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EPIDEMIOLOGY OF BRUCELLOSIS IN LIVESTOCK AND HUMANS IN MONGOLIA

By

Amanda Elizabeth Fine

A THESIS

Submitted to
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in partial fulfillment of the requirements
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ABSTRACT

EPIDEMIOLOGY OF BRUCELLOSIS IN LIVESTOCK AND HUMANS IN MONGOLIA

By

Amanda Elizabeth Fine

The focus of this thesis is a study of the epidemiology of brucellosis in livestock and humans in the Khangai mountain region of Bayankhongor Aimag (Province), Mongolia. The study was cross-sectional in design and conducted at the herd-level using a stratified random sample (n=105) of the 5,062 cattle (predominantly yak) herds in the region. The brucellosis status of the cattle herds was determined by using a combination of the Brucellosis Ring Test (BRT), on milk, and the Brucellosis Card Test (CARD), on serum, to detect *Brucella* antibodies. Livestock management practices, the occurrence of brucellosis-like symptoms in humans who herded the livestock, human/livestock contact, and practices of livestock product consumption were recorded with the use of two standardized questionnaires conducted as in-person interviews.

The estimated prevalence of brucellosis-seropostive cattle herds in the region was 53%; +/-9.5% (BRT or CARD-positive) and/or 24.3%; +/- 8.1% (BRT and CARD-positive). Increasing herd size and the occurrence of abortions in cattle were associated with brucellosis-seropositive herd status. The estimated prevalence of the occurrence of brucellosis-like symptoms in the human herder population was 24%. The practices of consuming raw milk, preparing dairy products from raw milk, and keeping newborn livestock in human living quarters, were associated with the occurrence of symptoms suggestive of brucellosis in the human herder population.

This thesis is dedicated to the livestock herders of Bayankhongor Aimag (Province), Mongolia, in appreciation of their generous hospitality, and in admiration of their skill and dedication to life on the open steppe.

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The Agricultural Cooperative Development International/Volunteers in Overseas Cooperative Assistance (ACDI/VOCA)[1] Project office in Ulaanbaatar, Mongolia, provided logistical and financial support for the study. I would like to thank ACDI/VOCA project director, Ms. Jeanne Bartholomew, Bayankhongor Aimag (Province) ACDI/VOCA office manager, Mr. Shagdar, and ACID/VOCA volunteer, Dr. Earl Thompson. Brucellosis testing kits and reagents were provided by the United States Department of Agriculture, Animal and Plant Health Inspection Service, National Veterinary Services Laboratory, Ames, Iowa, USA. The Mongolian State Central

[1] ACDI/VOCA is a private, non-profit organization that promotes broad-based economic growth in developing countries. ACDI/VOCA's work in Mongolia was in the agricultural sector and funded by the United States Agency for International Development.

Veterinary Diagnostic and Sanitary Laboratory volunteered their assistance with additional laboratory testing.

I would like to thank the faculty, students and staff of the Epidemiology Department, Large Animal Clinical Sciences Department, and Population Medicine Center at Michigan State University, for providing technical support and encouragement to write up this study. In particular I would like to thank: my advisor, Dr. John B. Kaneene for his patience, gentle guidance, technical expertise, and willingness to take on this extra responsibility; and the members of my thesis committee, Dr. C. A. Reddy and Dr. Mathew J. Reeves, for their careful reading of this thesis and expert advice.

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INTRODUCTION

MONGOLIA: A BRIEF HISTORY

Mongolia is located in North Central Asia, bordered by the Russian Federation to the north and the Republic of China to the south (See Figure 1). Mongolia is the 5th largest country in Asia in land area with one of the lowest population densities in the world. The nation's human population of 2.4 million is distributed across an area of 1.56 million km^2 (1.54 persons/km^2). Approximately 1/3 of Mongolians (750,000) live in Ulaanbaatar, the national capital. Another 1/3 of Mongolians live in smaller cities and towns, and 1/3 of Mongolians are nomadic, moving seasonally in search of pasture for their livestock (Mongolia, 2001c).

Present day Mongolia, formerly "Outer Mongolia", gained its independence from China in 1921. By 1924, after a series of power struggles involving Mongolia's Buddhist religious leaders, Chinese military commanders, the White Russians and the Russian Communists, the Mongolian People's Republic was founded as the world's second communist state. Mongolia continued to be closely aligned with Russia and the former Soviet Union until the late 1980's. In 1990, Mongolia began its latest economic and political transition with the democratization of the political system and a change from a centrally controlled economy to a market-driven system (Rossabi, 1999).

Extensive livestock agriculture has been the backbone of the region's economy, shaping its nomadic cultural tradition, since the 13th century when the Mongols established the largest contiguous land empire the world has ever known under the leadership of Chinggis Khan. Present day Mongols are predominantly (85%) of the

Khalkha ethnic group. Khalkha Mongolian is the language spoken by 90% of the population. The adult literacy rate in Mongolia is over 90% and the predominant religion (96%) is Tibetan Buddhist Lamaism (CIA, 2004).

The region's climate, characterized by extreme temperatures with short growing seasons, has forced Mongolian herders to migrate frequently (2-10 times/year) in search of sufficient pasture, water and minerals for their livestock for centuries (Rossabi, 1999). The grassland steppe in central Mongolia continues to support the majority of the region's present day population of >30 million head of livestock. Although this system of migratory, extensive, livestock agriculture has been in place for centuries, the organizational structure of herds and the system of livestock ownership has experienced changes that reflect the political and economic transitions of Mongolia's contemporary history.

Before 1924, secular or religious nobles controlled pasture use in Mongolia and the seasonal migrations of herders and the livestock they tended. Between 1924 and 1990, pasture use and livestock management was controlled by Mongolia's Soviet-influenced socialist government. By 1960, all livestock herders in Mongolia had joined livestock cooperatives and they received a salary for herding state-owned livestock. The collective administration directed livestock migrations and subsidized transportation, the building of wells and veterinary care. Democratic elections in 1990, and the liberalization of the pastoral economy, led to the dismantling of herder cooperatives in 1992, and the privatization of the majority of the state owned livestock. Pastureland (81% of Mongolia's land area) remains state-owned until this day, and privately-owned livestock are herded in common pastures defined by the same districts (soums) and sub-districts (baghs) used during the collective era (Fernandez-Gimenez, 2000).

Today roughly 1/3 of Mongolians are nomadic pastoralists, moving seasonally with their livestock. Half the nation's population relies, either directly or indirectly, on the pastoral economy, and the livestock sector accounts for 36% of Mongolia's gross domestic product. These numbers actually represent an increase in both the numbers of individuals who identify "livestock herding" as their primary employment activity (186% rise since 1990), and those living as nomadic pastoralists. Between 1990, and 2000, the number of households herding livestock in Mongolia increased by 156% (74,710 to 191,526). This increase was seen not only in the absolute numbers but also in the relative percentage of households involved in livestock herding, which rose from 16.6% in 1990, to 34.6% in 2000. The increased reliance on livestock agriculture at the household level, and the liberalization of the pastoral economy, led to an increase in the number of livestock in Mongolia from 25.9 million in 1990, to a record high of 33.6 million in 1999, and then down to 30.2 million in 2000, as a result of losses during the dzud (drought and severe snow storms) of 1999/2000 (Mongolia, 2001c).

The dramatic changes of the 1990's have had a significant impact on veterinary care delivery in Mongolia. The level of State support for the livestock health sector began to decline in the 1980's as the large scale Soviet subsidies (once 1/3 of Mongolia's G.D.P.) dried up. Government sponsored livestock vaccination campaigns, including efforts to control zoonotic disease such as brucellosis, were significantly downscaled. In addition, in 1999, soum (district) veterinary clinics and their staff were privatized. This forced an overnight transition from government provided veterinary care to a fee for service veterinary care delivery system. The impacts of these drastic changes in the provision of veterinary care delivery on livestock health and productivity have not been documented.

A number of authors have raised concerns regarding the impacts of the recent political and economic transition in Mongolia on both human (Foggin et al., 1997; Purevdawa et al., 1997) and livestock health (Roth et al., 2003) due to the changes in the health care delivery system which have occurred over the past 10-15 years. The study presented in this thesis examines the epidemiology of one disease, brucellosis, in a single region of Mongolia, the Khangai mountain region of Bayankhongor Aimag (Province), Mongolia. Brucellosis is a good example of a disease of economic and public health importance that would be affected by a breakdown in national veterinary and human health care provision. Additionally, Bayankhongor Aimag (Province) has experienced the same trends in human population shifts, increasing livestock numbers, and the transformation of the veterinary care delivery system seen across Mongolia.

BAYANKHONGOR AIMAG (PROVINCE)

Bayankhongor Aimag (Province) is one of 21 Provinces that make up Mongolia (See Figure 1). Located in the southwestern portion of the country, Bayankhongor covers an area of 116,000 km^2. It is home to 85,300 people and 2.4 million head of livestock. The Khangai mountain range traverses the northern third of Bayankhongor where the majority of the Province's yaks are herded. Bayankhongor contains all three of the major steppe grassland ecological zones found in Mongolia. The northern 1/3 of Bayankhongor is in the "mountain steppe" ecological zone, the central 1/3 in the "steppe" ecological zone, and the southern 1/3 of the Province, that stretches south to the Gobi desert and the Chinese border, is comprised of "desert steppe" (Fernandez-Gimenez, 2000).

