitensis* for total of 52 isolates (39 from sheep, 3 from goats, 10 from human) and *B.abortus for total of* 6 isolates: (2 from cattle, 3 from goats, and 1 from a patient).

Table 1 shows allelic diversity presented descending order: that bruce30 (0.78%), bruce18 (0.75%), bruce19 (0.72%) bruce04 (.68%), and bruce07 (0.61%).

Table 2 shows the allelic diversity of all loci with the following most variable loci in descending order: bruce30 (h=0.78), bruce16 (0.75), bruce19 (0.72), bruce04 (0.68), and bruce07 (0.61). Table 3 shows the genetic diversity of every locus, expressed as Hunter-Gaston diversity index (HGDI). There is considerable variation over many different loci indicating relatively high variability of Mongolian strains. The most discriminatory VNTR markers were in panel 2B; bruce30 with a diversity index of 75%, bruce04 with a diversity index of 68% and bruce07 with a diversity index 62% (Table 4). The discriminatory VNTR markers of panel 2A; bruce18 with a diversity index 26 and bruce21 with a diversity index 0.05. Among the VNTR marker of panel 1; bruce08 with a diversity index 51%, bruce42 with 51% and bruce43 with 29%.There was no diversity represented by bruce11 and bruce21.

Strains were divided into distinct groups of *B.abortus* and *B. melitensis*. *B.melitensis* is the dominant species among sheep and goats. *B.abortus* was found in cattle but no *B.melitensis*. *B.melitensis* strains isolated from sheep and goats were found to be closely related. Human strains formed two distinct clusters. The first group of human strains was closely related to the *B.melitensis* species isolated from sheep and goats (Figure 2).

Figure 3 shows a selection of strains used from the online data base http://mlva.u-psud.fr/mlvav4/genotyping/. *B.abortus* was at a distinct place in the tree. Mongolian strains grouped in very diverse ways. Some human strains related to the Near East and Mediterranean. Some of *B. abortus* strains were closely related to Mediterranean and European strains. Generally, Mongolian strains show mostly own features and do not share as much as expected.

7.5 Discussion

Small ruminants seem to be main reservoir hosts causing human brucellosis in Mongolia. Mongolians have the habit to eat partially cooked small ruminant liver and take fresh blood of goats in May. Herders provide obstetric assistance during lambing and take weak newborn in their ger (traditional mobile house). New-borns may stay with the family for a minimum of 4-weeks during extreme cold weather conditions. Herders and their family members are further exposed to weak infected new born animals through frequent handling during feeding animals. Such contact occurs several times each day and the risk exposure is compounded by young children playing with new-born “pet” animals [6, 7]. Also, consumers of livestock products are at risk of infection without having regular direct contact to infected livestock.

Field data collection from outbreaks is essential, but the lack of resolution with conventional biotyping does not allow accurate trace-back outbreaks in endemic brucellosis country. MLVA-16 offers the medical and veterinary sectors required a tool for accurate trace-back investigations and strengthens brucellosis surveillance and control.

The 58 Mongolian strains show very high genetic diversity compared with other countries. Allelic diversity in Kyrgyz and Chinese strains was lower than among Mongolian strains. In Kyrgyzstan only three loci were variable in panel 2B; bruce04, bruce16 and bruce30 [29]. Also, 18 Mongolian strains from a previous study by Kulakov et al. 2010 compared to 4 strains from Azerbaijan and 6 strains from Russia; using 11 locus-MLVA in 2009. HGDI were calculated for five loci: bruce16 (0.83), bruce04 (0.68), bruce07 (0.59), bruce14 (0.49) and bruce07 (0.37). No diversity was found for the remaining 6 loci. Bruce07 was used twice which provided the same number of repeats 3, 4 and 5 but it with two different HGDI values. BRU1250 (bruce07) was 0.59% but VNTR5A (bruce07) was 0.37%. These results were provided less variability than results of this study. It should be noted, that the Mongolian strains were older strains collected in 1970 (4 strains), in 1976 (1 strain), in 2001 (1 strain) and in 2009 (12 strains). Perhaps the different ages of the strains used by Kulakov et al. 2010 older and in this study explains some of the observed differences [23]. Four *B.melitensis* isolates from Mongolia appear to be closer to Kyrgyz strains the so-called Eastern Mediterranean group. Further comparisons with Chinese and Russian strains are pending. Full genome sequencing of Mongolian brucella strains is needed for a better understanding of strain characteristic.

Kyrgyz brucellosis strains might clonally expand by a dominant strain sweeping through Naryn oblast whereas; the Mongolian strain diversity indicates more endemic brucellosis transmission [34]. Sheep appear as the main source of human brucellosis infection in Mongolia. Sheep may be the main reservoir host spilling over to goats, some cattle and humans. *B.abortus* strains in cattle may spill over to goats.

Mongolian herders have mean herd size of 244 animals including 45% sheep, 43% goats, 6% horses, 6% cattle and 1% camels [33]. Several herds share same pasture lands and water points. This traditional way of herding facilitates cross infection between different livestock species. Threshold animal densities for brucellosis transmission in Mongolia were estimated at 1.2 (min. 0.6; max. 8) cattle/km^2 and at about 6.8 (min. 4.5; max. 21) small ruminants/km^2 [31]. Cattle and small ruminant densities in the study area are estimated at 1.8 for cattle/km^2 and 19.4 small ruminants /km^2; hence it can be reasonably assumed that brucellosis transmission is at an endemic stage within the study area [33-34].

The observed high diversity of *Brucella* strains confirms the assumption of endemic brucellosis transmission rather than an epidemic outbreak situation [32]. The forty seven livestock strains collected from a large geographical area; Dornod province covers 123,597 km^2, Khentii province 80,325 km^2 and Sukhbaatar province 82,287 km^{2}from 2011 to 2013 The national brucellosis vaccination campaign which has continued from 2000 to 2009 was not able to interrupt transmissions because vaccination coverage was not sufficient and monitoring of the vaccination coverage was

limited.During the period of 1998 to 2013, Mongolia's overall livestock population grew by 75% and number of small ruminants increased of 53%. Such increase, may have contributed to the reduced the vaccination coverage of livestock [35].

Data show this mass vaccination did not stop the circulation of Brucella field strains within different livestock species from Dornod, Khentii and Sukhbaatar provinces. None of the isolates were vaccine strains. Animal infections parallel with the high number of reported human cases in those provinces. Recent studies show that the vaccination coverage rates achieved were below the threshold coverage needed to interrupt transmission (80%) [20, 36, 37]. Rural veterinarians have limited resources to visit sparsely populated areas where nomadic herders live and work. Sometimes, these nomadic herders move across several provinces seeking better pastures for their livestock due to drought, desertification and establishment of mining projects located within their pasture areas. Herders from different administrative areas were not offered brucellosis vaccinations because private veterinarians are allocated vaccine supplies based on officially registered livestock numbers in the area. Herders register their livestock annual census of the local government. The result is livestock census numbers of the local areas are usually lower than the actual numbers of flocks at the herders' camp. This nomadic tradition therefore influences the planning of vaccination campaigns and leads to insufficient vaccination coverage among livestock herds residing in the providences.

Mongolians traditionally do not consume young animals [34]. Sheep and goats are consumed at the age of 4 or older. Older sheep [5 to 6 years old] are highly valued among Mongolians. Infected small ruminants shed large numbers of Brucella organisms in uterine discharges and in milk representing a major risk for humans and within the flocks [5, 7].

Figure 4 shows the livestock increases from 1930 to 2013.The total number of livestock increased 34.4% since 2000, and goat numbers almost doubled since 2000 due to the demand for cashmere which is the main source of income for herders [33,35].

The government of Mongolia has encouraged herders to increase livestock numbers and has given special prizes to herders who have increased their number of small ruminants above 1000 head [33].

The aforementioned reasons should be seriously considered when planning future mass vaccination programs, budget calculations and when implementing evidence based monitoring of the vaccination coverage.

7.6 Conclusion

The main reservoir host in Mongolia for B. melitensis is sheep which spills over to goats and humans. B. abortus species are found in cattle and may spill over to goats and humans. Mongolian strains have a high diversity indicating endemic brucellosis transmission rather than epidemic situation. Whole genome sequencing of isolated strains for a better understanding of Mongolian strain

characteristics, particularly to reconstruct outcomes of past brucellosis control activities. This will help us to better plan ongoing efforts.

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7.8 Disclaimer

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7.10 References

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Table 1. *Brucella* spp. Isolates analyzed in this study the following data are presented: strain identification code (ID), geographical origin (province, district), host, samples isolated from, urease, oxidase test carried out, species identification conducted by AMOS PCR

ID	Province	District	Year	Host	Isolated from	Urease	Oxidase	AMOS PCR	*Brucella* spp. identified
3	Dornod	Tsagaan-Ovoo	2013	Sheep	Stomach liquid of fetus	+	+	+	*Br.melitensis*
6	Dornod	Tsagaan-Ovoo	2013	Sheep	Stomach liquid of fetus	+	+	+	*Br.melitensis*
9	Dornod	Tsagaan-Ovoo	2013	Goat	Stomach liquid of fetus	+	+	+	*Br.melitensis*
14	Dornod	Tsagaan-Ovoo	2013	Sheep	Stomach liquid of fetus	+	+	+	*Br.melitensis*
16	Dornod	Tsagaan-Ovoo	2013	Sheep	Stomach liquid of fetus	+	+	+	*Br.melitensis*
18	Dornod	Tsagaan-Ovoo	2013	Sheep	Stomach liquid of fetus	+	+	+	*Br.melitensis*
21	Sukhbaatar	Asgat	2013	Sheep	Stomach liquid of fetus	+	+	+	*Br.melitensis*
22	Sukhbaatar	Asgat	2013	Sheep	Stomach liquid of fetus	+	+	+	*Br.melitensis*
23	Sukhbaatar	Asgat	2013	Sheep	Stomach liquid of fetus	+	+	+	*Br.melitensis*
25	Sukhbaatar	Asgat	2013	Sheep	Stomach liquid of fetus	+	+	+	*Br.melitensis*
53	Dornod	Tsagaan-Ovoo	2013	Sheep	Vaginal swab	+	+	+	*Br.melitensis*
125	Sukhbaatar	Munkhkhaan	2013	Goat	Vaginal swab	+	+	+	*Br.melitensis*
126	Sukhbaatar	Munkhkhaan	2013	Goat	Vaginal swab	+	+	+	*Br.melitensis*
130	Sukhbaatar	Uulbayan	2013	Cattle	Milk	+	+	+	*Br.abortus*
132	Sukhbaatar	Uulbayan	2013	Cattle	Stomach liquid of fetus	+	+	+	*Br.abortus*
64	Khentii	Bayankhutag	2013	Sheep	Milk	+	+	+	*Br.melitensis*
67	Khentii	Bayankhutag	2013	Sheep	Milk	+	+	+	*Br.melitensis*
68	Khentii	Bayankhutag	2013	Sheep	Milk	+	+	+	*Br.melitensis*
81	Khentii	Delgerkhaan	2013	Sheep	Vaginal swab	+	+	+	*Br.melitensis*
87	Khentii	Delgerkhaan	2013	Sheep	Vaginal swab	+	+	+	*Br.melitensis*
88	Khentii	Delgerkhaan	2013	Sheep	Vaginal swab	+	+	+	*Br.melitensis*
167	Khentii	Murun	2013	Goat	Stomach liquid of fetus	+	+	+	*Br.melitensis*
179	Khentii	Murun	2013	Sheep	Vaginal swab	+	+	+	*Br.melitensis*
182	Khentii	Murun	2013	Sheep	Vaginal swab	+	+	+	*Br.melitensis*

Table 1 *(continued)*

ID	Province	District	Year	Host	Isolated from	Urease	Oxidase	AMOS PCR	*Brucella* spp. identified
188	Khentii	Murun	2013	Sheep	Vaginal swab	+	+	+	*Br.melitensis*
183	Khentii	Murun	2013	Sheep	Vaginal swab	+	+	+	*Br.melitensis*
193	Khentii	Murun	2013	Sheep	Vaginal swab	+	+	+	*Br.melitensis*
185	Khentii	Bayankhutag	2013	Sheep	Vaginal swab	+	+	+	*Br.melitensis*
177	Khentii	Murun	2013	Sheep	Stomach liquid of fetus	+	+	+	*Br.melitensis*
12	Dornod	Tsagaan-Ovoo	2013	Sheep	Stomach liquid of fetus	+	+	+	*Br.melitensis*
10	Dornod	Tsagaan-Ovoo	2013	Sheep	Stomach liquid of fetus	+	+	+	*Br.melitensis*
51	Dornod	Tsagaan-Ovoo	2013	Sheep	Vaginal swab	+	+	+	*Br.melitensis*
58	Dornod	Choibalsan	2013	Sheep	Vaginal swab	+	+	+	*Br.melitensis*
13	Dornod	Tsagaan-Ovoo	2013	Sheep	Stomach liquid of fetus	+	+	+	*Br.melitensis*
11	Dornod	Tsagaan-Ovoo	2013	Sheep	Stomach liquid of fetus	+	+	+	*Br.melitensis*
20	Dornod	Tsagaan-Ovoo	2013	Sheep	Stomach liquid of fetus	+	+	+	*Br.melitensis*
sh-1	Sukhbaatar	Baruun urt	2012	Sheep	Spleen aborted fetus	+	+	+	*Br.melitensis*
sh-2	Sukhbaatar	Baruun urt	2012	Sheep	Spleen aborted fetus	+	+	+	*Br.melitensis*
go-1	Sukhbaatar	Baruun urt	2012	Goat	Spleen aborted fetus	+	+	+	*Br.melitensis*
go-2	Sukhbaatar	Baruun urt	2012	Goat	Stomach fluid of fetus	+	+	+	*Br.melitensis*
eks-1	Khentii	Delgerkhaan	2009	Sheep	Spleen aborted fetus	+	+	+	*Br.melitensis*
eks-2	Khentii	Delgerkhaan	2009	Sheep	Spleen aborted fetus	+	+	+	*Br.melitensis*
eks-3	Khentii	Delgerkhaan	2009	Sheep	Spleen aborted fetus	+	+	+	*Br.melitensis*
eks-5	Khentii	Delgerkhaan	2009	Sheep	Spleen aborted fetus	+	+	+	*Br.melitensis*
eks-6	Khentii	Delgerkhaan	2009	Sheep	Spleen aborted fetus	+	+	+	*Br.melitensis*
eks-7	Khentii	Delgerkhaan	2009	Sheep	Spleen aborted fetus	+	+	+	*Br.melitensis*
eks-8	Khentii	Delgerkhaan	2009	Sheep	Spleen aborted fetus	+	+	+	*Br.melitensis*

ID	Province	District	Year	Host	Isolated from	Urease	Oxidase	AMOS PCR	*Brucella* spp. identified
H-1	Ulaanbaatar	Nalaikh	2000	Human	Blood	+	+	+	*Br.abortus*
H-2	Ulaanbaatar	Bayanzurkh	2005	Human	Blood	+	+	+	*Br.melitensis*
H-3	Khentii	Bayankhutag	2005	Human	Blood	+	+	+	*Br.melitensis*
H-4	Selenge	Tsagaannuur	2007	Human	Blood	+	+	+	*Br.melitensis*
H-5	Dornogobi	Dalanjargalan	2007	Human	Blood	+	+	+	*Br.melitensis*
H-6	Sukhbaatar	Munkhkhaan	2009	Human	Blood	+	+	+	*Br.melitensis*
H-7	Selenge	Tsagaan-nuur	2011	Human	Blood	+	+	+	*Br.melitensis*
H-8	Khentii	Bayanmunkh	2011	Human	Blood	+	+	+	*Br.melitensis*
H-9	Khentii	Galshar	2011	Human	Blood	+	+	+	*Br.melitensis*
H-10	Khentii	Bayanmunkh	2012	Human	Blood	+	+	+	*Br.melitensis*
H-11	Ulaanbaatar	Bayanzurkh	2012	Human	Blood	+	+	+	*Br.melitensis*
H-12	Ulaanbaatar	Bayanzurkh	2012	Human	Blood	+	+	+	*Br.melitensis*

Table 2. Allelic diversity of the MLVA-16

No. of copies	Locus no. at MLVA															
	bruce 06	bruce 08	bruce 11	bruce 12	bruce 42	bruce 43	bruce 45	bruce 55	bruce 18	bruce 19	bruce 21	bruce 04	bruce 07	bruce 09	bruce 16	bruce 30
0	3	2	1	2	2	1	4	2	2	3	1	1	1	1	1	6
1	51	-	2	-	1	11	-	4	-	-	-	-	-	-	-	-
2	-	-	-	-	20	48	-	52	-	-	-	1	-	-	-	-
3	2	1	57	-	35	-	47	2	-	-	-	7	9	1	18	-
4	4	10	-	-	2	-	7	-	-	-	-	7	8	42	3	3
5	-	9	-	-	-	-	2	-	49	-	-	8	33	-	15	9
6	-	38	-	-	-	-	-	-	1	-	1	30	9	4	17	18
7	-	-	-	14	-	-	-	-	5	-	-	4	-	10	3	13
8	-	-	-	-	-	-	-	-	2	-	1	2	-	2	3	7
9	-	-	-	-	-	-	-	-	1	-	57	-	-	-	-	2
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	-	-	-	8	-	-	-	-	-	-	-	-	-	-	-	-
13	-	-	-	36	-	-	-	-	-	-	-	-	-	-	-	-
18	-	-	-	-	-	-	-	-	-	9	-	-	-	-	-	-
19	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
20	-	-	-	-	-	-	-	-	-	12	-	-	-	-	-	-
21	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-
22	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
23	-	-	-	-	-	-	-	-	-	25	-	-	-	-	-	-
25	-	-	-	-	-	-	-	-	-	6	-	-	-	-	-	-
n strains	57	58	59	58	58	59	56	58	58	57	59	59	59	59	59	59
Allelic diversi-ty* *h*	0.179	0.508	0.049	0.529	0.507	0.291	0.266	0.176	0.264	0.722	0.050	0.684	0.616	0.449	0.747	0.776

Table 3. Hunter-Gaston Diversity Indices (HGDI) of MLVA-16

	Locus						
		ATs[a]	**TRs[b]**	**HGDI[C]**	**CI[d]**	**K[e]**	**Max (pi)[f]**
Panel 1	Bruce06	2	1,3,4	0.250	0.108-0.391	4	0.864
	Bruce08	2	3-6	0.546	0.421-0.672	5	0.644
	Bruce11	2	1,3	0.067	0.000-0.155	3	0.966
	Bruce12	1	7,12,13	0.582	0.478-0.686	4	0.593
	Bruce42	2	1-4	0.541	0.455-0.626	5	0.593
	Bruce43	3	1-2	0.314	0.183-0.446	3	0.814
	Bruce45	1	3-5	0.355	0.208-0.503	4	0.797
	Bruce55	2	1-3	0.221	0.084-0.358	4	0.881
Panel 2A	Bruce18	2	5-9	0.334	0.183-0.485	6	0.814
	Bruce19	4	18-25	0.757	0.680-0.835	8	0.424
	Bruce21	1	6,8,9	0.067	0.000-1.555	3	0.966
Panel 2B	Bruce04	6	2-8	0.701	0.598-0.804	7	0.508
	Bruce07	4	3-6	0.652	0.550-0.754	5	0.542
	Bruce09	6	3-4,6-8	0.467	0.329-0.605	6	0.712
	Bruce16	9	3-8	0.774	0.730-0.819	7	0.288
	Bruce30	7	4-10	0.830	0.787-0.873	8	0.288

a. Allelic Type

b. Total number of repeat units each locus determined by the correlation with the amplicon size according to previously published reports

c. Hunter-Gaston Diversity Indices

d. Precision of the diversity indices, expressed as 95% upper and lower limits

e. K = Number of different repeats present at this locus in this sample set

f. Max (pi) Fraction of samples that have the most frequent repeat number in this locus (range 0.0 to 1.0)