Bayankhongor Aimag is divided into 19 administrative districts or soums. Each soum contains approximately 4 sub-districts, called baghs. The soum and bagh system was set up across the country during the collective era in Mongolia to direct grazing patterns. Today, the majority of Bayankhongor's nomadic population migrates within a particular soum and generally rotates pastures within a single bagh. The study described in this thesis includes the 5 northern Khangai mountain region soums of Gurvanbulag, Zag, Jargalant, Galuut, and Erdentsogt, and the sub-district or bagh of Shargaljuut.

As in all Provinces outside the "city-Provinces" of Ulaanbaatar, Darkhan-Uul, and Orkhon, livestock herding is the predominant occupation and means of livelihood in Bayankhongor. The Province has experienced the same increase in numbers of livestock (1.6 million to 2.4 million), individuals involved in herding (149% increase), households herding livestock (176% increase) and overall percentage of households involved in herding (33.6% to 68.2%), documented in Mongolia as a whole from 1990, to 2000 (Mongolia, 2001b).

Veterinary service delivery, at the soum or district level, in Bayankhongor was privatized in 1999, as it was across Mongolia. A state-sponsored veterinary laboratory and service center still exists in the Province center where a small staff collects records of livestock disease outbreaks in the Province. The facility is only semi-operational and very few diagnostic tests are performed. The Province veterinary center does serve as a distribution point for available livestock vaccines and veterinary products that are now purchased and administered by private veterinarians. Since 1990, state-sponsored preventive veterinary medicine has been significantly scaled back in Bayankhongor, as it has been across the country. As of 2002, the last year livestock were vaccinated for brucellosis in Bayankhongor was 1988.

STUDY RATIONALE

Brucellosis is an economically important agricultural disease and a significant public health concern in many regions of the world (Halling and Boyle, 2002) (Nicoletti, 1980b). Measuring the burden of disease, and understanding the epidemiology of brucellosis in a region, are essential components of informed livestock disease and public health policy.

Brucellosis is reported to be an endemic disease in Mongolia, however, neither the extent of brucellosis distribution, nor the economic and public health impact of the disease is known (OIE, 2002b). A number of authors have suggested that the rapid political changes of the early 1990's, and the subsequent economic crisis, severely disrupted the national health care system in Mongolia leading to a rise in the incidence of multiple infectious diseases (Foggin et al., 1997; Purevdawa et al., 1997). Health officials in Mongolia today have highlighted brucellosis, along with hepatitis (B & C) and tuberculosis, as one of their top three infectious disease public health priorities (Ebright et al., 2003; Mikolon, 2000). The veterinary community has responded to these concerns with the reinstitution of a livestock brucellosis vaccination program which was began in 2001.

This study sets out to collect data on the epidemiology of livestock and human brucellosis in the Khangai mountain region of Bayankhongor Aimag (Province), Mongolia. Bayankhongor Aimag was selected due to the predominance of yak and cattle herding in the region, and anecdotal reports of brucellosis-like disease in the area. The type of information collected in this study will be essential in establishing an understanding of the current state of brucellosis in the region, and will form a baseline for future evaluation of local and national disease control programs.

Extensive livestock agriculture has proven to be the economic "safety net" through multiple periods of economic and political transition in Mongolia. The maintenance of a healthy national livestock herd is important not only to the ¼ of Mongolian households who herd the country's 30 million head of livestock for a living, but also for the entire nation who rely heavily upon the grassland steppe and the livestock it supports for their health and well being.

PROBLEM STATEMENT

The epidemiology of brucellosis in Mongolia today, both the distribution and prevalence of disease in livestock, and the burden of disease in humans, is largely unknown. Brucellosis control in Mongolia, in the form of comprehensive livestock vaccination and systematic surveillance, ceased to exist in the late 1980's as a result of the abrupt loss of economic aid from the former Soviet Union. Brucellosis surveillance in Mongolia from 1990, until 2001, has consisted of low-level and sporadic herd level testing of bovine, caprine, and ovine herds. Data on the prevalence and incidence of brucellosis in humans is based primarily on hospital case reporting. Published information on brucellosis in Mongolia after 1990, consists primarily of summaries of reports made by Mongolian government officials to the *Office International des Epizooties* or the World Health Organization.

Well-designed population-based studies that assess current levels of brucellosis infection in livestock and humans in Mongolia, and/or identify risk factors associated with disease, do not exist. Recent publications have identified brucellosis as an important public health concern in Mongolia, (Ebright et al., 2003) (Roth et al., 2003; Zinsstag et al., submitted 2004), but little to no disease data is presented. The collection of comprehensive and robust brucellosis data should be an essential component of Mongolia's new national brucellosis control program. Systematic disease surveillance will enable the veterinary and public health community to evaluate and improve the effectiveness of the recently implemented livestock vaccination program in Mongolia.

OBJECTIVES

This thesis, therefore, focuses on the epidemiology of brucellosis in livestock and humans in the Khangai mountain region of Bayankhongor Aimag (Province), Mongolia, where cattle (primarily yaks) are the predominant livestock species. The specific objectives of this study were to:

1) Determine the prevalence of brucellosis-seropositive cattle herds in the Khangai mountain region of Bayankhongor Aimag (Province), Mongolia
2) Identify the herd management risk factors for brucellosis-seropositive cattle herds in the region
3) Identify clinical signs or changes in production measures associated with the brucellosis-seropositive status of cattle herds in the region

4) Determine the prevalence of clinical signs and symptoms suggestive of brucellosis in the human (livestock herder) population in the region
5) Identify livestock product consumption and/or livestock management practice risk factors for the occurrence of clinical signs and symptoms suggestive of brucellosis in the herder population

OVERVIEW

Each chapter in this thesis has an abstract, introduction, hypotheses, methods, results and discussion, with the exception of Chapter 1, which is a literature review and does not have a hypotheses or methods. Chapter 1 is a review of the literature published in internationally available journals on brucellosis in Mongolia dating back to the 1960's. The material covered includes studies and accounts published in the Russian and German language as well as reports published in the English language. The literature review provides a historical account of brucellosis epidemiology, research and control in Mongolia, describes current brucellosis surveillance and control programs, and identifies areas for further research. Chapter 2 describes the prevalence of brucellosis-seropositive cattle herds in the study area and provides a detailed description of the sampling and testing methods used. Chapter 3 uses the brucellosis prevalence data presented in Chapter 2 and identifies livestock management practices that are risk factors for cattle herd brucellosis-seropositive status in the study area. Chapter 3 also describes clinical signs of brucellosis in the cattle population and identifies changes in production parameters related to disease. Chapter 4 describes the prevalence of clinical signs and symptoms suggestive of brucellosis in the human herder population. Risk factors for the occurrence of brucellosis-like disease in humans are identified. The human disease occurrence data are also analyzed for relationships with cattle herd brucellosis seroprevalence data presented in Chapter 2. The thesis includes an overall summary and recommendations for future research.

Chapter 1

BRUCELLOSIS IN MONGOLIA (1950-2002): A REVIEW

1.1 INTRODUCTION

Brucellosis is a zoonotic disease with a global distribution caused by bacteria of the genus *Brucella* (Corbel, 1989). Major efforts have been undertaken around the world to control brucellosis due the significant economic losses associated with the occurrence of the disease in domestic livestock and the often debilitating disease associated with human infection (Halling and Boyle, 2002; Reddy, 2003). Clinical signs of brucellosis in domestic livestock include late-term abortions, decreased milk production and lowered fertility in both males and females (Drost and Thomas, 1996). The disease in humans is associated with a number of nonspecific complaints including irregular or intermittent fevers, night sweats and headaches. Untreated brucellosis in humans can lead to chronic osteo-articular complications manifesting as joint pain, joint effusion and debilitating arthritis (Benenson, 1995; Black, 2004a; Young, 1989). Particular biovars of *Brucella melitensis* are associated with pathogenicity in different domestic animals. *B. melitensis abortus* is the biovar most commonly associated with brucellosis diseasein cattl (Reddy, 2003). The vast majority of human infection is the result of direct or indirect transmission of the disease from infected livestock to humans through the ingestion of contaminated milk or dairy products or through direct contact with aborted material or uterine fluid from infected animals (Nicoletti, 1989; Shapiro, 2004; Slack, 2004a).

Brucellosis is classified as a "List B" disease by the *Office International des Epizooties* (O.I.E.). List B diseases are "transmissible diseases that are considered to be of socio-economic and/or public health importance within countries and that are significant in the international trade of animals and animal products" (OIE, 2003). Livestock agriculture accounts for a significant portion of Mongolia's economy producing 1/3 of the country's gross domestic product (G.D.P.) and employing over half its working-age population (Mongolia, 2001b; Mongolia, 2001c). In addition, the livelihoods of over half of Mongolia's population are linked either directly or indirectly to the livestock sector (Fernandez-Gimenez, 2000). The econmic and public health impacts of brucellosis on a country so heavily reliant on livestock agriculture are significant.

Data on the historical occurrence of brucellosis in Mongolia is limited to a series of articles published in the international literature over the last 5 decades that describe a number of population based epidemiological studies, vaccine field trials and the implementation of brucellosis control programs in the region (Beulig et al., 1969; Jezek et al., 1972; Jezek et al., 1974; Otgon, 1968; Pinigin et al., 1968a; Pinigin et al., 1968b; Tserendash, 1980, 1984; Tserendash and Shumilov, 1970; Zherikova et al., 1972). Published data on the current status of brucellosis infection in the region is also limited but a number of recent publications indicate that brucellosis occurrence and control is still a major concern (Ebright et al., 2003; Erdenebaatar et al., 2002; Roth et al., 2003; Zinsstag et al., submitted 2004). The publish studies addressing brucellosis in Mongolia are described below and summarized in Table 1.1.

The objectives of this literature review were to:

1. Explore the historical data available on the epidemiology of brucellosis in Mongolia, drawing primarily on material available in the non-English veterinary literature published between 1960 and 1990.
2. Identify and discuss reports and sources of data on the current status of brucellosis in Mongolia.
3. Highlight information gaps and areas for further research that would facilitate the collection of meaningful data and broaden our understanding of the epidemiology of brucellosis in Mongolia.

1.2 BRUCELLOSIS RESEARCH IN MONGOLIA

A significant effort to study brucellosis in Mongolia has been in place since the early 1960's. Mongolian veterinarians, physicians, scientific researchers, and government officials have collected information on the occurrence of brucellosis in domestic livestock and humans for decades. The availability of that information to the international scientific community, however, has been heavily influenced by the political climate of the times. It was, and still is, primarily through collaborative research efforts with international scientists that Mongolians have the opportunity to submit their work for publication in international journals. The availability and location of information on brucellosis in Mongolia in the international literature, therefore, reflects patterns of international aid, and economic and political agreements between Mongolia and her political allies over the decades.

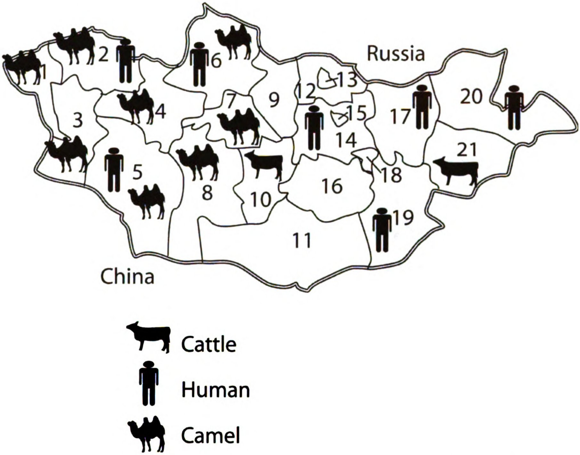
Publication of reports on studies of brucellosis in Mongolia conducted from the 1960's through the 1980's were the results of collaborative efforts between Mongolian and international scientists from the former Soviet Union and Eastern Europe. A few studies were published by Mongolian scientists in the Russian veterinary literature (Otgon, 1968; Tserendash, 1980, 1984). Other studies were published as co-authored manuscripts with scientists from the former Soviet Union (Pinigin et al., 1968a; Pinigin et al., 1968b; Tserendash and Shumilov, 1970; Zherikova et al., 1972) former Czechoslovakia (Jezek et al., 1972; Jezek et al., 1974), Hungary (Denes, 1997) and the former German Democratic Republic (Beulig et al., 1969).

More recent collaborative efforts in the field of brucellosis research in Mongolia have been between Mongolian veterinary specialists and scientists from Japan, Switzerland, South Korea, Germany and the United States. Literature published on brucellosis in Mongolia since 1990, includes an account of a veterinary assistance project administered by the Christian Veterinary Mission (JAVMA, 1998), a research note on the use of Agar Gel Immunodiffusion (AGID) testing to differentiate vaccinated from infected animals in Mongolia (Erdenebaatar et al., 2002), a study funded by the World Health Organization, Food and Agriculture Organization, Swiss Tropical Institute, Swiss National Science Foundation and Swiss Agency for Development and Cooperation (Roth et al., 2003) and an article on emerging infectious disease in Mongolia that highlights brucellosis as a disease of importance (Ebright et al., 2003).

1.3 BRUCELLOSIS SURVEILLANCE IN MONGOLIA

The presence of brucellosis in Mongolia was first reported in the international scientific literature in the late 1960's and early 1970's. Initial reports described the detection and identification of *Brucella* infection in yaks. These reports were followed by a series of epidemiologic studies carried out by the Mongolian National Veterinary Organization with assistance from the German Democratic Republic, Hungary, and the former Soviet Union. The early studies examined the distribution, prevalence, and incidence of brucellosis in the livestock and human population in various regions of Mongolia. Figure 1 indicates the specific aimags (provinces) and species for which historic brucellosis prevalence data are available.

Figure 1.1. The Aimags (Provinces) of Mongolia for which Historic Brucellosis Prevalence Data are Available for Livestock and Humans.



Key: List of Aimags (Provinces) and Cities (Ulaanbaatar and Darkhan-Uul)

1. Bayan-Olgi
2. Uvs
3. Khovd
4. Zavkhan
5. Govi-Altai
6. Khovsgol
7. Arkhangai
8. Bayankhongor
9. Bulgan
10. Ovorkhangai
11. Omnogovi
12. Selenge
13. Darkhan-Uul
14. Tuv
15. Ulaanbaatar (Capital)
16. Dundgovi
17. Khentii
18. Govisumber
19. Dornogovi
20. Dornod
21. Sukhbaatar

A. Brucellosis Testing

A number of internationally accepted methods of testing were used in the reviewed studies to diagnosis brucellosis in livestock and humans in Mongolia. The methods described included the following:

1. Allergic Skin Test: Also called the "Huddleson" reaction or the "Burnet" test. An intradermal (0.1 ml) or subcutaneous (0.5 ml) injection of brucellin allergen (an extract prepared from *Brucella*) is followed by injection site examination at 48-72 hours. A delayed hypersensitivity skin reaction develops specific to the genus *Brucella* in animals or humans sensitized by infection or recent vaccination. In livestock the injection site (usually the caudal tail fold) is observed and palpated for detect swelling. A 6-level scale for classifying the reaction in humans is used which ranges from a "++++ = red, indurated, grossly edematous lesion with necrosis, or a tendency to necrosis, at the center" to "0=absence of any lesion" (Alton and Jones, 1967).
2. Serum Tube Agglutination Test (STAT): The STAT is used to test for the presence of antibodies in the subject's serum produced in response to brucellosis infection. *Brucella* antigen is usually added to 5 narrow tubes containing 1:5, 1:10, 1:20, etc. of the serum under test and phenol saline. The degree of agglutination in the tubes is read after a 24-hour incubation at 37°C. The highest serum dilution showing 50% or more agglutination is recorded as the end-point (Alton and Jones, 1967).
3. Complement Fixation Test (CFT): The CFT is a serologic test designed to determine the *Brucella* titer of the serum under test. The CFT utilizes *Brucella*

antigen, complement and sheep red blood cells to measure the degree of hemolysis. The titer of the serum under test is read as the highest dilution showing 50% or less hemolysis. In cattle a titer of 1:40 indicates an infected animal and one of 1:20 is suspicious, indicating a need to retest in 2 weeks (Alton and Jones, 1967).

4. The Rose Bengal Test (RBT): A buffered *Brucella* antigen test used on serum. Standardized plates are used to observe the reaction between the subject's serum and the added standardized *Brucella* antigen. After a short period of rocking the plates are read and judged positive or negative based on the degree of agglutination (0=no agglutination to 3=coarse clumping, definite clearing) (Alton et al., 1988).

B. Livestock Brucellosis

The first international report describing the epidemiology of brucellosis in Mongolia was published in 1968 (Otgon, 1968). The author gives a brief history of brucellosis detection in Mongolia including the first laboratory confirmed case of brucellosis diagnosed in a cow from Selenge Aimag (a Mongolian Province bordering Russia) by a State laboratory in Songino, Mongolia, in 1932. In 1942, veterinary workers diagnosed brucellosis in the large "Orkhon" State sheep farm also located in Selenge Aimag and by 1951, brucellosis infection was confirmed in a number of State farms in several Aimags (Provinces) of Mongolia. Livestock were imported from both China and Russia during this time but the exact origin of brucellosis infection in Mongolia in unknown.

A national survey of livestock conducted by Otgon and the national veterinary organization in 1956, revealed that brucellosis infection was widely distributed across the country. The highly sensitive but less specific, allergic skin testing methods were used to identify infected livestock. The proportion of livestock with positive reactions was higher in the northern region of Mongolia (prevalence in cattle =4.7%; sheep and goats=3.7%) than in the south (cattle=2.1%; sheep and goats=0.02%). The author notes that there was a higher density of livestock in the northern regions of Mongolia (65% of the total population) and that livestock in the north where often clustered in and around open grazing areas. State farms were also more prevalent in the north where livestock were corralled during the winter months and provided harvested forage. Distribution of breeding stock from State farms in the north to smaller operations around the country also likely contributed to the spread of brucellosis in Mongolia (Otgon, 1968).

An article published in the German veterinary literature in 1969, describes brucellosis testing that was performed in Selenge Aimag (Province) as a collaborative effort between Mongolian veterinarians and veterinary specialists from the German Democratic Republic. The article provides details of the types of animals tested and the methods used for testing in Selenge. Cattle older than 1-year and rams were tested for brucellosis using the serum tube agglutination test (STAT) and complement fixation (CFT) test. Goats and camels were tested using STAT and sheep, other than rams, were tested with allergic skin tests. Brucellosis prevalence data is not presented but it was noted that positive animals were identified and extensive training on diagnostic techniques for brucellosis was provided to Mongolian veterinary specialists (Beulig et al., 1969).