Table 4. Hunter-Gaston Diversity Indices (HGDI) sorted by from the highest to the lowest value

Locus	Diversity Index[a]	Confidence Interval[b]	K[c]	Max (pi)[d]
Bruce 30 VNTR:16	0.83	0.787 - 0.873	8	0.288
Bruce 16 VNTR:15	0.774	0.730 - 0.819	7	0.288
Bruce 19 VNTR:10	0.757	0.680 - 0.835	8	0.424
Bruce 04 VNTR:12	0.701	0.598 - 0.804	7	0.508
Bruce 07 VNTR:13	0.652	0.550 - 0.754	5	0.542
Bruce 12 VNTR:4	0.582	0.478 - 0.686	4	0.593
Bruce 08 VNTR:2	0.546	0.421 - 0.672	5	0.644
Bruce 42 VNTR:5	0.541	0.455 - 0.626	5	0.593
Bruce 09 VNTR:14	0.467	0.329 - 0.605	6	0.712
Bruce 45 VNTR:7	0.355	0.208 - 0.503	4	0.797
Bruce 18 VNTR:9	0.334	0.183 - 0.485	6	0.814
Bruce 43 VNTR:6	0.314	0.183 - 0.446	3	0.814
Bruce 06 VNTR:1	0.25	0.108 - 0.358	4	0.864
Bruce 55 VNTR:8	0.221	0.084 - 0.358	4	0.881
Bruce 21 VNTR:11	0.067	0.000 - 0.155	3	0.966
Bruce 11 VNTR:3	0.067	0.000 - 0.155	3	0.966

a. Hunter- Gaston Diversity Indices

b. Precision of the diversity index, expressed as 95% upper and lower limits

c. K = Number of different repeats present at this locus in this sample set

d. Max (pi) fraction of samples that have the most frequent repeat number in this locus (range 0.0 to 1.0)

Table 5. List of all VNTR loci with allele size basepair and unit number

Panel	No	Sample name	Locus No	Alleles size basepair	Unit num-ber
Panel 1	1	-	bruce06	-	-
	2	sheep_8	bruce08	366	5
	3	sheep_9	bruce11	320	3
		sheep_10	bruce11	320	3
		sheep_11	bruce11	320	3
	4	sheep_25	bruce 12	392	13
		sheep_27	bruce 12	392	13
		sheep_28	bruce 12	392	13
	5	-	bruce42	-	-
	6	sample_38	bruce43	182	2
	7	-	bruce45	-	-
	8	-	bruce55	-	-
Panel 2A	9	sheep_1	bruce18	162	7
	10	-	bruce19	-	-
	11	-	bruce21	-	-
	12	-	bruce04	-	-
Panel 2B	13	sample_15	bruce07	166	6
		sample_27	bruce07	158	5
		sample_28	bruce07	150	4
	14	human_2	bruce09	164	8
	15	-	bruce16	-	-
	16	sample_29	bruce30	167	8

Figure 1. Map of Mongolia by provinces where strains were collected in *grey shade*

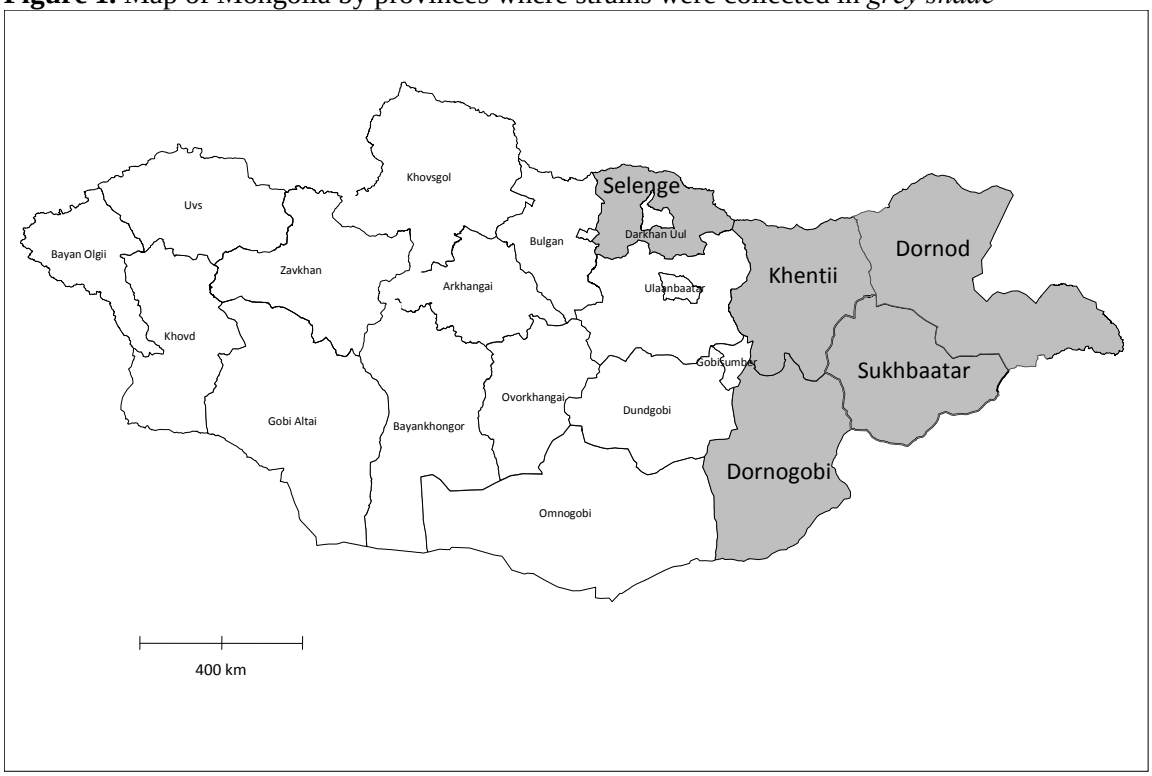


Figure 2. VNTR data using proc cluster UPMGA, SASTM

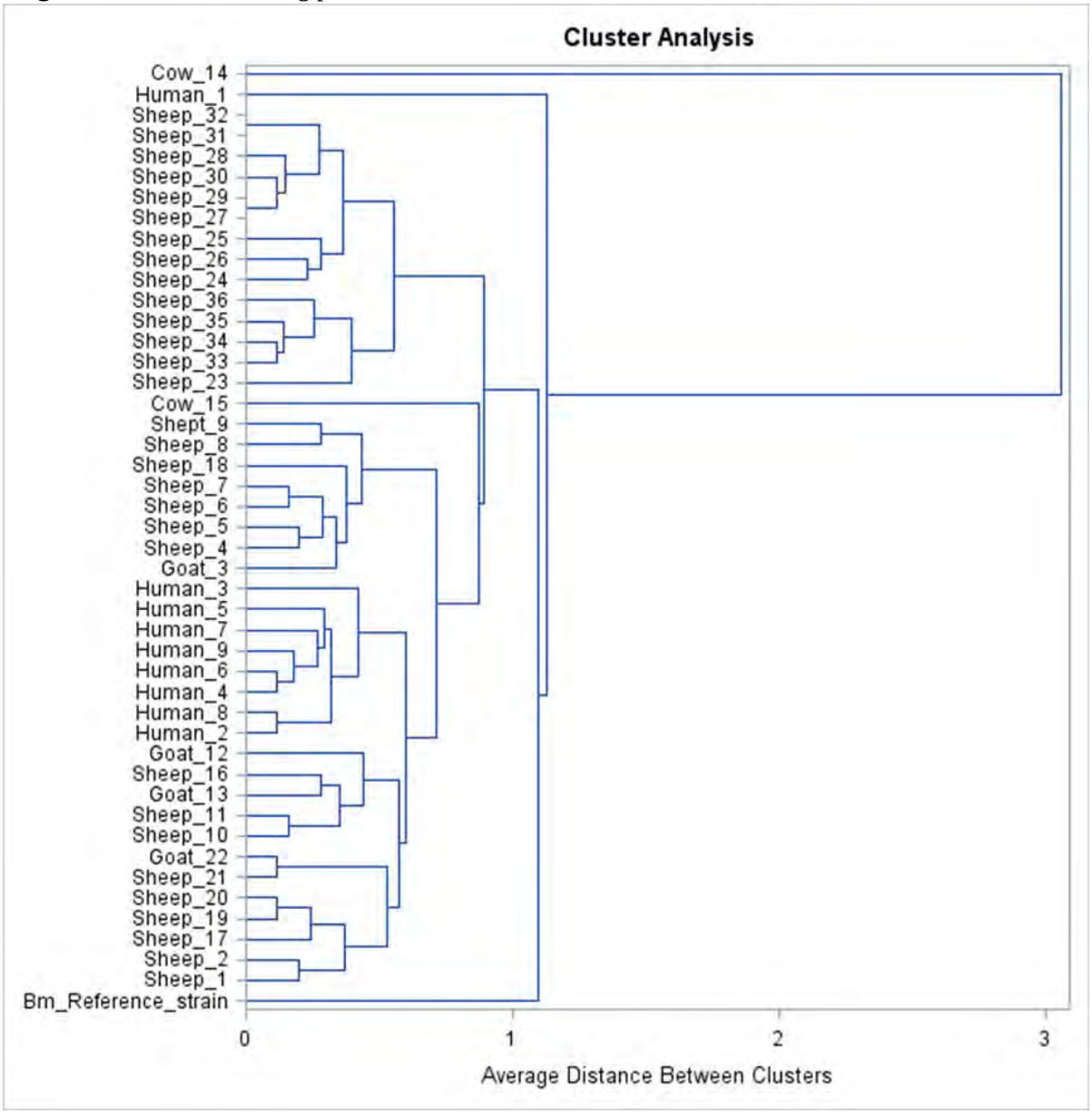


Figure 3. VNTR data using proc cluster UPMGA, with world strains SASTM

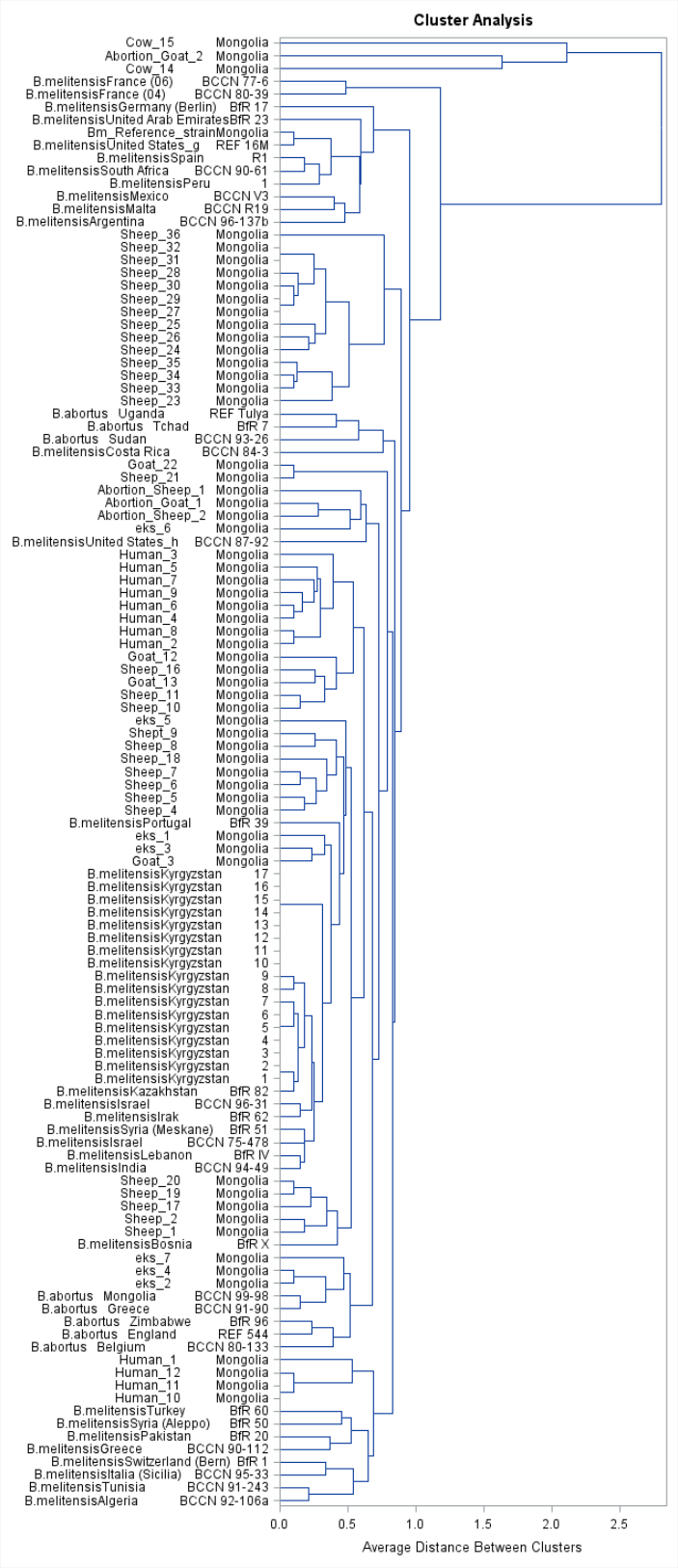
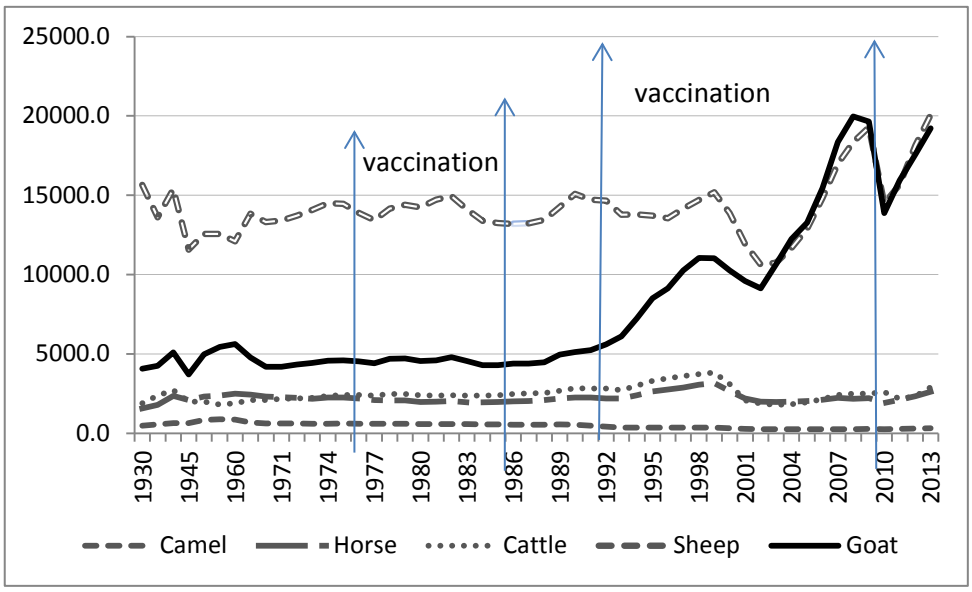


Figure 4. Livestock populations in Mongolia from 1930 to 2013 with the first and second mass vaccination (Mongolian national statistical office data)



8 Brucellosis Vaccine Cold Chain Situation in Mongolia

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8.1 Abstract

Background: A vaccine quality and cold chain maintenance are important factors for livestock mass vaccination campaigns to be effective. We tested the quality of locally manufactured batches of vaccines Rev 1 *Brucella melitensis* and S19 *Brucella abortu*s from the point of manufacturers to the point of administration of livestock in fields. We recorded the temperature of cool boxes carried by private veterinarains during the vaccination period.

Results: The colony-forming unit (CFU) counts were 17%-40% lower for vaccines that were stored at the provincial veterinary laboratory and 2.5-20% lower for vaccines stored at the private veterinary unit in soum according to the Mongolian laboratory result. OIE brucellosis reference laboratory results showed that CFU counts were 27- 37% lower for vaccines stored at the provincial veterinary laboratories, 6% lower for vaccines stored at the private veterinary units in soums and 1% further decreased at the point of administration during the vaccination campaign.

Conclusion: The vaccine cold chain should be documented and provided as a guideline for maintaining and managing it during livestock brucellosis vaccination campaign. The vaccine cold chain issues should be discussed among veterinarians and all relevant stakeholders for their consensus and approval.

Manuscript is not to be submitted yet

8.2 Introduction

The effectiveness of livestock mass vaccination is determined by a vaccination coverage and a vaccine efficacy of Rev1 (*Brucella melitensis*) and S19 (*Brucella abortus*) i.e. estimates of the number of animals that have received vaccines and the percentage reduction of brucellosis in vaccinated animals compared to unvaccinated animals (Zinsstag et al., 2005a). Vaccination coverage is estimated by the cross-sectional and post-vaccination surveys and the quality of vaccines has to be double-checked to ensure that its efficacy is high enough at the moment of vaccine administration. Within the framework of the Livestock Project funded by the Swiss Development Cooperation (SDC) in support of the Ministry of Food and Agriculture, the quality of the locally produced brucellosis vaccines and the maintenance of cold chain were assessed.

Attenuated brucellosis vaccines S19 and Rev1 have been produced by Biocombinat factory in Ulaanbaatar Mongolia since 1975. The vaccines are required to be stored for a year and the storage temperature requirements of the vaccines are between 4°C and 10°C and between -20°C and 4°C, respectively (Ulziitogtokh, 2008). Vaccines are produced in September to March and get transported to 22 provincial veterinary laboratories from March to May. The factory has several non-refrigerated trucks with a capacity of delivering vaccines within 5-48 hours from Ulaanbaatar to provincial centers. Vaccines are kept in non-refrigerated rooms at the Provincial Veterinary Laboratory (PVL) in April to September and get collected by private veterinarians who transport the vaccines by their own vehicles to the private veterinary units at the soum centers. The private veterinarians then administer brucellosis vaccines to livestock from September to November annually.

The objectives of this study were;

1. Assess vaccine quality from production at Biocombinat factory (June –August 2010) to its application in the field (October-November 2011).
2. Monitor vaccine storage at the private veterinary units during the vaccination campaign.
3. Review the current government policy and guideline on the vaccine cool chain

8.3 Materials and methods

The research team was consisted of veterinarians who worked in collaboration with the Veterinary and Breeding Agency, the Ministry of Food and Agriculture, the Veterinary Research Institute, Biocombinat factory (public enterprise) and the State Central Veterinary Laboratory. The quality of vaccines was tested by the independent State Veterinary Drug Testing and Confirmation Laboratory. Also, quality control of brucellosis vaccines was conducted at the Bacterial Zoonosis Unit at the Animal Health Laboratory of the French Agency for Food, Environment and Occupational Health Safety in Maisons-Alfort, France. It’s an OIE Reference Laboratory of the World Organization for Animal Health.