More extensive data on livestock brucellosis in Mongolia are provided by two reports by Pinigin et al., 1968 (Pinigin et al., 1968a; Pinigin et al., 1968b). The first report describes the typing of *Brucella* strains isolated from a number of yak raising regions in Central Asia and Siberia, including Mongolia. The second report contains seroprevalence data on yaks that originated in Mongolia and were imported into the Autonomous Soviet Socialist Republic (ASSR) of Tuva in 1961, as well as data on yaks from Mongolia delivered to two slaughterhouses in the Irkutsk region of Russian and the ASSR of Buriatia in 1962.

The first report by Pinigin, et al. 1968, describes the examination of 20 *Brucella* cultures, 17 isolated from yaks (aborted fetuses, internals organs and lymph nodes), 2 isolated from dogs and 1 isolated from a raven in the yak brucellosis "epicenters". One of the cultures described was obtained in Mongolia and the others were from the two former ASSRs of Tuva and Buriatia that border Mongolia to the north. The methods used to type the *Brucella* cultures were those approved by the *Brucella* taxonomy subcommittee and united expert committee of the Food and Agriculture Organization (FAO)/World Health Organization (WHO) on brucellosis. The morphological, serological and biochemical properties of each of the isolates were largely identical. They were all classified as *Brucella abortus*, biotype 3 (Pinigin et al., 1968b).

In a second article Pinigin et al., 1968, present slaughterhouse data from the testing of yaks of Mongolian origin in 1961 and 1962. The Mongolian yaks tested in 1961 were imported into the ASSR of Tuva. Serum tube agglutination testing (1:100 = positive) of 121 animals indicated that 44 or 37% were strongly positive. The same serum was tested with the Huddleson (allergic skin test) reaction and produced 109 positives, a prevalence of 90%. The author dismisses the Huddleson reaction results due to what was interpreted as an unacceptable number of false positive results. The STAT, however, is less sensitive and more specific than the allergic skin testing methods so the discrepancy between the results using the different tests (STAT=37% positive and Huddleson=90% positive) would be expected.

A second group of yaks from Mongolia were tested at two slaughterhouses in the Irkutsk region of Siberia in 1962. In this study the STAT and the complement fixation test (CFT) were used. The CFT is often used as a confirmatory test because it is generally more specific than the STAT. Of 1,404 animals tested with the STAT at one slaughterhouse, 308 (22%) were positive. The CFT was run on 1,378 of the same group of animals yielding 151 positives or a CFT seroprevalence of 11%. Testing preformed at the second Irkutsk slaughterhouse involved 394 Mongolian yaks. Of this group 254 of 394 yaks (64%) were classified as positive according to the STAT and 67 of 370 yaks (18%) were recorded as positive based on the CFT (Pinigin et al., 1968b). Although the exact origin of the Mongolian yaks is unknown, these initial reports confirm that brucellosis was present in the yak population of Mongolia by the early 1960's. The serologic evidence of brucellosis in this population was supported by the reported isolation of *Brucella abortus* from aborted material collected from Mongolian yaks delivered to the ASSR of Buriatia (Pinigin et al., 1968b).

Brucellosis in camels (*Camelus bactrianus*) appears to have been widespread in Mongolia in the late 1960's, with seropositive animals detected in each of the 8 Aimags (Provinces) surveyed (See Table 1.1). The relationship between the reported results of the testing of camels with the STAT vs. the CFT is contrary to what would be expected.

The data indicate that the CFT identified 4 times as many brucellosis-seropositive camels as the STAT when the opposite result is usually found when the two tests are used in other species. An explanation for this unexpected result is not given but Tserendash, *et al.*, suggest the use of both tests in combination for seroprevalence studies in camel populations. Clinical signs of brucellosis in the tested camels or attempts to isolate *Brucella* were not reported. A record of the isolation of *Brucella abortus* from camels in Mongolia was, however, found in other sources (Crawford et al., 1990).

C. Human Brucellosis

Mongolia is a country with a history of a significant level of brucellosis in both its livestock and human populations (Corbel, 1989; Joshi, 1991). Publications summarizing the distribution of brucellosis in the region imply that the burden of disease was similar to the pattern seen in the former Soviet Union where the incidence of brucellosis in humans was widespread by the 1930's and only began to decrease once livestock vaccination was instituted in the 1950's (Kolar, 1989). The collection of data on the epidemiology of human brucellosis in Mongolia began in the 1950's. Livestock vaccination did not begin until the 1970's as described in section 1.4. Although information specific to the epidemiology of human brucellosis in Mongolia is limited, the articles described below provide evidence of a significant burden of disease.

Medical clinics in Ulaanbaatar, the capital of Mongolia, have records of patients with brucellosis dating back to 1954 (Otgon, 1968). The first published population-based study of human brucellosis in Mongolia was conducted by Otgon in 1956, and involved the screening of 1,516 people. The population included slaughterhouse workers from Ulaanbaatar and livestock workers from 6 State farms in 6 Aimags (Provinces). The individuals were screened for brucellosis using allergic tests (Burnet and Huddleson). Thirty-four percent of the individuals tested had positive reactions to the test, indicating previous exposure to *Brucella.* Children as young as 6 showed positive reactions and both males and females were equally affected. On State farms where the prevalence of brucellosis infection in livestock was 3.7-4.7%, the prevalence of positive reactions in farms workers was 30.3%. Six percent of the individuals with positive reactions had clinical signs of brucellosis (Otgon, 1968). The observation that humans suffered from both mild and severe clinical disease and the fact that both small ruminants and cattle showed evidence of brucellosis infection, led the author to suggest that both *Brucella melitensis* and *Br. abortus* were present in Mongolia; however, isolation and typing of the agent was not performed. The study does establish the presence of significant brucellosis exposure in these two high-risk populations of farm and slaughterhouse workers.

Two studies published in the early 1970's represent the most complete data published on human brucellosis in Mongolia before disease control through livestock vaccination was instituted in the 1970's (Jezek et al., 1972; Jezek et al., 1974). The first study revealed that brucellosis was a serious public health concern in Mongolia, documenting evidence of *Brucella* antibodies in all age groups, in both sexes and across occupations in all regions of the country surveyed including the urban population. The first study consisted of a serologic survey of close to 5,000 individuals aged <4-60+ from the five Aimags or Provinces of Uvs, Govi-Altai, Khovsgol, Dornogovii, Dornod, and the national capital, Ulaanbaatar (See Figure 1). A multistage sampling method was used to select the clusters of 60-70 individuals. Approximately 500 individuals were screened in every age group (< 4, 5-9, 10-14, 15-19, 20-29, 30-39, 40-49, 50-59, 60+) with the exception of the 60+ age group that only contained 226 individuals. An additional 758 individuals with potentially high occupational risks for brucellosis infection were targeted and screened. The serum was tested with both the STAT and CFT. The prevalence of brucellosis-seropositive individuals of all age groups combined was higher in the rural population (4.4%) than it was in the urban population (1.7%). In both populations the prevalence increased with age with individuals in the 50-59 age group from the rural population having the highest seroprevalence (8.1%). There is also evidence of early childhood exposure in both the urban (1.1% prevalence in < 9 age group) and rural (2.4% prevalence in < 9 age group) populations. Individuals who identified themselves as shepherds (herding sheep and goats) had the highest prevalence of brucellosis seropositivity (9.9%).

A second study, designed to determine the incidence of brucellosis in Cenkhermandal soum (district) in Khentii Aimag (Province), supports the classification of brucellosis in Mongolia as a public health priority (Jezek et al., 1974). At the beginning of the study, serum samples were collected from over 1,000 individuals (ages 3 to 60+) of the 2,000 nomadic and settled Mongolians who made up the population of the Cenkhermandal soum (district). The serum was tested with both the STAT and the CFT. The initial overall seroprevalence of the Cenkhermandal cohort was 11.7% with a level of 10.2% in the settled and 13.5% in the nomadic population; similar to the national prevalence of brucellosis seropositivity reported in the first study (Jezek et al., 1972). However, prevalence levels higher than the national average (6.3% and 13.7%) were seen in both the settled and nomadic children ages <4-9 years. There were no differences in prevalence between the sexes and prevalence increased slightly with age. A higher prevalence (15.4%) was seen in individuals who classified themselves as agricultural workers vs. 10.7% prevalence in non-agricultural workers. The allergic skin test was also performed 989 of the 1164 individuals tested serologically. The overall prevalence of a positive skin reaction (raised edematous plaques of 10 mm or larger accompanied by erythema) was 21.4%.

The second examination of the Cenkhermandal soum cohort occurred 6 months later, which was 6-8 weeks after the end of lambing/kidding season. Of the 1,042 individuals who tested negative during the first examination, 901 were re-bled and their serum was tested with the STAT and CFT. The percent of converters in the 6 month period was 2.4% overall with 1.3% and 3.8% in the settled and nomadic population, respectively. The highest proportion of new positives was in the 20-29 year-old age category. The incidence of sero-conversion in the 20-29 year-olds in the nomadic population was 12.8%.

Recent studies of human brucellosis in Mongolia are not available. However, the World Health Organization (WHO) has indicated through various reports and publications that brucellosis remains a significant public health concern in Mongolia today (Joshi, 1991; WHO, 1998, 2003). Additionally, brucellosis has been highlighted as a re-emerging disease of public health importance in Mongolia by the WHO (WHO, 2001) and the Mongolian medical community (Ebright et al., 2003). The Mongolian Statistical Yearbook reported 850 cases of brucellosis 1995, 1,482 in 1999, and 992 in 2000 (Mongolia, 2001d). The method of reporting, case definition, and testing techniques used to identify these brucellosis cases is unknown. However, if they are new cases, the national incidence rate of human brucellosis in 2000, was 4.13 cases per

10,000. This indicates a return to the levels of brucellosis reportedly present in Mongolia (4.8 per 10,000) before livestock vaccination was begun in the 1970's (Mikolon, 2000).