Sampling and data collection

Biocombinat factory manufactured B. abortus S19 and B.melitensis Rev1 vaccines. S19 vaccine was presented as freeze dried and produced from 20 to 50 doses in 9.6 ml vials. Rev1 vaccine was presented as freeze dried and produced from 20 to 300 doses in 9.6 ml vials. All vials were kept inside of cardboard boxes for shipment to provincial veterinary laboratories. Of 22 provinces, Sukhbaatar and Zavkhan were selected for convenience at the request of the Ministry of Food and Agriculture. Within each aimag, four soums were selected through a randomly selection method. Conditions of vaccine storage were inspected at the Veterinary laboratories in both provinces: Sukhbaatar in April 2010 and Zavkhan in August 2010. On March 2010, Rev.1 and S19 vaccines were transported by a truck to Zavkhan province's veterinary laboratory. The Zavkhan veterinary laboratory returned vaccines to Biocombinat factory within four weeks. On May 2010, Rev.1 and S19 vaccines were transported by a truck to Sukhbaatar province's veterinary laboratory and the vaccines were returned to Biocombinat factory within a week.

The temperature loggers

Temperature data logger (TL-Series Loggers 3M^{TM} Hamburg, Germany) used in the study. The 3M™ TL20 Temperature Data Logger measures environmental temperature from -40°C to + 80°C. The temperature data logger was set to an hourly measurement for 125 days. The temperature data logger has a unique identifier and has a connection to a computer for transferring temperature data. Six temperature data loggers were programmed and inserted into boxes that stored Rev.1 and S19 vaccines for the shipment from Biocombinat factory to Zavkhan and Sukhbaatar provinces (*see* Table 1). Due to the outbreak of foot and mouth disease in Sukhbaatar province on 31st August, 2010 we were unable to conduct the study in Sukhbaatar. Therefore, the temperature data loggers were delivered to the private veterinarians in Zavkhan province only. Vaccine sample collection was done in the selected four districts (soums) of Zavkhan province. There were a total of 49 private veterinarians who administered brucellosis vaccination for 2 million livestock (cattle, sheep and goats).

Vaccine samples tested in Mongolia

Twelve batches of Rev.1 and four batches of S19 vaccines from Zavkhan and Sukhbaatar provinces stored at the Biocombinate. The vaccine samples were selected through a random selection method. Two samples were taken from each batch otherwise same batch of vaccine from both provinces selected. The control samples were taken from the storage of the vaccine production section of the Biocombinate (Table 2). Total of 44 samples selected which kept in the cool box between +4°C +10°C on 6 of July, 2010.The vaccine samples delivered to the Veterinary drug, biological testing and confirmation laboratory (VDBTCL) in Mongolia on 7 of July, 2010.Two inspectors, a technical advisor and a laboratory assistant worked on counting of the colony forming unit.

The vaccine samples tested at the Bacterial Zoonosis Unit, Animal Health Laboratory, OIE Reference Laboratory, in France

The OIE reference laboratory requested to send 10 samples from each batch. Total of 170 samples from 18 batches kept in the cooler at the +4°C+10°C. The each sample was packed with three layers fulfilled international requirement of shipment by International Air Transport Association. The brucellosis vaccine samples categorized under dangerous item required getting special permission went through different customs of the international airports. Samples transported to the OIE Reference Laboratory by DHL 21st of August, 2010. Unfortunately, the brucellosis vaccine samples did have cool chain from shipment until delivered to the OIE reference laboratory, Paris, in France. The OIE brucellosis reference laboratory conducted enumartion of colony forming unit per dose, and phase dissociation from 23 September to 8 October, 2010.

The second vaccine samples

Sukhbaatar province had outbreak of FMD and government stopped the brucellosis vaccination entire province. The field study carried out only in Zavkhan province. In total, 12 samples of 6 batches Rev.1 and S19 vaccines collected from Otgon and Aldarkhaan soums of Zavkhan provinces. Collected sample put into cool box and transported by car from Zavkhan province to Ulaanbaatar shortly after collection. The samples tested decrease of CFU at the SVDBTCL in Mongolia only.

Temperature loggers

Two brucellosis vaccines were transported by the lorry from Ulaanbaatar to Zavkhan province'sveterinary laboratory in autumn. The temperature loggers inserted when vaccine stored in the regular room of provincial veterinary laboratory. In total, 49 private veterinarians explained study purpose asked their permission to participate in the study and to keep the temperature logger with the vaccine during the vaccination period. All 49 private veterinarians agreed to participate. The private veterinarians received the temperature logger same time picking up brucellosis vaccines in September, 2010. Each veterinarian kept the temperature logger with vaccine during the brucellosis mass vaccination campaign started from 10 September continued until end of October, 2010. We received all 49 vaccines plus three loggers inserted at the provincial veterinary laboratory. 13 loggers were broken or unable to down load the data. We only analysed 40 temperature loggers' data. The field team consisted of two veterinarians, a driver and a local guide visited randomly selected practicing veterinarians during livestock vaccination from Ulaistai, Ider, Otgon and Aldarkhaan soums during 10 Sep- 9 Oct, 2010. The field team checked eight private veterinarians from selected soum that seven had cool boxes and presented it to the team. Cool boxes were still in the original packing never used it before. Veterinarians did not use the cool boxes that their carry the vaccines in the wooden or carton boxes.

The herd immunity after vaccination

The field team consisted of two veterinarians, a driver and a local guide visited randomly selected practicing veterinarians during livestock vaccination from Ulaistai, Ider, Otgon and Aldarkhaan soums. Random selection of four soums (district) from the province, the third stage sampling was 8 bags (village), the fourth stage were 10 hot ail (2-3 nomadic herder families camp together share same pasture, all species of livestock frequent mixing and contact at the water points) per bag and the fifth stage was individuals livestock sampling (Otte and Gumm, 1997). The selection of hot ails was done using the household lists of governmental livestock annual census from the governor's office of the 8 bags (Bennet et all.1991).The field team randomly selected 15 hot ail from vaccinated family list of private veterinarian and from each family selected 20 sheep, 20 goats and 5 cattle. The field team visited each family. Total 56 hot ails selected from four soums.

Bacteriological testing

Each batch of brucellosis vaccine samples cultured on the solid media (Trypcase Soja Agar) and incubated at the 37°C for 4-6 days at the SCVDBTCL. Each sample was counted for colony forming unit (CFU) from July to August, 2010 (Table 3).

Serological testing of livestock

All livestock serum tested by the Rose Bengal test (RBT) which is 1 serum: 1 antigen (*B. abortus* strain 99, Biocombinate, Mongolia) for small ruminants. One serum: three antigen ratio used for testing of large ruminants serum samples. Serum and antigen were mixed and rotated for 4 minute which agglutination recorded as negative and positive.

Data analysis

Forty temperature loggers data recorded from 15 September to 31 Octorber downloaded into computer which converted into the excel file. The Stata 10.1 was used for analysis of the temperature logger's data. The median time of temperatures outside of ideal range (+4°C +10°C) was analysed.

8.4 Results

The government policy on cold chain

The team did not find any cool chain guidelines or an official written document on policy level from 1990 to 2010. However, the Agency of Veterinary and Aninal Breeding distributed the cool boxes to the private veterinarians of 22 provinces in the country. The field team visited 8 private veterinarians working in Zavkhan province asked to show the cool boxes that 7 veterinarians presented the boxes with original packaging and a veterinarian told that he did not receive the cool box.

The team was found out that vaccine manufacturer transport vaccines directly to the provincial center due to the receiving the payment from the government. The government payment made only

after the vaccine distributed to the provincial veterinary laboratory of. This was only reason to transfer the vaccines from appropriate storage room of manufacturer to the regular office rooms at the 25°C and direct sun light which stored from March to September. This was the longest period where the vaccine colony forming unit decreased the most 17%-40% during storage period.

Storage condition at the production level

Rev.1 and S19 vaccines were stored two different storage rooms at the Biocombinate. The first storage room was at the+ 6°C and the second room, a cooler was broken and later it was fixed. Temperature data loggers were provided to monitor the cool chain system of the storage at the production level.

Storage condition at the provincial level

The team met with professionals who were in charge of the vaccine storage and vaccine distribution to private veterinarians. These professionals agreed that the cold chain is necessary for vaccine storage. There was no official manual or written instruction on storing and handling of vaccines at the provincial veterinary laboratory. There were very few vaccine instruction sheets. However, the instructions were not strictly followed. There were neither thermometer avialabe no did a temperature log exist in the vaccine storage. Brucellosis vaccines were stored regular office room without the protection from the sunlight.

Enumeration count of CFU result

The results obtained by SVDBTCL in Mongolia show that the CFU count was 17- 40% lower during the storage at Aimag as compared with the CFU count at the production level. CFU count was even lower 2.5-20% at the soum level (see Table 5). According to the results by the OIE Reference Laboratory, CFU counts were 27%- 37% lower at the provincial veterinary laboratory, 6% lower at the private veterinary unit storage at the soum level and 1% lower at the time of the brucellosis vaccination campaign (see Table 6). Furthermore, the OIE Reference Laboratory reported 852 batches of Rev1 vaccines failed due to vaccine phase dissociation and 855 batches of Rev1 vaccines had lower doses than the indicated.

The temperature data logger results

Thirty five out of 40 temperature data loggers used for the study and recorded the temperature throughout the vaccination period. The results showed that 81% (95% CI 76-88) of the median time vaccines were stored outside the recommended temperature range (from +4°C to +10°C). The temperature was above +20°C for the 5% of the time period. The lowest temperature recorded was -8°C and the highest temperature recorded was +41°C.

8.5 Discussion

Our results show that the occurrence of the loss of vaccine potency is highest at the distribution storage in provincial veterinary laboratory where there is lack of cold storages. Very little further loss occurs at the stage of vaccination campaign although the temperature requirement is not met. However, private veterinarians should be trained on the storage and handling of livestock live vaccines. There is a sufficient capacity as for the vaccine quality control and the results are comparable with the OIE Reference Laboratory's results. To maintain the vaccine quality control in the future, the subsamples should be crosschecked by the OIE Reference laboratory.

Livestock mass brucellosis vaccination was conducted for the first time in Mongolia during 1975-1985. According to the retired communist leaders, heads of veterinary services and private veterinarians who participated in the discussion, a cold chain was unbroken and managed at the highest standard during socialism because controlling brucellosis was a top priority for the communist party at that time. The communist leaders had to reach targets therefore allocated enough budget to achieve the highest level of quality control. At that time, Rev1 vaccines used to be transported by an airplane to provincial centres to avoid a decrease in CFU counts. The cold chain used to be a main discussion topic among all the stakeholders such as the Veterinary and Breeding Agency, Provincial Veterinary Department and the private veterinarians representatives. All stakeholders had the knowledge and understanding about the cold chain and its importance that vaccines need to be kept in a narrow temperature range at all stages, from the point of manufacturer to their use in vaccination campaign. Furthermore, each vaccine bottle used to have a manufacturer label which clearly specified a storage temperature requirement as between $+4^{\circ}C$ and $+10^{\circ}C$. Therefore veterinarians at that time made an extra effort to keep the vaccines within the permitted temperature range by covering it with a cloth to avoid its exposure to the direct sunlight. Although the cold chain was widely understood and practiced at that time, the process had never been officially documented and there was no written instruction of the cold chain until the present. As a result, the first generation of the post-communist veterinary seemed to have ignored the practice of cold chain. Therefore, today, the cold chain needs to be officially documented and provide as a guideline for all veterinarians. Also, relevant stakeholders need to discuss about this issue and reach a consensus. According to our study, Mongolian government has approved the budget. Interestingly, unlike the poor practice of the cold chain for veterinary vaccines, Mongolia is known for its best cold chain practice for childhood vaccines in the Western Pacific Region and this is because there was a continuous financial support from the Japanese government in the public health sector for over ten years. Therefore, there should be knowledge and experience sharing between public health and animal health sectors as encouraged in the "One Health" strategy (Zinsstag et al., 2005b). Our study report which described the poor practice of the cold chain in today's animal health sector was delivered to the relevant decision makers, professionals and international donors to aware them of

the current situation. As a result, the issue has received immediate attentions from donors and the country's veterinary authoritative bodies. For instance, thanks to the EU-funded project, there are now two refrigerated trucks available to transport livestock vaccines to provincial centers, and the VABA allocated a budget for establishing cool rooms in all of the 22 provinces in 2011.

8.6 Conclusions

There is a need for a vaccine cold chain in the veterinary sector. The cold chain should be documented and to develop standard operating procedures (SoP) for the ongoing livestock brucellosis vaccination campaign. The cold chain issue should be discussed among veterinarians and all relevant stakeholders for their consensus and approval. The Mongolian government should be allocated a budget for maintaining the cold chain of livestock vaccines. The veterinary sector should learn from the public health sector that is known for its best practice. It is further recommended that Rev1 and S19 vaccines should be stored at the manufacturers' plant until August to reduce vaccine quality losses. Similar studies on vaccine and cold chain quality should be conducted periodically at a larger scale in order to ensure the effectiveness of the ongoing livestock mass vaccination campaign.

8.7 Acknowledgements

The study was conducted as part of the SDC-funded Livestock Project. We would like to acknowledge the active participation of the Agency for Veterinary Services and Animal Breeding, the Ministry of Food, Agriculture and Light Industry, Agriculture Departments of Zavhan and Sukhbaatar Aimags, private veterinarians and herders from Aldarkhaan, Ider, Otgon and Ulaistai soums in this study.

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Photo1. 3M™ TL20 Temperature Logger

Photo 2. Temperature logger inserted to vaccines

Photo 3. Biocombinat cold room condition

Photo 4. Brucellosis vaccines stored at the office room in Zavkhan aimag in 2010

Figure.1 Temperature logger data converted to the graph
(Green line is required cold chain temperature

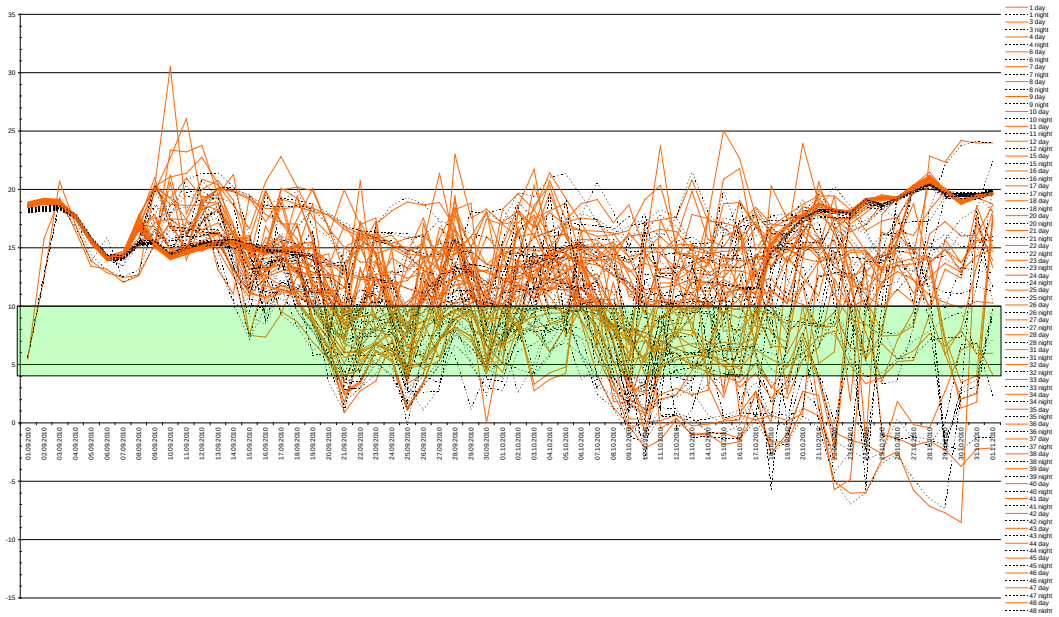

Table1. Sample collected for the OIE Reference Laboratory and Mongolian Laboratory in July, 2010

№	Brucellosis vaccine name	Batch number	Number of samples for OIE Reference Laboratory	Number of samples for SCVDT Laboratory
1		847	10	2
2		848	10	4
3	**Rev.1 vaccine**	849	10	2
4		850	10	4
5		851	10	2
6		852	10	2
7		853	10	2
8		854	10	2
9		855	10	2
10		856	10	2
11		857	10	2
12		858	10	4
13		275	10	2
14	**S 19 vaccine**	276	10	2
15		277	10	2
16		278	20	2
17	Rev.1 control			2
18	S19 control			2
	TOTAL		**170**	**44**

Table 2. Mongolian laboratory vaccine result sampled in aimag and soum level, 2010

Vaccine name	Storage level	Batch number	Produced dose	Reduced dose		Reduced dose rate	
				aimag	soum	aimag	soum
Rev.1	AVL [a]	848	200	150	150	25%	-
Rev.1	AVL	849	250	150	150	40%	-
Rev.1	AVL	851	100	100	100	-	-
Rev.1	AVL	852	150	150	137/127	-	8.6/7.6%
Rev.1	AVL	853	300	175	160	40%	8.50%
Rev.1	AVL	855	300	250	200	17%	20%
S19	AVL	277	50	40	40	20%	-
S19	Otgon[b]	277	50	40	40	20%	-
Rev.1	Otgon	852	150	150	147/135	-	2/10%
Rev.1	Uliastai[b]	852	150	150	130/140	-	13.3/6.6%
S19	Uliastai	277	50	40	39	20%	2.50%
Rev.1	Aldarkhaan[b]	849	250	150	150	40%	-

[a]Aimag Veterinary Laboratory [b] Selected soum of Zavkhan provinces

Table 3.OIE Reference Laboratory for Brucellosis quality test results

Vaccine	Batch number	Sample number	Expected CFU per dose	Doses per vial	Enumeration results (CFU/dose)	Phase dissocia-tion	Conclusion
	847	10	$1x10^9$	200	$5,93\text{-}6,72x10^8$	<5% phase R	passed
Rev.1	848	10	$1x10^9$	200	$6,36\text{-}7,11x10^8$	<5% phase R	passed
	849	10	$1x10^9$	250	$8,11\text{-}8,71x10^8$	<5% phase R	passed
	850	10	$1x10^9$	300	$4,24\text{-}5,21x10^8$	<5% phase R	passed
	851	10	$1x10^9$	100	$1,09\text{-}1,24x10^9$	<5% phase R	passed
	852	10	$1x10^9$	150	$0,977\text{-}1,18x10^9$	<5% phase R	failed
	853	10	$1x10^9$	300	$4,37\text{-}5,23x10^8$	<5% phase R	passed
	854	10	$1x10^9$	250	$5,68\text{-}6,45x10^8$	<5% phase R	passed
	855	10	$1x10^9$	300	$3,23\text{-}3,94x10^8$	<5% phase R	low titer
	856	10	$1x10^9$	200	$1,00\text{-}1,14x10^9$	<5% phase R	passed
	857	10	$1x10^9$	300	$4,66\text{-}5,24x10^8$	<5% phase R	passed
	858	10	$1x10^9$	250	$5,32\text{-}6,11x10^8$	<5% phase R	passed
	275	10	1x10	20	$6,27\text{-}7,34x10^9$	<5% phase R	passed
S19	276	10	1x11	20	$4,92\text{-}6,05x10^9$	<5% phase R	passed
	277	10	1x12	50	$4,98\text{-}6,70x10^9$	<5% phase R	passed
	278	10	1x13	50	$6,56\text{-}7,38x10^9$	<5% phase R	passed
	278	10	1x14	50	$5,57\text{-}6,60x10^9$	<5% phase R	passed

Table 4. Analysis of vaccine quality loss Aimag and Soum level

Variable	N	Mean	Standard error
Vaccine quality loss_mong	17	0.238	0.032
Vaccine quality loss_oie_low	17	0.371	0.053
Vaccine quality loss_oie_high	17	0.268	0.058
Vaccine quality loss at soum	7	0.058	0.030
Vaccine quality loss at soum	3	-0.011	0.0176

9 A Mathematical Model of the Dynamics of Mongolian Livestock Populations

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9.1 Abstract

Subsistence livestock herding is an important component of livestock production in Mongolia. However, pasture degradation, extreme weather, desertification, livestock overpopulation, infectious diseases and limited government support increasingly threaten this livelihood. To better assess these afflictions, understanding the population dynamics of livestock is critical. Towards this goal, we developed a model of Mongolian livestock populations. Using the Leslie– Gower difference equation competition model, a discrete analog of the continuous Lotka– Volterra 2-species model, Mongolian livestock population dynamics were simulated in MATLAB. The model encompasses four species and is stratified by age and sex. Calibration of parameters is accomplished using official population data from 1970 to 2010; a turbulent time period that includes the socialist to capitalist market transition and two growth periods both followed by two dzuds (severe winter storms). Herders were surveyed and herd structures were sampled for parameter and model initial value estimation. The current model simulates the Tuv province (province) goat, sheep, cattle and horse populations. However, with more data collection, the intention would be to simulate all species populations in any province or soum (province subdivision). A ten-year simulation of future livestock populations predicts a more than two-fold increase in goat and sheep populations, a slight increase in cattle populations and a slight decline in horse populations. Preliminary validation with 2011 population data shows accurate estimation. Furthermore, a stable future livestock population was attained with the implementation of more than double the current culling rate. The model can be integrated in infectious disease transmission modeling, used as a tool for predicting the economic potential and support requirements of the livestock sector and used to illustrate the urgency of fostering sustainable management of livestock populations in Mongolia. This story of Mongolian

pastoral life presents an excellent opportunity to study a social-ecological system as well as to contribute in creating a sustainable, healthy and efficient Mongolian livelihood.