1.4 BRUCELLOSIS CONTROL IN MONGOLIA

A. Historical Account

Vaccination of livestock against brucellosis in Mongolia began in the 1960's and 1970's with the use of the *Brucellosis abortus* Strain-19 vaccine in cattle and the *Br. melitensis* Rev-1 vaccine in small ruminants (Corbel, 1989; Tserendash, 1980). The vaccination program was deemed a success by Mongolian scientists who reported that there was a reduction in the number of livestock abortions recorded on farms (Tserendash, 1980) and by WHO officials who reported that the annual incidence of the disease in humans dropped to less than 1 case/10,000 according to Roth and Zinsdag et al., 2003. There are three scientific publications that evaluate the initial livestock vaccination program in Mongolia and explore the use of both the *Br. abortus* Strain-19 and *Br. melitensis* Rev-1 vaccine to control the disease (Denes, 1997; Tserendash, 1980, 1984).

The use of the *Br. abortus* Strain-19 vaccine in cattle in Mongolia was effective in controlling brucellosis but its use was associated with a short period of protection (not longer than 1 year) and the persistence of vaccine induced antibodies to *Brucella* that complicated subsequent testing efforts (Tserendash, 1980). The use of the *Br. melitensis* Rev-1 vaccine in cattle was investigated as an alternative to Strain-19 in hopes of showing equal protection but with a longer period of immunity and less vaccine-related false positive tests. This work, presented by Tserendash in a series of two articles

(Tserendash, 1980, 1984), provides data on a vaccine trial of Rev-1 in cattle, use of the Rev-1 vaccine in cattle in the field, and accounts of the relationship between brucellosis infection prevalence on farms and the percent of abortions recorded in the herd.

Tserendash compared the safety and effectiveness of the Rev-1 and Strain-19 vaccine for preventing brucellosis in cattle in a herd of 199 animals. He concluded that the two vaccines had similar characteristics in terms of safety since neither induced abortion in cattle when administered at 2-3 months of gestation. He also concluded that the Rev-1 vaccine was equal in efficacy to the Strain-19 vaccine because cows immunized with the two vaccines had similar abortion and calving rates when challenged with *Brucella*. In addition *Brucella* organisms were isolated from a similar percentage of challenged cows vaccinated with Rev-1 and Strain-19. It was unclear which strain of *Brucella* was used for the infection challenge though the use of both *Br. abortus* and *Br. melitensis* was mentioned. Further studies were performed in yaks and cattle of varying ages. Similar conclusions were drawn regarding safety and efficacy and it was also noted that post-vaccine serological reactions waned more quickly in calves vaccinated with Rev-1 than those vaccination with Strain-19 (Tserendash, 1980).

Despite the somewhat limited scope of the Rev-1 vaccine trial described above, the use of Rev-1 in cattle and yaks in Mongolia was extended to non-experimental herds in the mid 1970's through the early 1980's. Tserendash refers to the vaccination of over 100,000 head of cattle in multiple regions of the country. The use of the vaccine was associated with both a drop in the percent of reactors in the herds studied (14.3% to 5.3% after 2 years, to 0.2% after 3 years and to 0% after 4 years) and a parallel drop in the percentage of abortions in the herds (from 8.5% to less than 1%). There was also a drop in the percent of abortions attributed to brucellosis based on bacterial culture results (Tserendash, 1984). The reports by Tserendash also provide pre-vaccination seroprevalence data from multiple regions of Mongolia included in table 1.1.

A Hungarian sponsored livestock vaccination program implemented in collaboration with the Mongolian veterinary agencies to eradicate brucellosis from the cattle herds of agricultural cooperatives, State farms and small household stock, began in Mongolia in 1987, (Denes, 1997). The program consisted of brucellosis surveillance, the removal and slaughter of infected animals, and the systematic annual vaccination of healthy young cattle. The Hungarian project covered 7 Aimags in Mongolia including Bulgan, Dornod, Khentii, Selenge, Tov and 2 unlisted Aimags (Denes, 1997).

The Hungarian project was also concerned with the limited period of protection offered by the Strain-19 vaccine and the complications associated with vaccine-related false positive serological tests so opted to explore the use of Rev-1 in cattle. Citing encouraging findings reported by Tserendash (Tserendash, 1980, 1984), on the superior immunogenicity of Rev-1, Denes supported the large-scale use of the *Brucella melitensis* Rev-1 vaccine in cattle as an alternative to the *Br. abortus* Strain-19 product. The Hungarian vaccination protocol included the serological testing of all cattle, 20-25 days apart, before vaccination was begun. The cattle were tested with the Rose Bengal test (RBT), STAT and CFT. Brucellosis-seropositive cattle were marked for slaughter and healthy cattle were vaccinated. In the first year of the program all female cattle over 3 months of age (more than ½ a million head) were immunized with the Rev.1 vaccine. In the second year heifer calves older than 3 months, yearlings not vaccinated the first year, heifers older than 2 years and cows were vaccinated. The vaccine was administered subcutaneously at a dose of $5X10^9$ viable organisms in 2 ml of vaccine re-suspended in physiological saline. In herds formally vaccinated with Strain-19, the use of Strain-19 was continued using the same schedule as that outlined for Rev-1 above (Denes, 1997).

The prevalence of seropositive cattle observed by Denes ranged from 3.8% to 35%, and in the 20-25 days between the two initial testing periods an average of 10% more cattle sero-converted per herd (Denes, 1997). The combination of the high prevalence of brucellosis seropositivity and active disease transmission in these herds supported the institution of regular vaccination, and not a test and slaughter program, as the most effective and feasible method for controlling brucellosis in this population of cattle (Denes, 1997).

Denes followed the cattle herds vaccinated with Rev-1 as well as those vaccinated with the Strain-19 vaccine. The researchers found that cattle vaccinated with the Rev-1 had higher rates of post-vaccination seroconversion (92.7% in heifers and 90% in cows) than the cattle vaccinated with Strain-19 (68.8% in the heifers and 89.7% in the cows) though the authors note that this higher degree of antigenicity does not necessarily reflect a higher degree of protection from infection (Denes, 1997). Conversely, the positive serologic status of cattle 1 and 2 years after vaccination was greater in cattle vaccinated with Strain-19 (13.2% after 2 years) than in cattle vaccinated with Rev-1 (7% after 1 year). However, the presence of detectable antibodies to *Brucella* in proportion of the cattle tested by Denes 1 and 2 years after vaccination are likely a result of a recent natural *Brucella* challenge and not the result of previous vaccination.

Unfortunately a comprehensive evaluation of the livestock vaccination program begun in Mongolia in 1987, in cooperation with Hungarian veterinary experts, was never completed. The political and social changes that took place in Mongolia in the early 1990's effectively halted the brucellosis control program and prevented a final assessment of the Hungarian project (Denes, 1997).

B. Present and Planned Brucellosis Control Programs

Brucellosis control in Mongolia, in the form of comprehensive livestock vaccination and systematic surveillance, ceased to exist in the late 1980's as a result of the abrupt loss of Soviet economic aid (Mikolon, 2000). The brucellosis control program in Mongolia from 1990, until 2001, consisted of low-level surveillance and sporadic herd level testing for brucellosis in bovine, caprine, and ovine herds (Roth et al., 2003). In the early 1990's Mongolia reported to the OIE that the occurrence of bovine, caprine, and ovine brucellosis was "enzootic with a ubiquitous distribution" (OIE, 1990-1994), seemingly indicating that the Hungarian project described above had very limited success. Later in the decade the occurrence was reported as "low sporadic" and in 2001, and 2002, the reports of occurrence of bovine brucellosis were "based only on serological evidence of the disease" (OIE, 1996-2002). Data provided by the State Veterinary and Animal Breeding Department, Mongolian Ministry of Food and Agriculture, on the nationwide prevalence of brucellosis in cattle, sheep and goats between 1991 and 1999, indicated that the prevalence in cattle ranged from 0.80% to 3.04% and in sheep and goats between 0.50% and 1.11%. During this period (1990-2002) the Mongolian Ministry of Food and Agriculture reported a number of outbreaks of brucellosis in livestock as well as measures taken to control the disease in the form of slaughter, vaccination, quarantine and a modified stamping out policy (OIE, 1990-1994, 1996-2002).

Efforts to recreate and fund the latest brucellosis control program in Mongolia began in 1999. The World Health Organization (WHO) provided technical support for the preparation of a National Brucellosis Control Plan that called for the mass vaccination of ruminants and was due to begin in 2000 (Mikolon, 2000; WHO, 2000a; Zinsstag et al., submitted 2004). Mongolia's current brucellosis control strategy consists of a 10-year vaccination program designed to vaccinate all adult bovine, caprine, and ovine herds in the country twice within 6 years. One third of the total adult population will be targeted per year. All animals born during the 10-year program will be vaccinated once at less than 1 year of age (Zinsstag et al., submitted 2004). The 10-year vaccination plan has been set in motion by the Mongolian government despite significant set backs posed by the combination of drought and severe snowstorms in the 1999-2002 period, and a lack of significant foreign funding for the vaccination program. Although a recently published report indicated that "as of 2001, no uniform animal vaccination program existed in Mongolia" (Ebright et al., 2003) no data are yet available on the degree of implementation or impact of the current Mongolian brucellosis control program.