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9.2 Introduction

Pastoralism in Mongolia is a subject to the harsh environment of the continental northern latitudes. Additionally, in recent history erratic environmental conditions and human activity have increased pressure on the pastoral life. From around 1960 until 1990, the Mongolian livestock sector was organized into collective-based agricultural systems (Mongolian Society for Range Management, 2010). Planning, maintenance, training, infrastructure development and all general support were managed centrally. After the political change to a market economy in 1990, farming collective workers were given their share of the dismantled collectives in the form of livestock and jobless factory workers moved to the countryside. This sharp increase in inexperienced herders with little government support and oversight combined with universal constitutional rights of free access to all land and a new need for individual subsistence led to a rapid increase in livestock populations from 1992 until 1999 (Jamsranjav, 2009). A test of this fledgling livestock system came with two successive dzuds (harsh winters) during the winters of 1999–2000 and 2000–2001 where 11 million cattle, sheep and goats were lost. Most losses were due to a lack of access to food trapped under a frozen snow cover (Mahul and Skees, 2006). Too many animals were competing for scarce resources, especially after no institutions remained to manage reserve pasture grounds and emergency fodder (Jamsranjav, 2009). The effects of dzuds on the people of Mongolia are great, considering that 1 million people, or more than one-third of the total population, depend on livestock farming for their livelihood (Mongolian Society for Range Management, 2010).

These dzuds struck while overpopulation was beginning to accelerate pasture degradation and disease trans- mission. Pasture degradation is estimated to approach 70% (Trachtenberg, 2009). In 1991, 0.8% of the cattle population was infected with brucellosis and by 2002 the average prevalence was 2%. Zinsstag et al. (2005a) describe in their livestock–human brucellosis transmission model that in the year 2000, the Mongolian authorities started a mass vaccination of their ruminants. However, the onset of the vaccination campaign coincided with consecutive snow– storm disasters in the winters 1999–2002, the loss of millions of animals, and a break down in veterinary services.The herders rallied with the help of more mild weather and from 2002 to 2009 Mongolia's small ruminant and cattle population grew by 84% (National Statistical Office of Mongolia, 1970–2011). Another more devastating dzud during the 2009–2010 winter caused 9.7 million animal losses out of a record high of 44 million Mongolian livestock (Swiss Agency for Development and Cooperation in Mongolia, 2011). Many herders lost their entire herd and Mongolia‘s urban population has continued to swell, piling more stress on an already strained nation (National Statistical

Office of Mongolia, 1970–2011; Swiss Agency for Development and Cooperation in Mongolia, 2011).

The study of species population dynamics has accelerated since the mid-20th century. Competitive exclusion was studied by Thomas Park in respect to his experiments with two species of flour beetles (Park, 1948, 1954, 1957). The dynamics of fish populations were described mathematically in discrete, age-structured fashion by Beverton and Holt (1957). Leslie et al. (1968) and later Edmunds et al. (2003) honed the modelling of species competition in discrete time. More recently, interspecies competition was modelled by Pathikonda et al. (2009) in the context of wetland Irises.

Mongolian agriculture has been supported by models for vegetation growth, pasture productivity and brucellosis transmission (Center for Natural Resource Information Technology, 2011; Zinsstag et al., 2005a). The latter included a simple model for livestock population dynamics, which was fitted to official data from 1990 to 1999, but utilized no carrying capacity. A subsequent model analyzed the effect of mass vaccinations and carrying capacity on transmission dynamics (Alonso, 2007). Since infectious diseases such as brucellosis are transmissible between different livestock species, an understanding of multiple livestock population dynamics is important to understand disease transmission. Zinsstag et al. (2005a) extended this even to humans. In Mongolia, sheep, goats, cattle and horses usually graze together, using the same pasture. To assess the use of pastoral resources by livestock, a multi-species demographic model considering respective pastoral use in terms of reference livestock units are more appropriate than that of single species models. Our objective was to develop an age and sex specific demographic model to simulate past and predict future Mongolian livestock populations while considering varying socio-political and environmental factors.

9.3 Methods

Model description

We model livestock population dynamics with difference equations with discrete time steps of one year. The model is based on the Leslie–Gower competition model which uses a modification of the Beverton–Holt function and is a discrete analogue of the Lotka–Volterra two species differential equation competition model (Cushing et al., 2004; Leslie and Gower, 1958). The model includes four species (goats, sheep, horses and cattle) with a constant migration rate, constant per-capita birth and culling rates, and density- dependent mortality rates. We assume that the effect of a dzud on livestock populations is density dependent, while population dynamics during relatively stable environmental conditions are dominated by density independence. While normally population changes due to extreme weather are considered to be density independent, we believe that the dzud presents an exceptional case. Herders report a lack of access to food that was trapped under layers of ice and that animal losses were due to starvation. Therefore, most animal losses were as a result of competition over a constrained resource.We denote the state variables at time t for females and

males by X and Y respectively. We use two subscripts: the first subscript 1–3 denotes the age groups newborn, juvenile, and adult respectively and the second subscript (g; s; c; h) describes the species (goat, sheep, cow, or horse) respectively. Goat and sheep newborns, juveniles, and adults remain so from age 0–1, 1–2 and > 2 years old respectively. Cattle and horses spend two and three years, respectively, in the juvenile class, so cattle newborns are of age 0–1, juveniles of age 1–3, and adults are of age > 3, and similarly horse newborns are of age 0–1, juveniles of age 1–4, and adults are of age > 4. We represent the additional juvenile classes with a third subscript, (a; b; c) to represent the first, second, and third (for horses) year of the juvenile stage respectively. Therefore, for example, X2ca would represent female juvenile cattle of age 2, and Y 3 s would represent adult male sheep. Fig. 1 is an example graphical representation of the model dynamics of goats.

The four species follow a birth pulse pattern so that each age class models a cohort born around the same time. This is mainly due to the climatic conditions which favor

Figure 1. Goat model dynamics. Arrows represent flows of animals into or out of the population. Boxes represent state variables. The figure is meant to be a simple conceptualization of the model dynamics. Sheep are modeled with an equivalent schematic but cattle and horses have additional juvenile stages.

Synchronized birth in early spring. Furthermore, we assume that adult male populations do not limit reproduction so the number of newborn animals is proportional to the adult female population of that species. All four species live and feed on the same pastures so we assume competition between them and the density dependent competition between them and the density-dependent mortality is calculated from the total population of all species. Additionally, we assume that competitiveness and consumption rates vary for each species. However, we exclude camels and yaks from the model because they usually feed on separate pastures and there is less interaction between them and the four species considered in the model. The state variables for total population at time t of goats, sheep, cattle and horses are denoted G; S; C and H respectively

$$G(t) = \sum_{i}^{3} Xig(t) + Yig(t)$$
$$S(t) = \sum_{i}^{3} Xis(t) + Yis(t)$$
$$C(t) = \sum_{i}^{3} Xic(t) + Yic(t)$$
$$H(t) = \sum_{i}^{3} Xih(t) + Yih(t) \quad (1)$$

with juveline population of cattle given by

$$X(2c) = \sum_{\xi} \varepsilon_{i}\{a, b\}_{X} 2c\xi$$
$$Y(2c) = \sum_{\xi} \varepsilon_{i}\{a, b\}_{Y} 2c\xi$$

and the juvenile population of horses given by

$$X(2h) = \sum_{\xi} \varepsilon_{\{a,b,c\}_X} 2h\xi$$
$$Y(2h) = \sum_{\xi} \varepsilon_{\{a,b,c\}_Y} 2h\xi$$

We weight the animals' consumption of forage by its age and species type so that older animals consume more food than younger animals; and horses consume more than cattle, who consume more than sheep, who consume more than goats. No distinction is made between the consumption of males and females. The total food consumption at time t is denoted by A (t) and normalized to the consumption of sheep

$$A(t) = (\sum_{i}^{3} Cig(Xig(t) + Yig(t))x) + (\sum_{i}^{3} Cis(Xis(t) + Yis(t))x) + (\sum_{i}^{3} Cic(Xic(t) + Yic(t))x) + (\sum_{i}^{3} Cih(Xih(t) + Yih(t))x) \quad (2)$$

The model equations for goats are given by

$$x_{1g}(t+1) = b_g x_{3g}(t) + \frac{MgX1g(t)}{G(t)} \quad (3a)$$

$$x_{2g}(t+1) = \frac{X1g(t)}{(1+\alpha gdx12A(t)Kx1g)} + \frac{MgX1g(t)}{G(t)} \quad (3b)$$

$$x_{3g}(t+1) = \frac{X2g(t)}{(1+\alpha gdx12A(t)Kx1g)} + \frac{X1g(t)Kx1g}{(1+\alpha gdxDx3A(t)Kx1g)} + \frac{MgX1g(t)}{G(t)} \quad (3c)$$

$$Y_{2g}(t+1) = bgX3g(t) + \frac{MgY2g(t)}{G(t)} \quad (3d)$$

$$Y_{2g}(t+1) = \frac{Y1g(t)}{(1+\alpha gdY1gA(t)Ky1g)} + \frac{MgY2g(t)}{G(t)} \quad (3e)$$

$$Y_{3g}(t+1) = \frac{Y2g(t)}{(1+\alpha gdY2A(t)Ky2g)} + \frac{Y3g(t)}{(1+\alpha gdxDY3gA(t)Ky3g)} + \frac{MgY3g(t)}{G(t)} \quad (3f)$$

The equations for sheep are similar.

$$X_{1c}(t+1) = bcX3c(t) + \frac{McX1c(t)}{C(t)} \quad (4a)$$

$$x_{2ca}(t+1) = \frac{X1c(t)}{(1+\alpha cdx1cA(t)Kx1c)} + \frac{McX2ca(t)}{C(t)} \quad (4b)$$

$$x_{2cb}(t+1) = \frac{X2ca(t)}{(1+\alpha cdx2cA(t)Kx2c)} + \frac{McX2cb(t)}{C(t)} \quad (4c)$$

$$x_{3c}(t+1) = \frac{X2cb(t)}{(1+\alpha cdx2cA(t)Kx2c)} + \frac{X3c(t)}{(1+\alpha cdx3cA(t)Kx3c)} + \frac{McX3c(t)}{C(t)} \quad (4d)$$

The equations for cattle are given by

$$Y_{1c}(t+1) = bcX3c(t) + \frac{McY1c(t)}{C(t)} \quad (4e)$$

$$Y_{2ca}(t+1) = \frac{Y1c(t)}{(1+\alpha cdY1cA(t)Ky1c)} + \frac{McY2ca(t)}{C(t)} \quad (4f)$$

$$Y_{2cb}(t+1) = \frac{Y2ca(t)}{(1+\alpha cdY2cA(t)Ky2c)} + \frac{McY2cb(t)}{C(t)} \quad (4g)$$

$$Y_{3c}(t+1) = \frac{Y2cb(t)}{(1+\alpha cdY2cA(t)Ky2c)} + \frac{Y2ca(t)}{(1+\alpha cdY3cA(t)Ky3c)} + \frac{McY3cb(t)}{C(t)} \quad (4h)$$

The system of equations for horses is similar to that for cows but has two additional equations for the third juvenile horse stages, X_{2hc}, and Y_{2hc}.

The competition coefficient, α, represents the overall effect of environmental stress on that species. If the model were a homogeneous one-species model, α would be equivalent to the reciprocal of the carrying capacity. The parameter d modulates the effects of the competition coefficient for each age and sex class of that species. For example, younger animals are more susceptible to density-dependent mortality than older animals. The product of α and d is therefore the effect of competition on each individual of a particular age and sex class in a species. The parameter, k, is the reciprocal of the per capita survival of each age and sex class in the absence of density-dependent mortality. This per capita survival is usually dominated by the sale of animals to the market and slaughter for private use. As a reciprocal of a proportion, k1.Migration has become a considerable factor affecting the population dynamics of Mongolian livestock since the transition to a market economy. Today, herders are less constrained to their traditional seasonal pastures. Because of its proximity to Ulaanbaatar and the products and services provided there, Töv province has experienced increased immigration. Migration, m, represents the change in total animals by species per year due to the change in number of herder households. Multiplying m by the proportion of animals in a particular age/sex class gives the change in animals of that age/sex class due to migration. We ignore emigration of livestock because herders who leave Töv usually sell their herds to other herders in Töv so the livestock population does not decrease. The sale of animals to the market is captured by the parameter k. The main parameters of the model are summarized inTable 1.

Table 1

Description of types parameters of the model for livestock population dynamics. The model consist of five basic parameters that are further subdivided by species of which some are further subdivided by age and sex.

Parameter	Description	Level
b	Expected number of female offspring per one adult female over one year. Dimensionless	Species
m	Number of new animals entering the area in one year. Dimension: Animals	Species
α	Species-dependent resource availability. Dimension: 1/Animals	Species
d	Modulation of resource availability by age and sex of animal. Dimension	Species, sex, age
k	Reciprocal of per capita survival excluding the effects of density-	Species, sex, age
c	Weighting of food consumption by animals. Dimensionless	Species, age

Data collection

Livestock population and migration data were taken from the National Statistics Office of Mongolia (NSOM) to complete a population timeline from 1970 to 2010. Field- work was conducted during three separate vaccination and de-worming expeditions from May 18th–22nd, 25th– 27th and June 1st, 2011 to Töv province, Bayan-Önjüül and Sergelen soums and Ulaanbaatar. During the vaccinations, animals from 23 herds were counted by age and sex to acquire age/sex class distributions. A survey was conducted with each herder concerning topics such as slaughtering practices and animal losses from natural causes. Data was also used from focused interviews with veterinarians, a government census employee and lifelong herders.

Model calibration and simulation

The el was calibrated to available data for Töv province and the population dynamics of livestock were simulated for the period 1970–2020. Since the period from1970 to 2011 involved largely differences in socioeconomic and climatic factors, it was divided into seven separate periods, each with its own set of parameters values.

Table 2 Description of time periods separated for model simulation.

Period	Description	Level
(A)	1970-1991	Socialist
(B)	1992-1999	First post- Socialist growth
(C)	2000-2002	First post- Socialist dzud
(D)	2003	First post- Socialist dzud + 1
(E)	2004-2009	Second post-Socialist growth
(F)	2010	Second post-Socialist dzud
(G)	2011	Second post-Socialist dzud +1
(H)	2012-2020	Future growth simulation
(H')	2012-2020	Future stable population simulation

For example, due to extreme cold and poor nutrition, the percentage of live births is considerably reduced in the spring following a dzud winter. The periods are also delimited based on changing herder behaviour. For instance, as a result of heightened losses from dzuds, we observed very different age/sex class specific culling practices for dzud, dzud + 1 and normal years. We attempted to capture this behavior by gathering data from herders on their culling practices for years 2009, 2010, and 2011 deemed “growth” (normal), “dzud”, and “dzud + 1” respectively. The future period from 2012 to 2020 was simulated with two different assumptions. The period times and descriptions are summarized in Table 2.Parameter values were constant in each period but were allowed to vary across periods. However, weighting for species consumption, c, was kept constant across all periods. The parameter values for b, m, d, and c were determined from the survey data, NSOM, and expert. We attempted to capture this behavior by gathering data from herders on their culling practices for years 2009, 2010, and 2011 deemed “growth” (normal), “dzud”, and “dzud + 1” respectively. The future period from 2012 to 2020 was simulated with two different assumptions. The period times and descriptions are summarized in Table 2.Parameter values were constant in each period but were allowed to vary across periods. However, weighting for species consumption, c, was kept constant across all periods. The parameter values for b, m, d, and c were determined from the survey data, NSOM, and expert opinion. The parameter values for α and k were calibrated to data on a number of livestock from NSOM for some of the time periods and kept fixed for other periods. In two time periods, no calibration was conducted and these served as validation periods. Species forage consumption values are taken from the NSOM. Age class specific consumption is taken from the Food and Agriculture Organization's Livestock Development Planning System (LDPS2). Specifically, they are called System Specific Livestock Standard Units (sLSUs) (Lalonde and Sukigara, 1997). Cattle sLSUs were used for horses because of insufficient data. Consumption values are presented in Table 3.

Table 3

Consumption of forage by age class (x-axis) and species (y-axis). These parameter values are relative to the consumption of adult sheep and do not differ by sex.

Species by age class	1	2	3
Goats	0.45	0.63	0.9
Sheep	0.6	0.8	1
Cattle	2.4	4.2	6
Horses	2.8	4.9	7

Parameter values for birth rate, b, are taken from herder survey responses, expert opinion and the NSOM and represent live births per adult female animals of the respective species. The birth rate for newborn male and female animals is assumed to be equal. Data for migration, m, was collected from NSOM for each time period. Data for d was collected through herder surveys. Data on k for the dzud, dzud + 1, and second post-Socialist growth period was estimated from herder surveys. We describe the calibration of α and k (when not collected from herder surveys) in the order in which the calibrations were conducted (E, F, A, B, C) instead of in chronological order. These are followed by two periods of validation (G, D) and two periods of future simulations (H; H′). Period (E) 2004–2009: We chose to begin the model with 2003 for several reasons. Firstly, 2003 was a post-dzud year (dzud+1) and data was collected on age/sex class specific k, d and herd distribution in 2011, a dzud+1 year as well. This observed 2011 age/sex class herd distribution data was applied to the published Töv province population numbers for 2003 to estimate a herd distribution that could be used for this model as initial 2003 age/sex class specific population numbers. Secondly, a growth phase (for which the official annual population data is available) began in 2003 and ended in 2009, before the 2010 dzud.