1.5 CONCLUSIONS AND AREAS FOR FUTURE RESEARCH

Human and livestock populations in Mongolia have been affected by brucellosis from the 1950's through the 1980's and most likely continue to suffer from a significant burden of the disease today. Multiple studies performed in the late 1950's through the 1980's collected data on brucellosis in Mongolia that were published in the international literature (Beulig et al., 1969; Denes, 1997; Jezek et al., 1972; Jezek et al., 1974; Otgon, 1968; Pinigin et al., 1968a; Pinigin et al., 1968b; Tserendash, 1980, 1984; Tserendash and Shumilov, 1970). The authors also cite historical accounts of cases dating the recognition of brucellosis in Mongolia back to the early 1930's (Otgon, 1968). These published reports provide information primarily on the prevalence of brucellosis in humans and individual animals. Details of the testing methods employed are provided but the methods used for selecting individuals for testing are less clear.

Conclusions that can be drawn from the studies performed in the 1950's through the 1980's include the following:

1. Brucellosis was present in multiple livestock species in Mongolia.
2. Both rural and urban human populations were affected by brucellosis.
3. Brucellosis was distributed throughout Mongolia with some regional variation in the prevalence of the disease in livestock.
4. *Brucella abortus* was responsible for brucellosis infection in Mongolia. The presence of *Brucella melitensis* was suspected but undocumented.
5. Regions of the country with a higher density of livestock and State farms were associated with a higher prevalence of disease in livestock.
6. Higher risks of brucellosis-seropositivity in humans were associated with contact with livestock (veterinarians, slaughterhouse workers, and herders).
7. High rates of disease transmission were recorded in both the livestock and human populations.
8. Methods implemented to control brucellosis in livestock (primarily vaccination) led to a decrease in seroprevalence and clinical signs of brucellosis in livestock herds, though control programs implemented in the late 1980's were never evaluated.

Scientific studies assessing the effects of brucellosis control in Mongolia on the incidence of the disease in the human population are unavailable. Published information on brucellosis in Mongolia post-1990, consists primarily of summaries of reports made by Mongolian government officials to the *Office International des Epizooties* or the World Health Organization. The most recent publications explore the use of more discriminatory diagnostic methods for brucellosis testing (Erdenebaatar et al., 2002), highlight brucellosis as 1 of 4 most important infectious human diseases in Mongolia (Ebright et al., 2003), and outline the economic and human health benefits of a comprehensive program to control brucellosis in livestock in Mongolia (Roth et al., 2003; Zinsstag et al., submitted 2004).

The epidemiology of brucellosis in Mongolia today, both the distribution of disease in livestock and the burden of disease in humans, is largely unknown. Well-designed population-based studies are needed to assess the current level of infection in livestock and humans in Mongolia. Strain typing, disease incidence data and an analysis of risk factors associated with disease would greatly enhance the understanding of disease transmission and provide needed information for designing effective disease prevention programs. Ideally a nationwide brucellosis surveillance system should be put in place with the use of standardized testing methods, uniform case definitions and reliable reporting mechanisms. With limited funds and the logistical constraints associated with an extensive livestock system, the surveillance system would need to be adapted appropriately. Mongolia's move to implement its national brucellosis control program through the mass vaccination of its livestock is admirable. Coupling this disease prevention program with a streamlined yet functional brucellosis surveillance system would allow for an assessment of disease control progress, the identification of areas for more intensive measures, and a more successful program overall.

Table 1.1. Summary of Published Reports of Brucellosis Prevalence in Livestock and Human Populations in Mongolia by Year and Source.

Year of Data Collection			Estimated brucellosis prevalence % (# of samples tested)			
	Location	Test	Cattle	Sheep/Goat	Camel	Human
1956						
Otgon (1968)	Northern	Skin	**4.7**	**3.7**		**34.0** (1516)*
	Southern	Skin	**2.1**	**0.02**		
1961						
Pinigin and Kokourov (1968)	Mongolia	STAT	**37.0** (121)			
		Huddleson	**90.0** (121)			
1962						
Pinigin and Kokourov (1968)	Mongolia	STAT	**22.0** (1404)			
		CFT	**11.0** (1378)			
		STAT	**64.0** (394)			
		CFT	**18.0** (370)			
1968						
Tserendash and Shumilov (1970)	Bayan-Olgi	STAT			**1.1** (6954)	
		CFT			**6.5**	
	Uvs	STAT			**1.8** (24002)	
		CFT			**2.3**	
	Khovd	STAT			**0.7** (10871)	
		CFT			**0.7**	
	Govi-Altai	STAT			**1.2** (16693)	
		CFT			**1.6**	

* Human data collected from Ulaanbaatar and 6 Aimags (Provinces) including the "Orkhon" and "Zhargalant" state farms (exact location unknown)

Table 1.1. Continued

Year of Data Collection	Location	Test	Estimated brucellosis prevalence % (# of samples tested): Cattle	Sheep/Goat	Camel	Human
1968						
Tserendash and Shumilov (1970)	Zavkhan	STAT			**0.6** (10661)	
		CFT			**2.5**	
	Khovsgol	STAT			**1.1** (3703)	
		CFT			**14.9**	
	Arkhangai	STAT			**0.6** (909)	
		CFT			**9.0**	
	Bayankhongor	STAT			**1.0** (15753)	
		CFT			**2.3**	
1972						
Jezek et al. (1972)	Ulaanbaatar	STAT/STAT or CFT			Slaughter house workers	**13.7/21.9** (233)
					Dairy workers	**2.3/20.6** (131)
					Agricultural Institute	**2.1/8.9** (237)
					Skin-tanning workers	**1.9/7.0** (157)
					TOTAL (Urban)	**1.7/3.8** (1058)
	Uvs	STAT				**2.0** (685)
	Govi-Altai	"				**4.7** (730)
	Khovsgol	"				**4.5** (705)
	Dornogovi	"				**5.0** (873)
	Dornod	"				**5.2** (761)
					TOTAL (Rural)	**4.4** (3758)

Table 1.1. Continued

Year of Data Collection	Location	Test	Cattle	Sheep/Goat	Camel	Human
			Estimated brucellosis prevalence % (# of samples tested)			
1974						
Jezek et al. (1974)	Khentii	STAT/CFT			Settled population	**10.2** (619)
	"	"			Nomadic population	**13.5** (562)
					TOTAL	**11.7** (1181)
	Khentii	STAT/CFT (1 year incidence)			Settled population	**1.3** (556)
	"	"	"		Nomadic population	**3.8** (486)
					TOTAL	**2.4** (1042)
	Khentii	Skin				**22.4** (989)
197?						
Tserendash (1980)	Ovorkhangai	STAT	**14.3** (~8000)			
1973-1982						
Tserendash (1982) Prospective data collected after the vaccination program on "Khalzan" farm, Sukhbaatar Aimag (Province)						
1973	Sukhbaatar	STAT	**11.0** (~9000)			
1974	"	"	**2.5**			
1975	"	"	**5.2**			
1976	"	"	**3.5**			
1977	"	"	**2.5**			
1978	"	"	**1.8**			
1979	"	"	**1.1**			
1980	"	"	**0.3**			
1981	"	"	**0.1**			
1982	"	"	**0.0**			

Table 1.1. Continued

Year of Data Collection			Estimated brucellosis prevalence % (# of samples tested)			
1973-1982	**Location**	**Test**	**Cattle**	**Sheep/Goat**	**Camel**	**Human**
Tserendash (1982) Prospective data collected after the vaccination program on "Bat-Ulzi" farm, Sukhbaatar Aimag (Province)						
1973	Sukhbaatar	STAT	**3.8** (~9000)			
1974	"	"	**5.1**			
1975	"	"	**2.9**			
1976	"	"	**2.8**			
1977	"	"	**2.7**			
1978	"	"	**4.0**			
1979	"	"	**1.2**			
1980	"	"	**1.5**			
1981	"	"	**0.8**			
1982	"	"	**0.1**			
1991-1999						
Zinsstag et al. (2004)**						
1991	Mongolia	Serology	**0.8** (2765217)	**0.5** (11468330)		
1992	"	"	**0.9** (2897395)	**0.6** (11937672)		
1993	"	"	**3.0** (2968915)	**0.8** (12393552)		
1994	"	"	**2.0** (3147373)	**0.8** (12873452)		
1995	"	"	**1.2** (3276856)	**0.9** (13595133)		
1996	"	"	**1.6** (3422561)	**1.1** (13411433)		
1997	"	"	**1.2** (3571865)	**0.8** (14105744)		
1998	"	"	**1.0** (3715283)	**0.7** (15344730)		
1999	"	"	**1.3** (3776780)	**0.8** (15069770)		

** Original source: National Brucellosis Surveillance; Mongolian State Veterinary and Breeding Department, Ministry of Food and Agriculture

Chapter 2

THE PREVALENCE OF BRUCELLOSIS-SEROPOSITIVE CATTLE/YAK HERDS IN THE KHANGAI MOUNTAIN REGION OF BAYANKHONGOR AIMAG (PROVINCE), MONGOLIA

2.0 STRUCTURED ABSTRACT

Objective: To determine the prevalence of brucellosis-seropositive cattle herds in the Khangai mountain region of Bayankhongor Aimag (Province), Mongolia, and describe livestock management and human/livestock interaction in the region.

Design: Cross-sectional study

Sample Population: A stratified random sample (n=105) of the 5,062 livestock herds in 5 contiguous soums (districts) and 1 sub-district in the Khangai mountain region of Bayankhongor Aimag (Province), Mongolia.