Since it was not possible to gather cattle and horse intra- juvenile population distributions, they were estimated by assuming a 95% progression rate between juvenile years.

In period (E) we fit α to observed population data while fixing all other parameters. Period (F) 2010: A severe dzud occurred in 2010. The model represents this refitting α to the suddenly reduced there was no reliable data on k. Period (B) 1992–1999: For the first socialist growth period we used α, b, and d from period (E) and official migration data for period (B). We estimated k since in this period of upheaval and change, culling could not be estimated from other periods. Period (C) 2000–2002: For this period of dzuds, the values for k, d, and m were taken from period (F). Birth rates specific for period (C) were taken from NSOM. The values for α were estimated.

Although it is said that there were dzuds for only two consecutive winters, the NSOM indicates three consecutive years of increased animal losses. Therefore, dzud population dynamics are simulated for 3 years from 2000 to 2002.

Period (G) 2011: A post-dzud (dzud+1) period was simulated to predict the population of 2011 as a validation exercise. The parameter values for α, b, m, and d were used from period (E), while values for k were collected from herder surveys.

Period (D) 2003: The post-dzud period of 2003 is also used as a validation with parameter values from period (G) and initial population values from 2002 of period (C).

Period (H) 2012–2020: The first future simulation uses all parameter values from period (E) and assumes there are no further dzuds.

Period (H′) 2012–2020: The second future simulation estimated k to maintain population sizes at the levels of period (G) levels while fixing all other parameters at values from period (E). The period-dependent parameter values for all four species are shown in Tables 4–7. Simulations were run

with MATLAB and parameters were estimated using non- linear optimization to find the minimum difference between the squares of the difference of the logarithms of the simulated and observed populations.Table4, Table 5

Table 4 List of parameters, their values and units for the goat subsystem

Goats	Values for period								
Parameter	(A)	(B)	(C)	(D)	(E)	(F)	(G)	(H)	(H')
b_g	0.95	0.95	0.54	0.95	0.95	0.40	0.95	0.95	0.95
m_g	0	14789	0	0	14401	0	0	14401	14401
α_g [a]	9.27E-09	9.27E-09	6.97E-09	9.27E-09	9.27E-09	8.30E-09	9.27E-09	9.27E-09	9.27E-09
d_{x1g}	3.0	3.0	14.0	3.0	3.0	14.0	3.0	3.0	3.0
k_{x1g}	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
d_{x2g}	1.5	1.5	2.0	1.5	1.5	2.0	1.5	1.5	1.5
k_{x2g} [a]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
d_{x3g} [a]	2.0	2.0	13.0	2.0	2.0	13.0	2.0	2.0	2.0
k_{x3g}	1.69	1.18	1.10	1.09	1.18	1.10	1.09	1.18	1.61
d_{y1g}	3.0	3.0	14.0	3.0	3.0	14.0	3.0	3.0	3.0
k_{y1g}	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
d_{y2g}	1.5	1.5	3.0	1.5	1.5	3.0	1.5	1.5	1.5
k_{y2g} [a]	1.13	1.10	1.02	1.29	1.10	1.02	1.29	1.10	1.11
d_{y3g}	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
k_{y3g} [a]	1.39	1.55	1.20	1.24	1.25	1.20	1.24	1.25	1.89

[a] values were optimzed for respective period

Table 5 List of parameters, their values and units for the sheep subsystem

Sheep	Values for period								
Parameter	(A)	(B)	(C)	(D)	(E)	(F)	(G)	(H)	(H')
bs	0.90	0.90	0.64	0.90	0.95	0.40	0.90	0.90	0.90
ms	0	48289	0	0	18868	0	0	18868	18868
αs [a]	3.45E-09	3.45E-09	9.11E-09	3.45E-09	3.45E-09	2.52E-09	3.45E-09	3.45E-09	3.45E-09
dx1s	3.0	3.0	21.0	3.0	3.0	21.0	3.0	3.0	3.0
kx1s	1.00	1.00	1.00	1.00	1.02	1.00	1.00	1.02	1.02
dx2s	1.5	1.5	7.0	1.5	1.5	7.0	1.5	1.5	1.5
kx2s [a]	1.44	1.48	1.00	1.07	1.19	1.00	1.07	1.19	1.22
dx3s [a]	2.0	2.0	15.0	2.0	2.0	15.0	2.0	2.0	2.0
kx3s	1.37	1.45	1.07	1.10	1.21	1.07	1.10	1.2	1.52
dy1s	3.0	3.0	21.0	3.0	3.0	21.0	3.0	3.0	3.0
ky1s	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
dy2s	1.50	1.50	8.00	1.50	1.50	8.00	1.50	1.50	1.50
ky2s [a]	1.57	9.50	1.37	2.25	1.57	1.37	2.25	1.57	3.00
dy3s	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
ky3s [a]	8.81	2.13	1.15	1.54	1.70	1.15	1.54	1.70	10.00

[a] values were optimzed for respective period

9.4 Results

Fig. 2 shows population changes from periods (E) to (H). Based on the same parameter values as period (E) and in the absence of dzuds, the model predicts that we will again see rapid growth in period (H) that produces a more than two-fold increase in goat and sheep populations, a slight increase in cattle populations and a slight decline in horse populations. No stable population is found before the year 2020. Fig. 3 shows population changes from periods (A) to (D). The most important output from this time period is the resulting fitted culling, k. It is a rough estimate of the culling practices employed with central oversight. The model has been able to capture the post-socialist livestock population explosion that resulted from simply slightly decreasing culling practices. Increasing competition by re-fitting α to the sudden decrease in livestock populations during dzud winters or periods (C) and (F) (Figs. 2 and 3) produced a matching simulated decrease. Total and age/ sex class specific culling for goats are provided in Table 8 as well as the project culling rate needed to remain a stable population. Tabe6, Table 7 Fig 2 Fig 3 Table 8 Fig 4 When reliable parameter data is specific to maintain a stable population.

When reliable parameter data that is specific to the target Mongolian district is gathered, this optimized culling will be a very useful target for sustain able herding. For each species modeled, the summed percentages of slaughtering across all age/sex classes for Töv province are slightly less than the NSOM national data on slaughtering. Fig. 4 shows an example of the simulated age/sex class herd distribution of goats for the entire time frame of the model. The effects of socialist, dzud and dzud+1 culling practices on age/sex class herd distribution can be seen. The plot also shows the rate at which the age/sex class distribution returns to normal after a dzud strickes. We believe our model overestimates the size of the Y 3g population.

Table 8 as well as the project culling rate needed to remain a stable population. Tabe6, Table 7 Fig 2 Fig 3 Table 8 Fig 4 When reliable parameter data is specific to maintain a stable population.

One validation time point has already been reached with the publication of 2011 population numbers. Our model estimated a total goat population of 1,124,462 compared to the officially recorded 1,088,900 (National Statistical Office of Mongolia, 1970–2011). Further validation of the model is only possible with annual publications from the NSOM.

Table 6 List of parameters, their values and units for the cattle subsystem

Cattle	Values for period								
Parameter	(A)	(B)	(C)	(D)	(E)	(F)	(G)	(H)	(H')
bc	0.90	0.90	0.53	0.90	0.90	0.42	0.90	0.90	0.90
mc	0	9987	0	0	2344	0	0	2344	2344
αc a	1.28E-08	1.28E-08	1.28E-08	1.28E-08	1.28E-08	1.28E-08	1.28E-08	1.28E-08	1.28E-08
dx1c	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
kx1c	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
dx2c	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
kx2c a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
dx3c a	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
kx3c	1.39	1.39	1.02	1	1.08	1.02	1	1.08	1.29
dy1c	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
ky1c	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
dy2c	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
ky2c a	2.39	3.00	1.08	1.09	1.30	1.08	1.09	1.3	1.96
dy3c	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
ky3c a	1.22	2.99	1.28	1.29	1.21	1.28	1.29	1.21	2.31

[a] values were optimzed for respective period

Table 7 List of parameters, their values and units for the horse subsystem

Horses	Values for period								
Parameter	(A)	(B)	(C)	(D)	(E)	(F)	(G)	(H)	(H')
bh	0.9	0.9	0.41	0.9	0.9	0.51	0.9	0.9	0.9
mh	0	11345	0	0	3571	0	0	3571	3571
αh a	1.54E-08	1.54E-08	6.99E-08	1.54E-08	1.54E-08	3.07E-08	1.54E-08	1.54E-08	1.54E-08
dx1h	3.0	3.0	2.0	3.0	3.0	2.0	3.0	3.0	3.0
kx1h	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
dx2h	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
kx2h a	1.10	1.06	1.00	1.10	1.06	1.00	1.00	1.00	1.00
dx3h a	2.5	2.5	2.0	2.5	2.5	2.5	2.5	2.5	2.5
kx3h	1.21	1.21	1.07	1.14	1.21	1.07	1.14	1.21	1.22
dy1h	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
ky1h	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
dy2h	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
ky2h a	2.17	1.77	1.00	1.06	1.00	1.00	1.00	1.06	1.09
dy3h	2.0	2.0	1.3	2.0	2.0	1.3	2.0	2.0	2.0
ky3h a	1.26	1.29	1.13	1.18	1.26	1.13	1.18	1.26	1.35

[a] values were optimzed for respective period

Table 8 Percentage of total and age/sex class culling for goats. The parameters for culling, k were recalculated as percentages. Age/sex class cullingg percentage will not necessarely add up to total because these values represent culling from that age/ sex class specifically.

Period	culled from age / sex class (%)						
	X_{1g}	X_{2g}	X_{3g}	Y_{1g}	Y_{2g}	Y_{3g}	Total
(A)	0.00	0.00	40.89	0.00	11.56	35.42	22.01
(B)	0.00	0.00	15.55	0.00	9.31	27.8	12.33
(C)	0.00	0.00	9.26	0.00	2.25	16.53	7.00
(D)	0.00	0.00	8.5	0.00	22.2	19.55	9.54
(E)	0.00	0.00	15.11	0.00	9.17	19.94	10.29
(F)	0.00	0.00	9.3	0.00	2.3	16.53	7.00
(G)	0.00	0.00	8.51	0.00	22.24	19.55	9.54
(H)	0.00	0.00	15.1	0.00	9.17	19.94	10.3
(H')	0.00	0.00	37.98	0.00	9.84	47.08	23.78

[a] values were fit for that period

9.5 Discussion

We provide a four species demographic model of live- stock production in Mongolia. The model uses a resource constraint in the form competition and is fitted to official livestock population data. As well as estimating livestock population trends, we believe the strength of our model will lie with the use in augmenting other simulations. By integrating this model, accurately planning development interventions, environ- mental and pasture management, livestock production or ever, a simplified model is better than no model and an optimum balance between parsimony, available data and complexity should be found.

Our model predicts excessive growth and displays no sign of stabilization during the next 10 years. We believe a more than two-fold population increase cannot be sustained. More thorough data collection and further refinement of the parameter calibration process is needed before implementing this model on an official basis. Further improvement of the model includes exploration of stochasticity and spatial and environmental effects such as pasture degradation and disease. Statistical and sensitivity analysis should also be conducted, though increasing the model's complexity may provide difficulties. Parameter estimates may be biased because of the relatively small cross-sectional surveys. Ideally a livestock reporting system, as it is established in many industrialized countries should be considered, which allows tracing animals from birth to slaughter. Under Mongolian conditions, livestock cohorts of hundred thousand animals would possibly allow estimating productivity parameters with sufficiently high accuracy. For this purpose, modern mobile communication technologies could be combined with field validation at a lower cost when compared to field surveys only.

Our eventual goal is to use the model to predict population dynamics of all species at the province and/or sum level. Some of our most important results are in the form of knowledge of what data needs to be collected, and how it can be collected. More time needs to be spent with more herders to gather complete, thorough, statistically valid and accurate data on slaughtering, natural losses, migration, birth rates and herd distribution before more reliable projections can be made. It takes time, a good 4 x 4, a command of the model, and a command of the Mongolian language to gather the relevant responses from herders and data from the herds. Introduced by Zinsstag et al. (2005b) the concept of "one health" includes not only human and animal health but also environmental health. Nomadic herding in Mongolia is a scenario where health in a social-ecological system (HSES) is of importance (Zinsstag et al., 2011). Mongolia's situation represents an excellent example where human behavior, human and animal health, and environmental forces must all be considered together.

9.6 Conclusion

We attempted to capture the fluctuating socio-political and environmental factors of the last four decades to use in a comprehensive model of Mongolian livestock populations to predict future population trends. Using an expansion of the Leslie–Gower difference equation model, our model predicts that assuming mild conditions, Mongolia's livestock population will continue to grow exponentially throughout the near future. As a result and just as before, pasture degradation and disease burden will increase unless action is taken. As the recent decades have shown, dzud frequency has increased placing more urgency on addressing Mongolia's situation. Another dzud of the same magnitude as that of the winter of 2009–2010 may produce even more drastic losses because higher populations will depend on more taxed and fragile pastures. Our Mongolian colleagues are full of enthusiasm, energy and ideas and wish to improve the state of their country. With tragedy comes change. Mongolia's chief livelihood is again in the limelight and this awareness should be used to spur reform.

9.7 Conflict of interest

The authors declare no conflict of interest.

9.8 Acknowledgments

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10 Discussion

10.1 Human brucellosis

The simultaneous assessment of human and livestock using a multistage cluster sampling method was conducted in Sukhbaatar and Zavkhan province in 2010. The estimate findings of seroprevalence were: 27% (95% CI: 23.6-31.3) among herders (n=574), 6.2% (95% CI 5.5-7.1) in sheep, 5.2% (95% CI 4.4-5.9) in goats, 16% (95% CI: 13.7-18.7) in cattle, and 2.5% (95% CI: 0.8-7.6) in camels. More female than male participants were found to be seropositive (OR=1.7; P <0.0014).

A total of 2,856 rural people ranging in ages from 4 to 90 years participated in the study.The median age is 38. These participants were from Arkhangai, Govi-Altai, Khuvsgul, Selenge, Sukhbaatar, Umnugobi, Uvs, and Zavkhan provinces. The study was conducted during the period of November, 2011to January, 2012.

The overall apparent seroprevalence among the rural people was estimated to be 11% (95% CI: 10.0-12.1) ranging from 2.3% to 22.6% within the eight provinces. Seroprevalence was higher in females than in males (11.2% compared with 10.9%, p=0.029) High seroprevalence reflects re-emergence of human brucellosis in Mongolia since 1990. This increase is primarly due to the severe decline in government-run disease surveillance and control programs and; the simultaneous establishment of a private health system and privatization of livestock ownership [22].

Human serums were tested using the Rose Bengal Test (RBT) and indirect IgG and IgM ELISA. Studies showed that a titre of 1:160 diagnoses of active brucellosis non-endemic areas but titre of 1:320 and 1:640 or higher considered diagnosis of brucellosis in endemic areas [14].
In endemic areas similar to Mongolia subclinical infection with *Brucellae* organisms may lead to the presence of a high titre of *Brucella* agglutination in otherwise asymptomatic individuals. Rural people who herd livestock or have contact with livestock have a high titre of *Brucella* agglutinins without symptoms or signs of active diseases[14].

In a brucellosis endemic country like Mongolia, medical and veterinary sectors should use standardised diagnostic tests and reagents according to internationally accepted standards. Brucellosis diagnostic tests have to be validated with gold standard reference sera of the national serum bank. Therefore, it is of paramount importance to establish a national collection of gold standard reference sera from culture positive and culture negative humans and animals.

The national human and animal serum bank is essential for validation of the applied laboratory tests for conducting simultaneous human and livestock serosurveys in Mongolia. This would ena-

ble implementation of internal quality control of medical and veterinary laboratories, validation of different serological tests and improvement of overall quality of the national diagnostic system.
No such gold standard serum exists in medical and veterinary sectors in Mongolia. Medical and veterinary diagnostic systems have been using the competitive and indirect ELISA classified as positive and negative according to the manufacturer's recommended cut-off ranges set by a brucellosis free country serum. This is not suitable to interpret an endemic situation in Mongolia. Therefore, in the frame of this thesis the first livestock standard positive serums collected are the basis for a future national gold standard serum [31].

10.1.1 Human serological tests

In the absence of specific symptoms, human brucellosis is difficult to be distinguished from other febrile conditions such as: tuberculosis, typhoid fever, tularaemia and rheumatoid arthritis. Clinical suspected case has to be confirmed using laboratory tests. There is a lack of human brucellosis incidence data in Mongolia reflecting the difficulties of recognising a disease that lacks pathognomonic symptoms with the absence of a diagnostic capacity in soum health centre and provincial hospitals [15, 32]. The diagnosis of brucellosis depends on a history of occupational or consumer exposure and including travel history combined with significantly raised *Brucella* antibody titres [14].
Cultures should be performed when possible in pyretic phase of suspected case. Two or three 2-3 blood samples taken from different veins or body fluid from the patients. Blood samples should be used for isolating *Brucella* using Castaneda's biphasic solid and liquid media. In the acute brucellosis phase it is the agglutinating activity which dominates and superseded by the non-agglutinate activity as the disease progresses.

Non-agglutinating and blocking antibodies are common in brucellosis becoming agglutinating at pH $\leq$5. This can be detected by employing the Rose Bengal Test (RBT). RBT is rapid spot agglutination assay using antigen stained with Rose Bengal and suspended to low pH, usually 3.6-3.7 to detect IgM, IgG and IgA. It is often considered a qualitative test (classified as positive or negative) not effective in discriminating exposure from active infection in endemic areas. The RBT is very sensitive and like all other serological tests, it could sometimes give a false positive result [1,15]. Therefore, World Health Organisation is concerned that the low specificity of RBT results needed to be confirmed by indirect and competitive ELISA or Polymerase Chain Reaction (PCR). However, this recommendation is not suitable for an endemic country such as Mongolia because these tests are complex requiring a sufficient budget to run and trained laboratory staff. In a developing country like Mongolia, a simple confirmatory test is needed within the limited budget of small rural laboratories.

The modified RBT meets these requirements. For example; Serum dilutions allowing for a diagnostic titre established using five drops of 25 microliter saline dispensed on white tiles. Drop of 25 microliter saline the first mixed with an equal volume of the positive plain serum (1/2 serum dilution). Then 25 microliters of mixture transferred to the second drop of saline with help of a micropipette and mixed it to obtain ¼ dilutions. It means dilution from 1/8 to 1/32 dilutions is obtained by successive transfer and mixing start from 1/32 to ½ reverse ways. Finally, each drop test with an equal volume of (25 microliter) RBT reagent. A titre which is higher or equal than 1/8 provides 87-95% sensitivity in truely infected patients but increases specificity of the test. RBT is used again, with 25 microliter of the 1/4 serum dilution mixed with 25 microliter of RBT reagent (resulting in the 1/8 dilution). After mixing on a white glossy tile, it is rotated gently for up to 4-8 minutes before reading. Read for agglutination immediately after 4 minutes. Test result will classified as positive and negative based on visible spot agglutination. A positive test result indicates infection which will confirm diagnosis. Negative sera at ¼ should be confirmed using a different test, such as the complement ELISA. All patients according to this case definition have to be treated following proper treatment regime [32].

The modified RBT was used the testing the serum of 453 participants which had tested by competitive ELISA. By the standard RBT 138 participant serums were positive. Serum of 136 participant tested by modified RBT at ½ dilution, ¼ dilution, 1/8 dilution and 1/16 dilution. Twenty five serum of all participants were positive at 1/16 dilution.The modified RBT is increased test sensitivity for 100% and specificity for 97% [15].