Procedure: Cattle herd brucellosis status was determined by testing pooled milk samples from each herd with the Brucellosis Milk Ring Test (BRT). Additionally, serum from a subset of individual cattle in each herd was tested with the Brucellosis Card Test (CARD). A structured questionnaire was administered to collect data on livestock management, livestock/human interaction and human health.

Results: The prevalence of brucellosis-seropositive cattle herds in the region was 41.3% (95% C.I.; +/- 9.8%) based on the results of the BRT testing. Additional herds detected with the CARD test increased the proportion of positive herds to 53%. The proportion of

seropositive individual animals was 14.9%. Bovine abortions were reported in 21% of the herds and 84% of herds experienced some degree of neonatal mortality. The majority of herder households (75%) herded multiple species of livestock and 100% of the households herded and milked cattle (predominantly yaks). Twelve percent of the herders interviewed reported consuming raw milk and 33% reported preparing dairy products from raw milk. Twenty-four percent of the herders interviewed reported clinical signs suggestive of brucellosis.

Conclusions: The high prevalence of brucellosis-seropositive cattle herds in the Khangai mountain region of Bayankhongor Aimag is consistent with data from other regions of the world where there is an absence of brucellosis control programs and established records of economic losses and serious public health implications due to zoonotic transmission of the disease. The proportion of seropositive individual cattle (15%) found in this study was higher than that officially reported for Bayankhongor (5.4% overall, 8.8% in female cattle). Livestock management factors, livestock/human interaction behavior, and the occurrence of human illness reported in this study can be used in a future evaluation of risk factors associated with livestock and human brucellosis in this region of Mongolia.

Relevance: This study provides data that support the anecdotal reports of brucellosis as a re-emerging disease in Mongolia and a potential major public health concern. The data will also serve as important baseline information on the cattle herd brucellosis-seroprevalence in the region before the implementation of the national brucellosis control program.

2.1 INTRODUCTION

Brucellosis is an infectious disease of livestock and one of the most widespread zoonotic diseases in the world (Corbel, 1989). The worldwide distribution of endemic brucellosis includes Mongolia, a sparsely populated country (1.5 persons/km^2) in Central Asia where livestock herding is the backbone of the county's economy and an important part of its cultural heritage (Mongolia, 2001c; OIE, 2002b). Major efforts have been undertaken around the world to control brucellosis due the significant economic losses associated with the occurrence of the disease in domestic livestock and the often debilitating disease associated with human infection (Halling and Boyle, 2002; Ragan, 2002; Reddy, 2003). Clinical signs of brucellosis in domestic livestock include late-term abortions, decreased milk production and lowered fertility in both males and females (Drost and Thomas, 1996). The disease in humans is associated with a number of nonspecific complaints including irregular or intermittent fevers, night sweats and headaches. Untreated brucellosis in humans can lead to chronic osteoarticular complications manifesting as joint pain, joint effusion and debilitating arthritis (Benenson, 1995; Young, 1989).

Particular biovars of the species of *Brucella melitensis* are associated with pathogenicity in different domestic animals. The disease in cattle is most commonly caused by the biovar *abortus* (Reddy, 2003). The vast majority of human infection is the result of direct or indirect transmission of the disease from infected livestock through the ingestion of contaminated milk or dairy products or through direct contact with aborted material or uterine fluid from infected animals (Nicoletti, 1989).

Historically, Mongolia has had a high incidence of brucellosis in its human population (Otgon 1968; Jezek Rusinko et al. 1972; Jezek, Rusinko et al. 1974) as a consequence of the high prevalence of the disease in domestic livestock (Beulig et al., 1969; Jezek et al., 1972; Jezek et al., 1974; Otgon, 1968; Pinigin et al., 1968a; Pinigin et al., 1968b; Tserendash, 1980, 1984; Tserendash and Shumilov, 1970; Zherikova et al., 1972). Brucellosis prevention, through livestock vaccination, began in Mongolia in the 1960's (Corbel, 1989; Tserendash, 1980). A second prevention program, lead by the World Health Organization (WHO), began in the mid-1970's and focused on the vaccination of small ruminants (Corbel, 1989). The former Soviet Union continued to support a brucellosis livestock vaccination program in Mongolia (*Brucellosis abortus* strain-19 vaccine in cattle and *Br. melitensis* Rev-1 vaccine primarily in small ruminants) through the late 1980's. Initially the control programs were deemed a success by Mongolian scientists (Tserendash, 1980) and by WHO officials who reported that the annual incidence of the disease in humans dropped to less than 1 case/10,000 in the early 1980's (Mikolon, 2000; Roth et al., 2003).

Despite evidence in the early 1980's of a significant reduction in the incidence of brucellosis in Mongolia, concerns have been raised recently about the re-emergence of the disease in the country (Ebright et al., 2003; Joshi, 1991; Roth et al., 2003; WHO, 2000b, 2001, 2003). Brucellosis surveillance in Mongolia from 1990, until 2001, has consisted of unsystematic sampling of individual livestock and sporadic herd-level testing (Roth et al., 2003). These data do not allow one to quantify the regional prevalence of disease or confirm the re-emergence of brucellosis, however, they do demonstrate that livestock brucellosis continues to persist in Mongolia. In the early 1990's Mongolia reported that the occurrence of bovine, caprine and ovine brucellosis was enzootic with a ubiquitous distribution (OIE, 2002b). Later in the decade the occurrence was reported as "low sporadic" and in 2001, and 2002, the reports of occurrence of bovine brucellosis were based only on serological evidence of the disease (OIE, 2002b). Data provided by the State Veterinary and Animal Breeding Department, Mongolian Ministry of Food and Agriculture, on the nationwide prevalence of brucellosis in cattle, sheep and goats between 1991 and 1999, indicated that the prevalence in cattle ranged from 0.80% to 3.04% and in sheep and goats between 0.50% and 1.11%. During this period (1990-2002) the Mongolian Ministry of Food and Agriculture reported a number of outbreaks of brucellosis in livestock as well as measures taken to control the disease in the form of slaughter, vaccination, quarantine and a modified stamping out policy (OIE, 2002b).

The persistence and possible re-emergence of brucellosis as a major public health problem in Mongolia has been blamed on the collapse of the veterinary service delivery system and public health infrastructure in Mongolia which began in the late 1980's (Ebright et al., 2003; Mikolon, 2000). The Mongolian veterinary system in the 1990's reflects the changes that were experienced by the country as a whole as Mongolia transitioned from a centrally planned economy and socialist system of government to a democratic system with a market economy (Ebright et al., 2003; Rossabi, 1999). The combination of the abrupt loss of economic aid from the former Soviet Union, the privatization of livestock in 1990, and the privatization of veterinary service delivery in 1999, meant the end of large scale, government subsidized, livestock health campaigns including comprehensive livestock vaccination programs and systematic surveillance for brucellosis (Mikolon, 2000).

Recently collected data on the prevalence, incidence and distribution of brucellosis in Mongolia's livestock and human population is largely unavailable. Official government reports provide some information on disease occurrence but the sampling methods employed are unknown and little insight into the level and distribution of disease can be gained. The aim of this study was, therefore, to systematically collect data on the prevalence of brucellosis in livestock in Mongolia. The decision was made to focus on the Khangai mountain region of Bayankhongor Aimag (Province), where brucellosis was reported to occur and to estimate the prevalence of brucellosis-seropositive cattle herds in the region. The specific aims were the following:

1. To use the brucellosis ring test (BRT) on milk samples and the Brucellosis Card (CARD) agglutination test on individual serum samples to determine the brucellosis status of cattle herds in the study area.
2. To demonstrate the efficiency of using stratified random sampling to estimate disease prevalence in an extensively managed (nomadic) livestock population.
3. To conduct 2 questionnaires designed to characterize livestock management practices, human/livestock interaction, and the occurrence of clinical signs suggestive of brucellosis in the human population in the region.

2.2 HYPOTHESES

1) The prevalence of brucellosis-positive cattle (yak) herds in the Khangai mountain region of Bayankhongor Aimag (Province), Mongolia, can be estimated by field testing randomly selected herds in the region.

2) Structured questionnaires can be used to collect information on livestock management practices and the occurrence of clinical signs and symptoms suggestive of brucellosis in the human herder population in the study region.

2.2 MATERIALS AND METHODS

A. Study Site: Bayankhongor Aimag (Province), Mongolia

This study took place in Bayankhongor Aimag (Province) in southwestern Mongolia. Bayankhongor is the third largest of Mongolia's 19 Aimags or Provinces. The country is made up of the 19 Aimags and 2 cities, the national capital, Ulaanbaatar, and an industrial center to the north, Darkhan-Uul (See Appendix A). Each Aimag is divided into an average of 20 soums or districts. The soums are further divided into the soum centers (small settled communities) and an average of 4 smaller administrative units called baghs or sub-districts. This study area covered approximately 1/3 of the 116,000 km^2 of Bayankhongor Aimag (Mongolia, 2001a).

Bayankhongor is one of the most ecologically diverse provinces in Mongolia. The southern 1/3 of the province is part of the Gobi desert where it forms a section of Mongolia's border with China. The central 1/3 of the province is less arid and transitions from the desert to the grassland steppe. The northern 1/3 of Bayankhongor is in the mountain steppe zone and contains part of the Khangai mountain range where elevations reach 4,000 meters and the primary livestock species is the yak. The five soums (districts) and one sub-district (Shargaljuut) that make up the study area contain 41% of the cattle (*Bos taurus* and *Bos grunniens*), 25% of the herder households, and 15% of the total number of livestock in Bayankhongor (Mongolia, 2001b). Bayankhongor was the leading province in number of livestock in 1999, with 2,475,100 head of camels, cattle, horses, sheep and goats (Mongolia, 2001b).