For this reason, the modified RBT used as a confirmatory test and the standard RBT is a primary test in the Mongolian small rural laboratories at the soum. The modified test helps to identify infected individuals exposed over the years through their occupation or from direct contact with animals. This helps to identify diagnose asymptomatic infected people from infected symptomatic patients.

The RBT antigen used for routine diagnosis in Mongolia was tested independently at the CITA OIE/ EU Brucellosis reference laboratory in CITA, Zaragoza, Spain. The result showed that Indian produced Tulip™ RBT antigen had pH =3.62, sensitivity 88.5%, and specificity 100% which tested agglutination titer against the OIE International standard serum (OIEISS) international standard serum, RBT antigen should give clear positive reaction with 1/44.7 International Unit (IU) and it was positive reachtion with1/55 which supposed to be negative (not adequate) in these conditions. The sensitivity of the EU antigen fulfilled the OIE/EU standardization criteria and used as control resulted in 80.7% sensitivity and 100% specificity. The test was over-sensitive in detecting brucella non-agglutinating IgM, IgG and IgA among humans. This means that people who do not have the

disease but are classified as an infected person could be psychological affected by the positive test result and might receive the combined antibiotic treatment when it is not necessary.

10.1.2 The reported main symptoms in humans with seropositivity

All 572 participants from Sukhbaatar and Zavkhan provinces reported symptoms that occurred during the previous month. Participants reported that joint pain, back pain, weakness, night sweat, sleep disturbance, and neuralgia. These are significantly associated with seropositivity in the univariable analyses. Joint pain, muscle pain, weakness, night sweat, sleep disturbance, neuralgia and pains in legs and arm were reported by 93 participants who had experienced at least one of these symptoms.

Overall, 15.3% (95% CI: 11.3-20.0) of participants had seropositive results with reports of at least two of the main symptoms.

Reported clinical symptoms of at the time of the study were compared to the serostatus of particpants. Overall, 165 of the 316 (52.2%) brucellosis seropositive participants and 1186 of the 2540 (seronegative) participants reported symptoms. Among all seropositives, 36.7% reported more than three symptoms ($P < 0.001$). Headache, joint, back and muscle pain; night sweats and sleeping disturbances were significantly associated with brucellosis seropositive.

10.1.2.1 Estimate the proportion of clinical illness among the seropositive

The overall apparent seroprevalence among the rural people was estimated at 11.1% (95% CI: 10.0-12.1%) ranging from 2.3% to 22.6% in the eight provinces. The average duration of brucellosis seropositivity was estimated at 10.9 years using Kyrgys human data [33].

Keeping this constant, human incidence of apparent seroconversion is estimated to be 0.18% (95% CI 0.17-0.19) per year for the RBT. This means that on average 2000 (95% 1879-2110) persons per 100.000 are exposed to brucellosis annually in Mongolia.

On average 400 cases (95% 376-422) per 100.000 populations become as clinical brucellosis cases each year in Mongolia.

10.1.3 Estimation of underreporting of brucellosis situation

This is based on Zavkhan and Sukhbaatar's reports of new case data for underreported cases. In Zavkhan provinces there were no new cases registered in 2008 and 2 cases per 10.000 were reported in 2010. If only 10% of serological positive cases become clinically ill this means the Zavkhan province will have 23 new cases per 10.000 per year representing a ten fold underreporting compared to the official reported cases.

If Zavkhan province's results were extrapolated to the whole of Mongolia this would means that there would have been on average 6.650 new brucellosis cases (95% CI 5180-8370) in 2010. The

Sukhbaatar province reported 15 human incidences n 2010.It represents a 15 fold underreporting of human brucellosis cases in Mongolia.

10.1.4 Childhood brucellosis

In Sukhbaatar province, children under 10 years had 15.7% (95% 3.4-39.6) and those children from 10 year to 17 years old had 5.9% (95% 0.1-26.0%). Childhood brucellosis is a common indication- of an endemic situation. Transplacental transmission of *Brucellae* spp. from mother with active brucellosis may pass to the fetus. It was reported that preterm delivery of infants with active brucellosis was confirmed via culture. Nursing mothers with active brucellosis may transmit *Brucella* spp. to their infants via breastmilk. There was a reported case of *Brucella* spp isolated from nursing mother's milk. Children are more prone to many different diseases especially brucellosis when they live in rural areas but often have fewer or milder symptoms [14].

Seminomadic herders who live away from community centres do not have access to children's day care. Herder children often adopt new born livestock as pets. Typically, weak-born kids (goats) and lambs are brought in to the house and usually remain over 2 months.
Herders often place their young children [2 to 4 years in age] into sheep pens with stronger new born livestock, when both parents go to collect water or bring their herds from the pastures. A child with milder presentation of brucellosis is misdiagnosed sometimes in Mongolia. Three years old girl misdiagnosed but serological test results finally confirmed her diagnosis and after which she received appropriate treatment at the Sukhbaatar province hospital.

10.1.5 Risk factors of brucellosis

Seroprevelance of female participants (18.2%) were slightly higher than male (17.5%). A Kyrgyzstan study showed male participants had higher seroprevelance than the female participants [31]. In Arab countries, the males usually have a higher prevalence than females. The Mongolian situation is different to rest of the world. This may be probably due to the fact that nomadic herder families have different roles for males and females. Female roles revolve around the home and mainly involve milking, making dairy products, obstetric assistance for animals during lambing, and caring newborn animals to the house for protection from the extreme cold. Females also feed new-born animals in their homes, process internal organs of slaughtered animals for household consumption and are responsible for the feeding and caring of herder dogs.

Male roles involve caring of the herds, slaughtering animals for household consumption, skinning animals, shearing sheep and combing cashmere goats. Univariable analysis showed a significant association that 20-45 year old herders were at a higher risk than 10-20 year old participants. This means the older groups had a longer exposure to *Brucella* spp. The reason is younger groups live away from home while studying at secondary schools in the soum or provincial centers from Sep-

tember to June each year. Therefore, direct contact with infected livestock plays a very important role - especially during the lambing season where livestock shed *Brucella* spp. in large numbers to the environment through excretion [6].

Herders traditionally provide obstetric assistance during lambing. Also, they feed of weak newborn lambs and kids [goats] in their ger (traditional dwelling place) during the cold winter seasons [6, 7]. However, these exposure risks are not found to be significantly associated with seropositivity. Mongolian herders traditionally practice home slaughtering of animals for household consumption which includes a particular tradition of consuming partially cooked liver. The consumption of partially cooked liver was found to be significantly associated with the seropositivity of the people [14].

10.1.6 Human treatment and diagnostics at the province and soum level

Overall, 95 participants reported that they had been previously tested at the province or a tertiary health centres from Sukhbaatar and Zavkhan province. Thirty five people had brucellosis confirmed by serological test but only 57% of people received specific treatment- mainly (70%) at province hospitals.

Of the study participants, 2,7% reported (n=76) receiving treatment for human brucellosis in the past; the median time since past brucellosis treatment was 14 years (Q1=3.3 and Q3=20 years). There were significant differences between age groups in reporting clinical symptoms; the age groups of 20 to 44 tears and 45 years and older group reported more clinical symptoms. Female participants reported more headaches, joint, back and muscle pain, fatigue and sleeping disturbance than males.

Reported clinical symptoms at the time of study were compared to the sero status of the participants. Overall, 165 of the 316(52%) brucellosis seropositive participants and 1186 of the 2540 (47%) seropositive participants reported symptoms. Among all seropsoitives, 36, 7% reported more than three symptoms; among the seronegatives, 23% reported more than three symptoms.

Mongolian Ministry of Health revised the brucellosis case definition to increase access of rural populations to receive diagnosis at the soum health centres. The standard RBT is a screening test and the modified RBT is used as a confirmatory test at the soum level. However, Sukhbaatar and Zavkhan province's eight soum hospitals did not have RBT diagnostic reagents during the field visit. The local soum hospital's limited resource does not have a budget line for laboratory diagnostics.

Any person visiting a soum medical doctor complaining of symptoms; fever, chills, malaise, generalised aches and pains all over the body, joint and low back pain, headache, and general tiredness is not given serological test. Instead, a diagnosis is based on the obvious symptoms and is prescribes medicine for these symptoms-only. Therefore, this patient is not properly diagnosed and given a serological test and they are not registered as a brucellosis patient. Only serologically confirmed

case of brucellosis is entitled receiving the specific treatment which includes hospitalization for 10 days. Hospitilization costs are covered by health insurance but any outpatient treatment is an out of pocket expenses for the patient. As a result, the acute brucellosis patient becomes a chronic case because of not receiving adequate treatment.

The recommended treatment regime for an outpatient is 100mg of doxycycline to be taken orally twice a day plus 15mg/ kg of gentamycin given intravenously everyday for 7-14 days. This treatment can be combined with other antibiotics for 42 days for adults and children over 8 years old.

Pregnancy poses special problems with tetracycline and streptomycin, as those must be avoided. Instead a rifampin monotherapy is the considered the best treatment regime. Children under 8 yearss in age, have fewer and milder symptoms. The recommended childhood brucellosis treatment is a regime of rifampin-cotrimoxazole combination**.** Alternatively rifampin or cotrimoxazole plus gentamicin can be prescribed [1].

10.1.7 Prevention of human exposure through educational campaign

A mass vaccination of livestock has been conducted since the year 2000 up to the present. However, the best prevention for humans exposured to brucellosis is through educational campaign using mass media, new information and communication technologies. Thirty five percent of participants (n= 573) from Sukhbaatar and Zavkhan reported, TV is the main source of information followed by information received from doctors, veterinarians and others.

Brucellosis educational materials, brochures, and leaflets in the Mongolian language are collected and reviewed by a team of medical and veterinary experts. However, the educational materials and related hand-outs are formatted similar to a school text book providing facts about the *Brucella* spp. bacteria, and describing main symptoms in scientific jargon. Usually, these materials are not easily understood by the general public. The educational material consists mainly of text with poor quality photos and deficient in explaining means of prevention and where to get diagnosis and treatment. Only 1.000-1.500 copies were published due to a limited budget allocation.

An overall assessment showed the educational materials were produced only because of the financial support received from donors. The educational materials were not appealing to the general public because prevention, access to diagnosis and treatment information was not included.

Also, the materials had no instructions specifically for school children to understand. Governmental agencies do not give emphasis to educational programs for the general public. The educational material is usually provided only to local medical doctors with limited to distribute among people who are already sick.

The study team developed a brucellosis picture book with simplified information for school children. The book was reviewed by selected school children and it was edited the based on children’s

comment. A total of 500.000 copies of educational book were made available for distribution through the school system of 22 provinces.

The team reviewed brucellosis case reports from 1958-2010 which revealed the seasonality of brucellosis [spring and autumn] reflecting the traditional and cultural aspects of the Mongolian people. Herders and their family members are exposed to a high number of *Brucella* spp. during the lambing season of Feb to April and of May to June. The traditional home slaughtering of large numbers of livestock during November–December, further increases herder family exposure to infected animals and their meat products.
Seasonality provides the best timing for mass media information which should focus on brucellosis risks especially during January to April and October to December.
The educational materials need to be distributed to rural populations every year before the lambing season which means from the middle of January until April and during October -December. Generally, rural children who live in school dormitories return home at the end of May with many urban children live with grandparents during the summer months. Children's material should be distributed in May.

Currently, there are no online Brucellosis educational materials available in Mongolian language. The only online accessible material is related to donor funded projects available only on their project websites. Mongolia has been changing as with other countries such that majority of people have access to the internet and are able to search for information online. Therefore, brucellosis educational material should be put into the public domain for access by the general public.

Participatory approaches are more likely to engage the herder families, hot ails and opinion leaders and create awareness and motivation for people to change their behaviour and seek health care when needed [22]. The participatory approach stimulated herders and rural leaders to get involved in discussions of the brucellosis problem and to attempt to solve some issues, and to increase disease awareness by sharing and exchanging their opinions during field visits in Sukhbaatar and Zavkhan provinces. For example, during a participatory meeting with herders discussed about how to limit direct contact with aborted foetus and placenta during the lambing season. This resulted in decisions to make metal hooks to pick up placenta, to handle aborted foetuses with a shovel, to dig a pit for all aborted foetuses and to cover the pit adequately to prevent dogs from accessing the contents.

10.2 Stepwise brucellosis elimination approach

A step wise brucellosis elimination approach was developed by NCCR north and south for control of brucellosis in Central Asia. This approach was adapted to the Mongolian condition to control brucellosis.

10.2.1 Brucellosis reporting and data systems in Mongolia

A monitoring and surveillance system is of little value if the information results are not distributed to decision makers or directed toward control action. A standardized reporting system would allow policy makers and authorities to make rapid and accurate evaluations, analyse existing situations, and take immediate decisions and implement required actions to prevent and control brucellosis [34].

10.2.1.1 Public health reporting system

The Ministry of Health has primary, secondary and tertiary levels of surveillance which report monthly from soum, intersoum health centres to aimag health departments. These reports are sent from provincial health departments and the national centre for communicable diseases (NCCD) which in turn report to the national centre for health development [35]. Reports of brucellosis cases confirmed by a secondary serological test were only available if there were brucellosis diagnostic facilities at the reporting soum health centres and provincial hospitals.

Of the randomly selected 8 soum hospitals assessed by field team visited in 2010, none had diagnostic capacity for brucellosis. Zavkhan and Sukhbaatar provincial hospitals were also assessed during the field team visit and only Sukhbaatar provincial hospital had brucellosis RBT antigen for diagnosis.

Sukhbaatar provincial health department reported human brucellosis cases in 2009, 2010 and 2012 were 302, 412, and 384 respectively. Government brucellosis incidence estimates were 11, 15, and 14 per 100.000 populations in the same years [36].

The recent global burden of human brucellosis'systematic review assessing study design, sampling method, study level and diagnostic methods on human seroprevalence and incidence rate were not found in Mongolia [16].

The Zavkhan and Sukhbaatar's study result showed an apparent seroprevalence of humans 26% and 28.5% respectively. In Sukhbaatar, 170 new cases per 100,000 were reported in 2008 and 280 cases per 100,000 in 2010. In Zavkhan province, there no new human case reported in 2008 and two new cases reported in 2010.

An extrapolation of the results from Zavkhan to the whole country would mean that Mongolia had on overage 6650 (95% CI 5180-8370) new brucellosis cases in 2010.This would represent an incidence of newly reported cases of 2370 per 100,000 (95% CI 1850-2990).
By using the data from Zavkhan rather than Sukhbaatar province, a more conservative estimate is calculated. Assuming that 50% of the seroprevalence have clinical symptoms (which were the case in our study) the incidence of clinical brucellosis in Sukhbaatar would be 1310 (95% CI 1100-1540) per 100,000 and 1190 (95% CI 930-1500) in Zavkhan province. These results would incidate an underreporting of the annual incidence of clinical brucellosis by a factor of 4.6 (1307/280) in Sukhbaatar and by a factor of 59 (1188/20) in Zavkhan.

Against the national incidence of 150 humans per 100,000 populations in Mongolia in 2010, this represents a 15 fold underreporting. We took this conservative estimate to report in the study. The current reporting masks the current situation in Mongolia which results in decision makers not prioritizing a sufficient budget for soum hospitals.

Our simultaneous assessment of humans and livestock estimates showed seroprevalence was 27% (95% CI 23.6%-31.3%) among herders (n=574) in Sukhbaatar and Zavkhan in 2010. The same study design was used to assess human brucellosis in other 6 randomly selected aimags. The seroprevalence assessed among 2856 study participants from 609 nomadic camps from 31 districts in the eight provinces. The overall seroprevalence among rural people was 11% (95% CI 11.0-12.1%) ranging from 2.3% to 22.6% in the eight provinces. Within nomadic camps, 39% had at least one seropositive members reported. This equated to an annual incidence of seroconversion of 1145 per 100,000 and overall annual incidence of 229 clinical cases per 100,000.

10.2.1.2 Veterinary services reporting system

The Veterinary and Animal Breeding Agency (VABA) of the Ministry of Agriculture is responsible for reporting on livestock disease data, disease control, prevention, laboratory services, providing the information and communication. The VABA has a primary (village), secondary (province) and tertiary level information system reporting on a quarterly basis. A total of 723 private veterinary units are at the soum level reporting to 22 Provincial Veterinary Department and they report to VABA. Also, State Central Veterinary Laboratory reports to the VABA. The VABA reported 784 livestock brucellosis cases in year 2012 and 2011 which included: 1495 in cattle, 43 in sheep and 24 in goats [34].

In 2010, the simultaneous assessment of humans and livestock study result showed seroprevalence was 27% (95% CI 23.6%-31.3%) among herders (n=574), 6.2% (95% CI 5.5%-7.1 %) in sheep, 5.2% (95% CI 4.4 %-5.9 %) in goats, 16.0% (95% CI 13.7%-18.7%) in cattle/yaks, and 2.5% (95%

CI 0.8%-7.6%) in camels in Sukhbaatar and Zavkhan. Herd seroprevalence levels in the sampled population was 26.2% of sheep, 14.3% of goats, and 7.4% of the cattle herd were positive. The discrepancy in the diseases status information is explained by the fact that the study used in Mongolia was a cross sectional multistage cluster sampling method. There is no clear standardized system in the veterinary system set up.

Mongolia became a member World Animal Health Organization (OIE) in year1989. The OIE sent mission to evaluate Mongolian veterinary sector in 2008.The OIE evaluation team assessed that the Mongolian veterinary sector scored 1.8 out of 5 points. The OIE evaluation team reported veterinary technical decisions made based on non-scientific information, informal or irregular coordination of the information and lacked effective communication at the central and local levels [35]. This confirmed the lack of scientific evidence necessary to make correct decisions in disease control strategies. Private veterinarians reported disease outbreaks without standardized methods. Their reports are mix of active surveillance and inactive surveillance data. Most of the brucellosis endemic countries lack of scientific evidence on the prevalence of infected livestock [36].

Therefore, decisions of the control policy in Mongolia are based on raw data collected annually since the 1960s. Veterinarians are lack proper training on how to do data analysis and provide accurate evidence for the current status of brucellosis. Brucellosis control with herds is of paramount importance than only single cases reports from a specific soums or province. This situation is directly connected with the epidemiological curriculum missing at the School of Veterinary Medicine. For this reason, Mongolian veterinarians have a very limited understanding of basic epidemiology concepts. They do not know how to collect data, assign values, and lack skills lacking data analysis. Moreover, the chief veterinary officer is appointed by political parties. As a result, substantial changes occur during each political change in the country. The Mongolian veterinary system must be independent of political influence and veterinary school circulums should be updated to include a epidemiology.

10.2.2 Vaccination

Mongolia received WHO assistance when conducting brucellosis surveillance and reported the alarming situation of human and livestock infection during the 1960s. The estimated livestock prevalence was 17% in cattle, 3.5% in sheep and 2% in goats. The government of Mongolia took immediate action and implemented a test-slaughter strategy without WHO expert opinion. The control program was selected without expert opinion therefore it lacked scientific knowledge and understanding of proper control strategy. The test-slaughter strategy was stopped after two years because of the high seroprevalence of livestock required testing of 37.5 million animals. The testing strategy lacked specific sensitivity and specificity of the serological test, movement control of

nomadic herds, constant mixing of livestock. Also, no “brucellosis free” replacement animals existed in the country. The WHO team recommended adopting a vaccination strategy using Rev.1 vaccine for the small ruminants. The government of Mongolia implemented the mass livestock vaccination from 1975 to 1986. Over 30 million small ruminants were vaccinated during a ten year campaign. Human brucellosis cases declined from 48 (1974) to 0.23 case per 100,000 population in1985. Small ruminant seroprevalence dropped below 0.01% by1985.

Ninety percent of the livestock was owned by the government. The control of brucellosis was a government’s and communist party’s agenda. Communist party members mobilized during the vaccination campaign. A forementioned factors contributed to the success of vaccination campaign during the 1980s.

International experts recommended to the WHO that Mongolia commence mass livestock vaccination against brucellosis again. In 2000, the Ministry of Agriculture had budget the equivalent of US$ 10.5 million for a 10 year mass livestock vaccination campaign. A cross-sector economic analysis showed the mass vaccinations would be largely profitable to the Mongolian society (benefit-cost ratio of 3.1), if costs would be shared between the public health sector and the agricultural sectors. Cost-effectiveness could be at US$ 20 per averted Disability-Adjusted Live Years (DALY) [17]. However the mass livestock vaccination campaign which started in year 2000 apparently lost traction as reported cases of human brucellosis did not further decline after 2004 [37].
There was not any official report regarding the vaccination campaign, coverage rate or existing monitoring. Moreover, the vaccination program planned for only 25.7 million livestock in 1998. However, the small ruminat number doubled during the period of 1998 to 2009. As a result, small ruminant vaccination coverage did not reach the expected 80% livestock coverage during the campaign.
The OIE evaluation team (2008) emphasized the veterinary system had only 1.400 veterinarians providing the veterinary services which did not meet existing demand of the rapidly expanding number of livestock. Forty-five million livestock requirs approximately 25.000 veterinarians [35]. The field study result showed that during the vaccination campaign a veterinarian would need to vaccinate approximately 43.000 livestock during the vaccination campaign which belong to over 700-1200 nomadic herders -within little over one month. The current mass vaccination program has been extended until 2020 [38].

Mongolia’s vast territory was divided into three zones based on the estimated number of livestock, local brucellosis vaccine production capacity and the required number of available personnel and their capacity. Within the first zone, 14.2 million small and large ruminants were vaccinated in

2011. Approximately, 23.2 million livestock were vaccinated within the central zone in 2012. In 2013, 6.6 million livestock were vaccinated within the eastern zone [39].

The success of any immunisation process depends on several factors: vaccine efficacy, assurance the cold chain system is intact, adequate rehydration of the vaccine and the application of the vaccination individual animal. Therefore, a quality control of the vaccines and the cold chain from production to administration to the individual animal is critical to the control of brucellosis. Control of the cold chain establishment is needed production, during transportation, storage at the province, and soum level, during the vaccination process and application to the invididual animals.

Therefeore, creation of a surveillance system would generate information to confirm and document systemic quality management thereby; assuring vaccine and vaccination qualityare implemented at all critical steps from production, during transportation, and storage up to the point of administration to animals.

The brucellosis vaccine control study results confirmed that showed that the vaccine CFU decreased the most during province level storage period over the three months. The problem can be solved by having the manufacturer store all brucellosis Rev.1 and S19 vaccines until end of July. The vaccines would then be transported by the cold chained trucks to the province storage facilities. Provincial veterinary services have cooling storage rooms to accommodate vaccines until the middle of August.

Moreover, it should be stressed to private veterinarians that they must use cool boxes during the vaccination period.The vaccine requires rehydration period of 15-20 minutes to make certain all vaccine cells are completely rehydrated.To assure success of the cold chain requires continuous monitoring and for quality assurance every year.

Annual mass vaccinations must be assessed to confirm all vaccination have been conducted properly. For this reason, a serological survey is needed and conducted 2 to 3 weeks after livestock vaccinations (the immunological response induced is highest during this period) [31]. A representative samples should be collected to accuratedly assessing the coverage of the livestock population targeted during the mass vaccination campaign. A representative sampling method must be published as a handout material and distributed to all team members responsible for assessing the coverage of the mass vaccination. To confirm success, the overall livestock vaccination immunization rate should reach 80% to ensure the disease transmission between animals can be interrupted. RBT reactors after vaccination [2- 3 weeks] should range 70% to 80% if animals are vaccinated properly [31].

10.3 Main circulating *Brucella* spp

The study results showed that *B.abortus* and *B. melitensis* is circulating between different livestock species and humans. *B.melitensis* is the dominant species among sheep and goats. *B.melitensis* strains isolated from sheep and goats were relatively close to human strains.This indicates that small ruminants are likely to be the main reservoir hosts and sources of human disease in Mongolia.

B.melitensis isolates from Mongolians appears to be closer to Kyrgyz strains-the so–called Eastern Mediternean group [40]. Study results also showed small ruminants brucellosis control must became higher priority than exists in the current national brucellosis program.

These collected strains from humans and livestock are the basis and justification for establishing a national brucellosis gold standard for serum in Mongolia.

11 Conclusion

Study results confirmed that a high seroprevalence exists among herders and the rural populations and indicate that annual reported cases are significantly underreported. This situation is the leading reason why brucellosis is not perceived as an important risk to public health thereby, limiting budget allocations for brucellosis treatment and diagnosis especially for primary and secondary hospitals.

Brucellosis is endemic countries similar to Mongolia medical and veterinary serological tests must become standardised accordance with internationally accepted reagents and standards. Medical and veterinary brucellosis diagnostic tests have to be validated with respect to adequate gold reference sera from the national serum bank [31]. This should be implemented to validate all serological tests including RBT. In rural Mongolia, the standard RBT (that is 25 microliter serum & 25 microliter RB reagent) is used for screening. The modified RBT is used for confirmation purposes at 1/8 or 1/16 dilution. A positive result indicating infection (confirmed) and clinic should document patient symptoms together with patient's history of exposure. Adult patients with confirmed acute brucellosis should be treated as outpatient with a doxycycline-streptomycin or a doxycycline-gentamicin combination for 42 days.

Livestock serosurveillance has shown that sheep, goats and cattle have higher than 0.01% incidence. Therefore, mass livestock vaccination is the best option of control for brucellosis. However, mass vaccination must be implemented with a vaccine cold chain quality control from production to application to assure, vaccine efficacy during the vaccination process. The government of Mongolia is dedicated to share the cost of mass vaccinations until the year 2020.

The *B.melitensis* isolates have indicated that small ruminants are likely the main reservoir hosts causing human disease in Mongolia. *B.melitensis* isolates from Mongolia appear to be closely related with Kyrgyz strains; the so–called Eastern Mediternean group [40]. Study result showed that small ruminants' brucellosis control is must become high priority than the current nationwide brucellosis program.

The importance of brucellosis mass vaccination, the monitoring of vaccination coverage and the requirement for a cool chain needs to be communicated with MoH, MoAI and MoF.

The livestock brucellosis control program should always be accompanied with disease awareness raising and communication programs. Herders must receive need to participate educational and intervention materials. The brucellosis educational material needs to be distributed to rural populations before the lambing season- middle of January until May. Materials should also be distributed

to the general public before May and June as a majority of urban people spend their vacations in rural areas

12 Research Outlook

12.1 One Health surveillance

The results of the simultaneous assessment of humans and livestock provided a better understanding of the current disease status in Mongolia. Thie simultaneous assessment of humans and livestock facilitated shared logistics and transportation costs, and provided medical and veterinary services for the herders who needed any assistance during the visit. This approach can be adapted for the study of the other zoonotic diseases like Q fever, rabies, and anthrax in Mongolia.

12.2 Enhance access to diagnosis of brucellosis patients

The under reporting of human brucellosis has caused true burden of brucellosis in Mongolia to be underestimated. For this reason, the decision makers did not allocate an adequate budget for diagnosis and treatment of human brucellosis. Also, soum health centres do not have an adequate budget to diagnose brucellosis in the community. Therefore, the rural people and herders do not have access to the diagnostic tests at the soum health centers. The standard RBT is a simple and cheap for diagnosing human brucellosis in rural settings. The modified RBT can be used as a confirmatory test at the soum health centres. MoH adapted this test to diagnose human brucellosis in rural settings. However, this requires training of the local medical staff in how to use the standard and the modified RBT. Therefore, the training of staff in Mongolia needs to be planned and implemented in nationwide.

12.3 Improve treatment regimes

The Mongolian medical sector puts more emphasis on inpatient care than outpatient care. With the current strategy, confirmed brucellosis cases or relapsed episodes require hospitalization for 10 days. The internationally recommended treatment is doxycycline-streptomycin or doxycycline-gentamicin combination for 42 days as an outpatient. This regime has been discussed with MoH that it be included in the human brucellosis diagnosis and treatment standard however, it has not been practiced widely. The current strategy needs to find a solution so that confirmed brucellosis cases are treated as an outpatient. This will require continue discussion with MoH, provincial hospitals and soum health centres staff.

12.4 Analyse the spatio-temporal change of MLVA

Geographical information on the Mongolian 60 strains is available. This information will used to analyse the spatio–temporal change of the brucellosis strains in different geographical locations. The several *Brucella* spp. strains collected in the 1970s are stored at the Hymalya Institite, Moscow, Russian Federation. The Russian and Mongolian research collaboration used stored old strains to study how *Brucella* spp has evolved over time.

12.5 Cross-border transmission dynamics with China

Mongolia borders with China in the south and the Russian Federation in the north. Sukhbaatar and Dornod provinces border with Inner Mongolia which reports the highest incidence of human brucellosis in China. Livestock and gazelles often cross the border from Mongolia in to Inner Mongolia and vice versa.

In China, Suis 2 vaccine has been used to control livestock brucellosis, however; Inner Mongolians are considering shifting to Rev 1 and S19 vaccines. Therefore, livestock and wild life cross border issues need to be addressed with Chinese research collaboration.

13 Research Outcome and Implementation

The results herein were presented to the medical and veterinary joint meetings at Ulaanbaatar, Mongolia in 2011 and 2012. A total of 218 rural veterinarians and medical doctors participated in "One health conference" which focused on how to collaborate on disease control and surveillance in 2011, 2012 and 2013.

The brucellosis vaccine cool chain results were presented to the decision makers in 2011 lead to action in setting up the cold chain in the veterinary sector in nationwide. Ministry of Food and Agriculture allocated funds and established vaccine storage rooms in 22 provinces and distributed cool boxes to private veterinarians in 2012 and 2013.

Since 2013, brucellosis vaccination monitoring and vaccine coverage survey have been implemented by MoFA.

MoFA adapted the brucellosis control strategy according to the internationally recommendation. National livestock brucellosis control strategy was approved by MoFA in 2015.

MoH changed and approved standard on human brucellosis diagnosis and treatment in 2015. As a result of the study human brucellosis confirmed cases have been treated as an outpatient for 42 days.

Medical and veterinary staff from 357 districts of 22 provinces attended training on how to diagnose human brucellosis using the standard RBT and the modified RBT from 2013 to 2015. Medical and veterinary staff from 22 provinces attended training on how to collaborate brucellosis control using "One health" approach.

Since 2011, brucellosis educational material was developed over 500,000 copies distributed to the secondary schools of Mongolia. Human brucellosis four minute programs developed and it has been broadcasted through national television channels from 2013 up to present during the lambing season.

14 References

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15 Curriculum Vitae

Zolzaya Baljinnyam was born 1975 in Ulaanbaatar, Mongolia.

Education

2011-2014	PhD student in the Human and Animal Health Unit, Swiss Tropical and Public Health Institute, University of Basel
2003-2004	Master's student at the Sydney School of Public Health, the University of Sydney, Australia
1998-1999	Master's student of Faculty of Veterinary Medicine, Mongolian State University of Agriculture, Ulaanbaatar, Mongolia

1993-1998	Bachelor of Veterinary Medicine, Faculty of Veterinary Medicine, Mongolian State University of Agriculture, Ulaanbaatar, Mongolia
1983-1993	Primary and secondary school at the School N0 10, Songino, Ulaanbaatar, Mongolia

Professional experience

2009-2016	Public health officer, Animal Health Project, Swiss Agency for Development and Cooperation SDC, Ulaanbaatar, Mongolia
2004-2008	Project manager of V.E.T Net NGO, Ulaanbaatar, Mongolia
1999-2003	Veterinarian of Vetnet project, JCS International, Ulaanbaatar, Mongolia

16 Appendices

Figure 1. Map of Mongolia study areas in *grey shade*

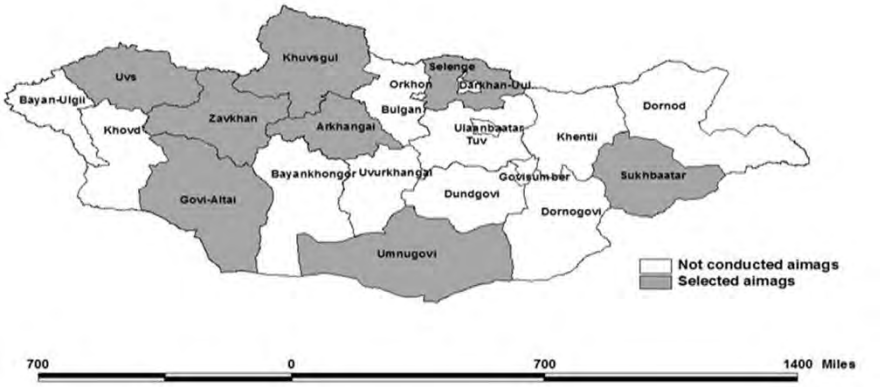


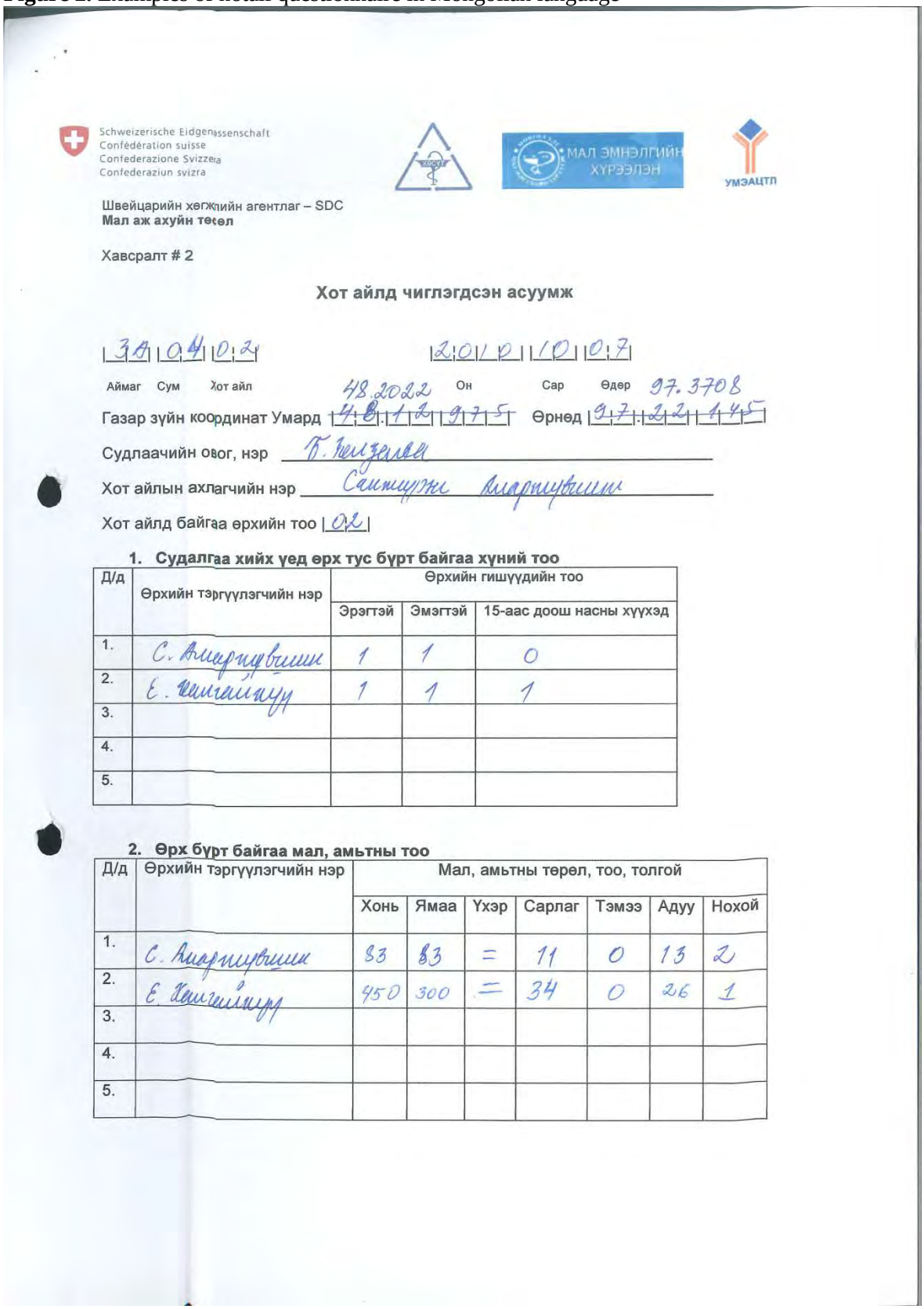
Photo 1. Collecting money bills by study team

Photo 2. Drawing money bills for random number

Photo 3. Randomly selected number

Photo 4. Field material distribution

Figure 2. Examples of hotail questionnaire in Mongolian language



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УМЭАЦТП

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Мал аж ахуйн төсөл

Хавсралт # 2

Хот айлд чиглэгдсэн асуумж

3	0	4	0	2		2	0	1	0	1	0	7

Аймаг Сум Хот айл 48.2022 Он Сар Өдөр 07.3708

Газар зүйн координат Умард | 4 | 8 | 1 | 2 | 9 | 7 | 5 | Өрнөд | 9 | 7 | 2 | 2 | 1 | 4 | 5 |

Судлаачийн овог, нэр Б. Энхзаяа

Хот айлын ахлагчийн нэр Сайнжаргал Амарсүрэнгийн

Хот айлд байгаа өрхийн тоо | 02 |

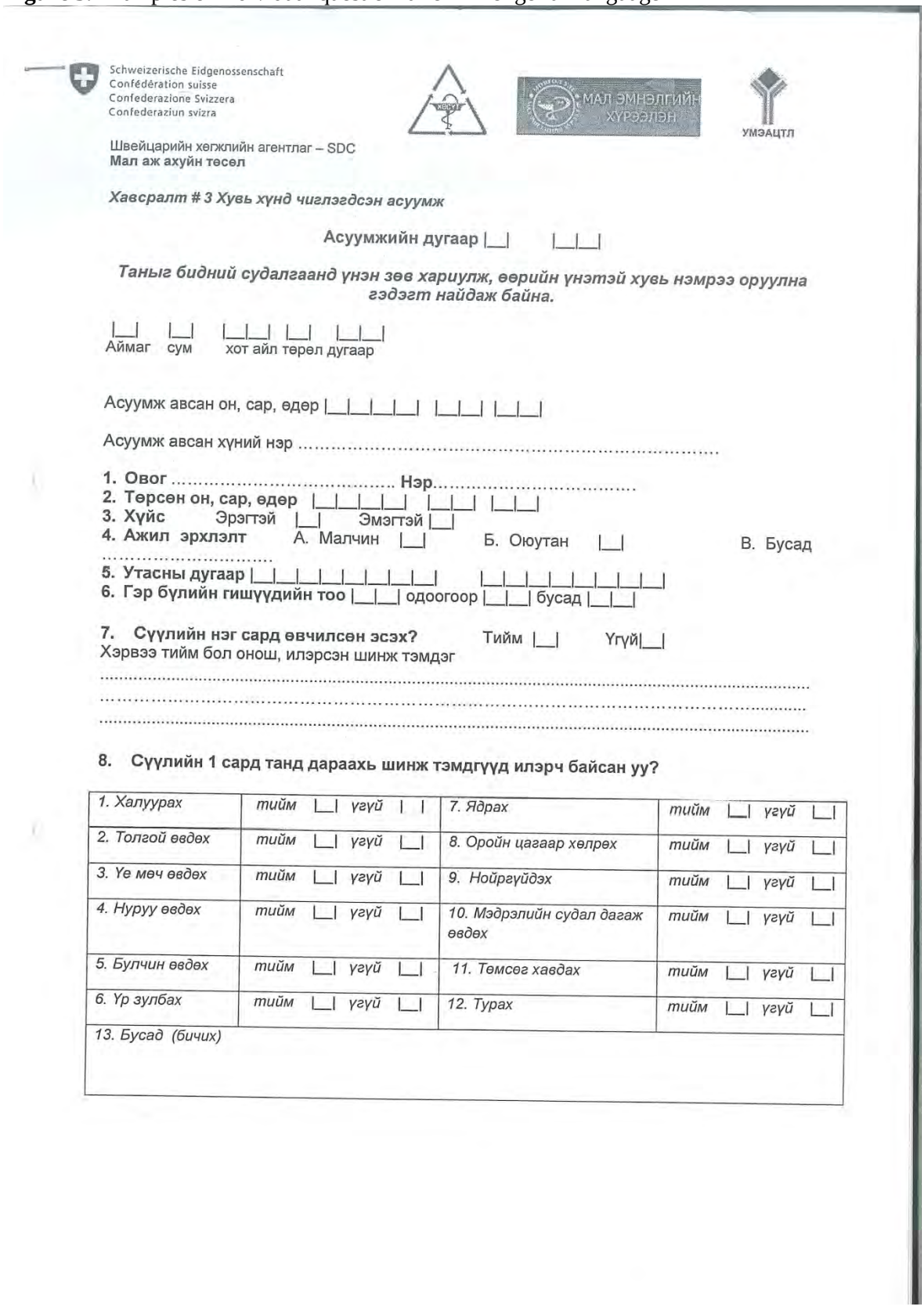
1. Судалгаа хийх үед өрх тус бүрт байгаа хүний тоо

Д/д	Өрхийн тэргүүлэгчийн нэр	Өрхийн гишүүдийн тоо		
		Эрэгтэй	Эмэгтэй	15-аас доош насны хүүхэд
1.	С. Амарсүрэнгийн	1	1	0
2.	Е. Цэнгэлмаа	1	1	1
3.				
4.				
5.				

2. Өрх бүрт байгаа мал, амьтны тоо

Д/д	Өрхийн тэргүүлэгчийн нэр	Мал, амьтны төрөл, тоо, толгой						
		Хонь	Ямаа	Үхэр	Сарлаг	Тэмээ	Адуу	Нохой
1.	С. Амарсүрэнгийн	83	83	=	11	0	13	2
2.	Е. Цэнгэлмаа	450	300	=	34	0	26	1
3.								
4.								
5.								

Figure 3. Examples of individual questionnaire in Mongolian language



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УМЭАЦТЛ

Хавсралт # 3 Хувь хүнд чиглэгдсэн асуумж

Асуумжийн дугаар |__| |__|__|

Таныг бидний судалгаанд үнэн зөв хариулж, өөрийн үнэтэй хувь нэмрээ оруулна гэдэгт найдаж байна.

|__| |__| |__|__| |__| |__|__|
Аймаг сум хот айл төрөл дугаар

Асуумж авсан он, сар, өдөр |__|__|__|__| |__|__| |__|__|

Асуумж авсан хүний нэр .....

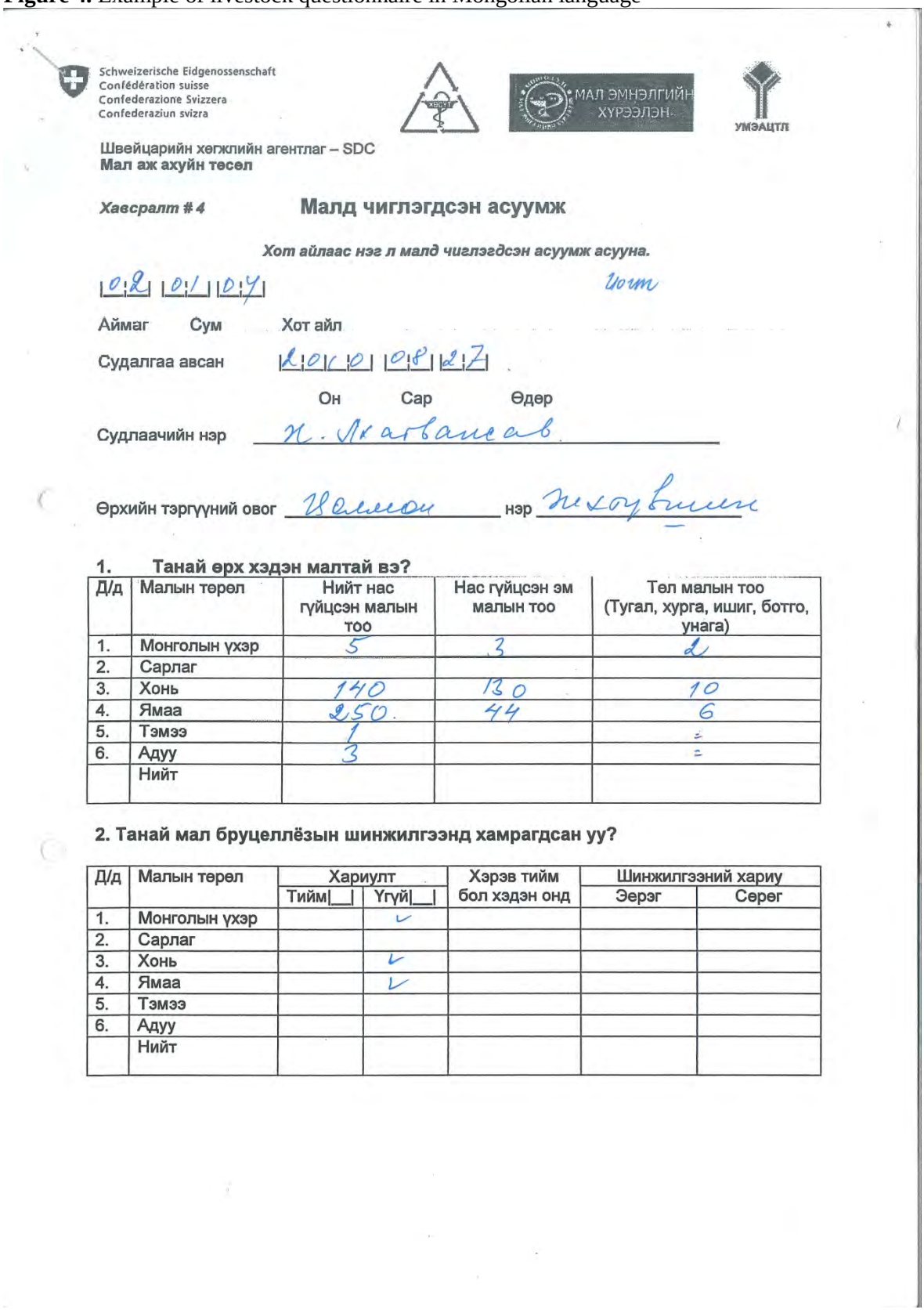
1. Овог ..... **Нэр**.....
2. Төрсөн он, сар, өдөр |__|__|__|__| |__|__| |__|__|
3. Хүйс Эрэгтэй |__| Эмэгтэй |__|
4. Ажил эрхлэлт А. Малчин |__| Б. Оюутан |__| В. Бусад
.....
5. Утасны дугаар |__|__|__|__|__|__|__|__| |__|__|__|__|__|__|__|__|
6. Гэр бүлийн гишүүдийн тоо |__|__| одоогоор |__|__| бусад |__|__|

7. Сүүлийн нэг сард өвчилсөн эсэх? Тийм |__| Үгүй|__|
Хэрвээ тийм бол оноош, илэрсэн шинж тэмдэг
.....
.....
.....

8. Сүүлийн 1 сард танд дараахь шинж тэмдгүүд илэрч байсан уу?

1. Халуурах	*тийм*	__	*үгүй*		*7. Ядрах*	*тийм*	__	*үгүй*	__	
2. Толгой өвдөх	*тийм* |__| *үгүй* |__|	*8. Оройн цагаар хөлрөх*	*тийм* |__| *үгүй* |__|
3. Ye мөч өвдөх	*тийм* |__| *үгүй* |__|	*9. Нойргүйдэх*	*тийм* |__| *үгүй* |__|
4. Нуруу өвдөх	*тийм* |__| *үгүй* |__|	*10. Мэдрэлийн судал дагаж өвдөх*	*тийм* |__| *үгүй* |__|
5. Булчин өвдөх	*тийм* |__| *үгүй* |__|	*11. Төмсөг хавдах*	*тийм* |__| *үгүй* |__|
6. Үр зулбах	*тийм* |__| *үгүй* |__|	*12. Турах*	*тийм* |__| *үгүй* |__|
13. Бусад (бичих)								

Figure 4. Example of livestock questionnaire in Mongolian language



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УМЭАЦТЛ

Швейцарийн хөгжлийн агентлаг – SDC
Мал аж ахуйн төсөл

Хавсралт # 4 **Малд чиглэгдсэн асуумж**

Хот айлаас нэг л малд чиглэгдсэн асуумж асууна.

| 0 | 2 | | 0 | 1 | | 0 | 7 |

Хот

Аймаг Сум Хот айл

Судалгаа авсан | 2 | 0 | 1 | 0 | | 0 | 8 | | 2 | 7 |
Он Сар Өдөр

Судлаачийн нэр Х. Лхагвасэв

Өрхийн тэргүүний овог Цэлмэн нэр Энхбилэг

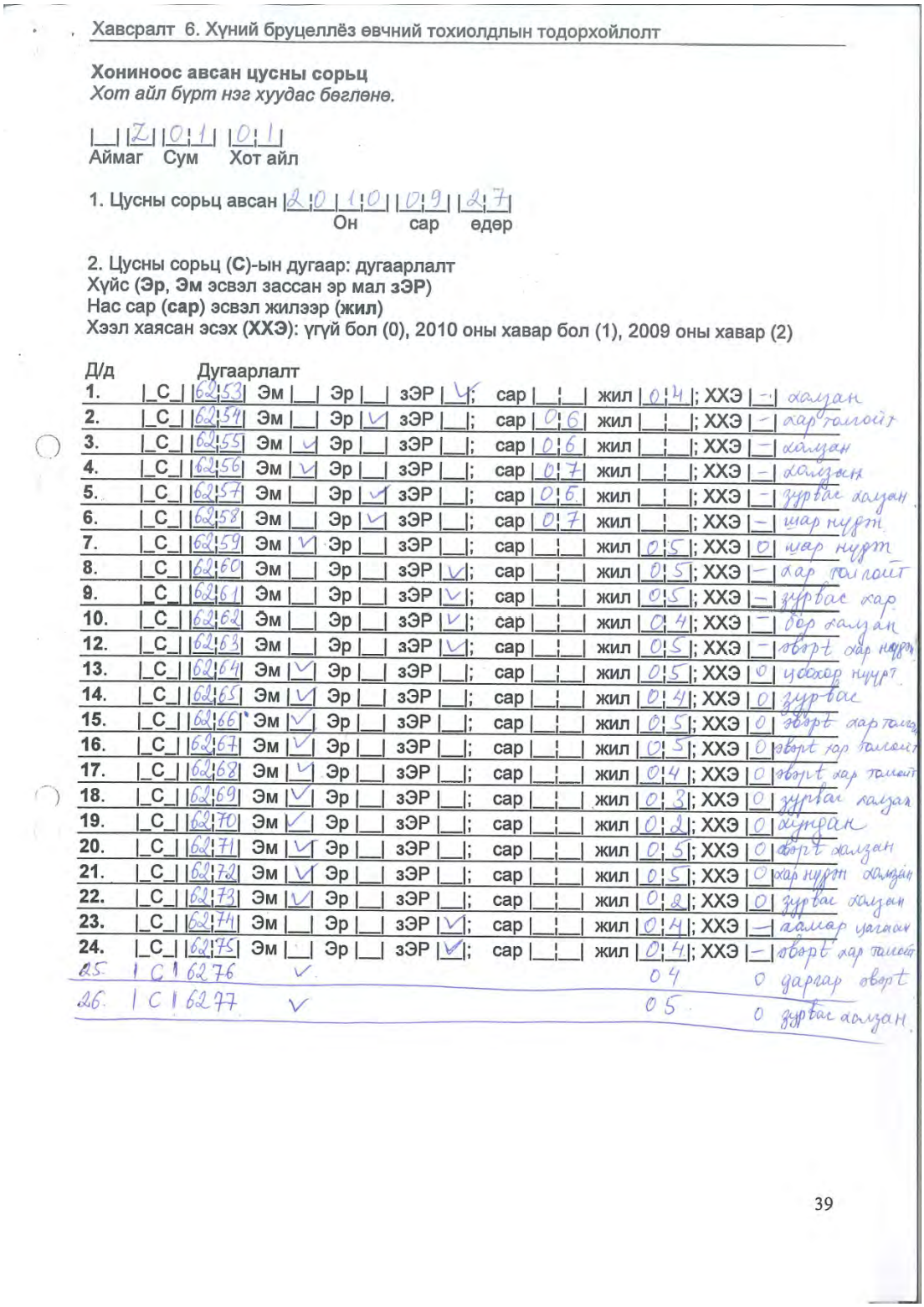
1. Танай өрх хэдэн малтай вэ?

Д/д	Малын төрөл	Нийт нас гүйцсэн малын тоо	Нас гүйцсэн эм малын тоо	Төл малын тоо (Тугал, хурга, ишиг, ботго, унага)
1.	Монголын үхэр	5	3	2
2.	Сарлаг			
3.	Хонь	140	130	10
4.	Ямаа	250	44	6
5.	Тэмээ	1		=
6.	Адуу	3		=
	Нийт			

2. Танай мал бруцеллёзын шинжилгээнд хамрагдсан уу?

Д/д	Малын төрөл	Хариулт		Хэрэв тийм бол хэдэн онд	Шинжилгээний хариу	
		Тийм__	Үгүй__		Эерэг	Сөрөг
1.	Монголын үхэр		✓			
2.	Сарлаг					
3.	Хонь		✓			
4.	Ямаа		✓			
5.	Тэмээ					
6.	Адуу					
	Нийт					

Figure 5. Example of livestock blood sample collection form in Mongolian language



Хавсралт 6. Хүний брүцеллёз өвчний тохиолдлын тодорхойлолт

Хониноос авсан цусны сорьц
Хот айл бүрт нэг хуудас бөглөнө.

	2	01	01
Аймаг	Сум	Хот айл	

1. Цусны сорьц авсан 20 10 09 27
Он сар өдөр

2. Цусны сорьц (**С**)-ын дугаар: дугаарлалт
Хүйс (**Эр, Эм** эсвэл зассан эр мал **зЭР**)
Нас сар (**сар**) эсвэл жилээр (**жил**)
Хэзээ хаясан эсэх (**ХХЭ**): үгүй бол (0), 2010 оны хавар бол (1), 2009 оны хавар (2)

Д/д	Дугаарлалт							
1.	С 62:53	Эм	Эр	зЭР ✓	сар	жил 04	ХХЭ –	халуан
2.	С 62:54	Эм	Эр ✓	зЭР	сар 06	жил	ХХЭ –	хар толгойт
3.	С 62:55	Эм ✓	Эр	зЭР	сар 06	жил	ХХЭ –	халуан
4.	С 62:56	Эм ✓	Эр	зЭР	сар 07	жил	ХХЭ –	халзсэн
5.	С 62:57	Эм	Эр ✓	зЭР	сар 06	жил	ХХЭ –	зүрвэс халуан
6.	С 62:58	Эм	Эр ✓	зЭР	сар 07	жил	ХХЭ –	шар нүүрт
7.	С 62:59	Эм ✓	Эр	зЭР	сар	жил 05	ХХЭ 0	шар нүүрт
8.	С 62:60	Эм	Эр	зЭР ✓	сар	жил 05	ХХЭ –	хар толгойт
9.	С 62:61	Эм	Эр	зЭР ✓	сар	жил 05	ХХЭ –	зүрвэс хар
10.	С 62:62	Эм	Эр	зЭР ✓	сар	жил 04	ХХЭ –	бор халуан
12.	С 62:63	Эм	Эр	зЭР ✓	сар	жил 05	ХХЭ –	эвэрт хар нүүрт
13.	С 62:64	Эм ✓	Эр	зЭР	сар	жил 05	ХХЭ 0	цэхэр нүүрт
14.	С 62:65	Эм ✓	Эр	зЭР	сар	жил 04	ХХЭ 0	зүрвэс
15.	С 62:66	Эм ✓	Эр	зЭР	сар	жил 05	ХХЭ 0	эвэрт хар толгойт
16.	С 62:67	Эм ✓	Эр	зЭР	сар	жил 05	ХХЭ 0	эвэрт хар толгойт
17.	С 62:68	Эм ✓	Эр	зЭР	сар	жил 04	ХХЭ 0	эвэрт хар толгойт
18.	С 62:69	Эм ✓	Эр	зЭР	сар	жил 03	ХХЭ 0	зүрвэс халуун
19.	С 62:70	Эм ✓	Эр	зЭР	сар	жил 02	ХХЭ 0	хунран
20.	С 62:71	Эм ✓	Эр	зЭР	сар	жил 05	ХХЭ 0	эвэрт халуан
21.	С 62:72	Эм ✓	Эр	зЭР	сар	жил 05	ХХЭ 0	хар нүүрт халуун
22.	С 62:73	Эм ✓	Эр	зЭР	сар	жил 02	ХХЭ 0	зүрвэс халуан
23.	С 62:74	Эм	Эр	зЭР ✓	сар	жил 04	ХХЭ –	халар унагаж
24.	С 62:75	Эм	Эр	зЭР ✓	сар	жил 04	ХХЭ –	эвэрт хар толгойт
25.	С 6276	✓				04	0	дарлаг эвэрт
26.	С 6277	✓				05	0	зүрвэс халуан

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Photo 5. Chocolate bar given for participant

Photo 6. Blood sample taking from camel

Photo 7. Sampling dog for study

Photo 8. Veterinary care for livestock

Photo 9. Centrifuging blood samples

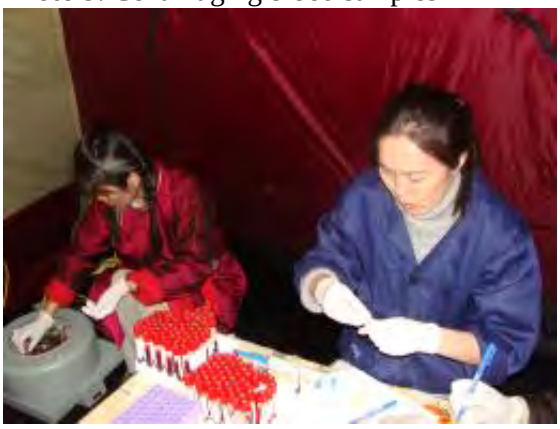

Photo 10. Field mobile laboratory