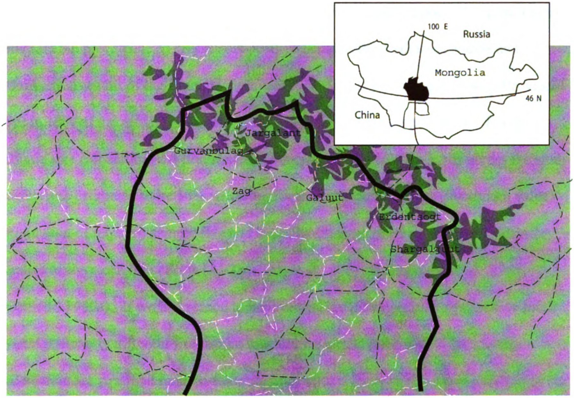
B. Study Design and Sampling Frame

A cross-sectional approach was used to determine the prevalence of brucellosis-seropositive cattle herds in the Khangai mountain region of Bayankhongor Aimag (Province), Mongolia. The unit of interest was the herd and all herds located in the 5 contiguous Khangai soums (districts) of Gurvanbulag, Zag, Galuut, Jargalant, Erdenstogt, and the sub-district of Shargaljuut, were chosen for inclusion in the study (See Figure 2.1). This region was selected based on the predominance of cattle (mostly yaks) in the composition of livestock herds in the region (Bayankhongor, 1999).

A livestock census is performed in Mongolia on a yearly basis. The herd, or herder household, is considered the economic unit in rural areas and forms the basis for measuring agricultural output in Mongolia's extensive livestock management system (Mongolia, 2001f). Livestock census data available at the Aimag (Province) level include the total number of herder households in each soum (district) and the total number of each livestock species per soum. These data from the most recent livestock census (1999) were obtained and used to determine that the 6-strata (5 soums and 1 sub-district) sampling frame contained 5,062 herder households, or sampling units, and close to 200,000 head of cattle (See Table 2.1).

Livestock census data available at the soum (district) level include the number of herder households per soum (district) as well as the number and species of livestock in each herd, the bagh or sub-district in which the herd is grazed, the name of the herd owner and the number of household members associated with the livestock herd. These data were available in the form of lists obtained from the soum (district) government offices.

Figure 2.1. The Study Region. Inset: The Khangai Mountain Region of Bayankhongor Aimag (Province), Mongolia. Below: The Five Contiguous Soums (Districts) of Gurvanbulag, Zag, Jargalant, Galuut, Erdentsogt and the Bagh (Sub-District) of Shargaljuut.



C. Sample Size Calculation

Equation 1 (Thompson, 1992) was used to calculate the number of herds or sample size required to estimate the proportion (prevalence) of brucellosis-seropositive cattle herds in the finite population of 5,062 herds in the study region. The goal was to estimate the prevalence of brucellosis-positive herds in the 5 adjacent soums and 1 administrative unit of the Khangai mountain region in Bayankhongor Aimag (Province) within 10% of the actual prevalence and a 5% probability of Type I error. For the sample size calculation, the prevalence of brucellosis-seropositive herds across the study region was estimated to be 50%, allowing for the most conservative (maximum value) calculation of required sample size and based on findings of other population-based studies of livestock brucellosis (Al-Talafhah et al., 2003; Kabagambe et al., 2001; Nicoletti, 1980a). If we had reason to believe that the prevalence of seropositive herds was significantly different from soum to soum we would have used a sample size calculation for stratified random sampling that adjusts for both the relative weight of the stratum and the expected prevalence of each stratum. When the expected prevalence is equal across strata, the calculated sample size will be identical to that calculated for a simple random sample using the formula below.

Equation 1:

$$\text{Sample Size} = n = \frac{Np\,(1-p)}{(N-1)\, d^2/z^2_{(1-\alpha/2)} + p\,(1-p)}$$

Where, "N" is the total number (5,062) of herds in the study; "p" is the estimated prevalence of brucellosis-seropositive cattle herds in the region (0.5); "d" is the precision or maximum acceptable error rate (0.1); and "α" is the probability of Type I error (0.05).

Using the formula in **Equation 1**, it was determined that brucellosis status data would need to be collected from 95 herds to estimate the prevalence of brucellosis-seropositive herds in the 6-strata study area with the desired level of precision. The calculated sample size of 95 was increased by 10, to 105, to account for the potential failure to collect data due to the inability to locate herds, the absence of cattle in the herd, refusal of herders to participate or diagnostic test failure.

D. Herd Selection

The selection of the105 herds was performed using a stratified random sampling technique. A simple random sample of herds was selected within each of the 6 stratum (soum/district or bagh/sub-district). The number of herds selected per stratum was based on the relative proportion of herder households in each stratum in the study area (See Table 2.1). The stratification and proportional allocation of sampling was done for practical reasons. The census reports containing the individual herd identifiers were only available at the soum (district) government offices. It was most efficient to calculate the overall sample size based on the aimag-level census data, which provided the number of sampling units (herder households/soum), and then obtain the soum-level census data with the herd identifiers upon arrival in the soum (district) centers.

The simple random sample of individual herds was selected from each soum list by first assigning a number to each herder household and then using a hand held calculator to generate random numbers for herd selection (See Table 2.2). The location and testing of the herds selected in each stratum began as soon as the herds were identified. The herds were located with the assistance of the local soum (district) veterinarians and the bagh (sub-district) governors.

E. Data Collection Team and Informed Consent

The data collection team consisted of two veterinarians, a physician, a driver who also assisted with animal restraint, and a volunteer data recorder. In each soum the data collection team met with local officials and the soum (district) veterinarian. The soum veterinarian accompanied the data collection team on all herd visits. The local soum veterinarian made introductions to the herder household members. The Mongolian bilingual (English and Mongolian) collection team member explained the project and informed the herders that their participation in the study was voluntary. All team members were available for questions. A record of informed consent was kept and data collection proceeded only if consent was obtained.

F. Specimen Collection and Testing

Data collection took place in July and August of 2000. The summer months in Mongolia are considered peak milk production periods. All of the livestock species are seasonally bred and offspring are born in the spring and early summer.

Milk testing:

The Brucellosis Milk Ring Test (BRT) was used to test raw milk samples collected from the selected herds in the study area. The BRT is an internationally recognized serological method for detecting brucellosis in a herd of cattle and classifying it as a positive or negative herd (OIE, 2002a). Blue haematoxylin-stained *Brucella abortus* antigen is used to detect *Brucella* antibodies in the milk of affected cows (Alton and Jones, 1967). The BRT was developed as a tool to use at the herd level to screen dairy cattle for brucellosis. The sensitivity of the test was reported to be 72.2% (95% C.I.; 58.1%-83.1%) with a specificity of 90.5% (95% C.I.; 76.5%-96.9%) when used to test dairy cattle herds of varying size (11-399), (Vanzini et al., 2001). The number of cows contributing to the mixed bulk milk samples tested in this study was never exceeded 30 cows. Samples were stored at 4° C and tested using standard international protocols for herds of less than 100 milking cows (OIE, 2002a).

Milk samples were collected soon after the morning or evening milking had been completed. Individual cattle were routinely milked into buckets that were then emptied into aluminum milk cans or an equivalent small bulk storage container. The raw milk in the milk cans was mixed thoroughly and a single sample was withdrawn for testing. The milk samples were stored in a portable refrigerator powered by a 12-volt car battery and tested within 24 hours[2]. The milk samples were tested for the presence of *Brucella* antibodies using standard *B. abortus* strain 1119-3 BRT antigen provided by the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), National Veterinary Services Laboratories (NVSL), Ames, Iowa, USA. One ml of whole milk from each sample was transferred to a narrow, sterile plastic test tube. BRT antigen (0.03 ml) was added to each of the samples and mixed well. The milk samples were then incubated for 1 hour at 37 ^{0}C in a portable incubator powered by a 12-volt car battery. Concentration of dyed antigen in the cream layer, forming a dark blue ring, indicated a positive reaction (Alton et al., 1988; OIE, 2002a). Results were read at the conclusion of incubation and recorded.

<u>Serology:</u>

Ten ml of blood were collected from approximately ½ of the female cattle over the age of 6 months in each of the selected herds. Sterile 20-gauge, 1-inch needles and a

[2] The standard minimum storage time of 24 hours was reduced to 12-24 hours in this study. The protocol was adjusted due to the difficulty in maintaining the samples at the required 4° C for a 24-hour period in the field. The samples were tested in less than 24 hours to avoid curdling of the milk.

10 ml Vacutainer tube or syringe were used to draw blood from the jugular or middle coccygeal vein. The samples collected by syringe were transferred to clean test tubes and the test tubes and Vacutainer tubes were centrifuged for 3-5 minutes to separate the red blood cells from the serum, using a portable centrifuge powered by a 12-volt car battery.

Serum samples were tested for the presence of antibodies against *Brucella* using the commercially prepared Brucellosis Card Test Kit with Rose-Bengal stained, buffered *Brucella abortus* strain 1119-3 antigen produced by the USDA, APHIS, NVSL, Ames, Iowa, USA. The brucellosis card test (CARD) is a modified serum agglutination test that was developed in the United States to meet the need for a rapid field test for the serological diagnosis of brucellosis in the ranch or stockyard setting (Prior et al., 1975). The test was well suited to field conditions in Mongolia.

The CARD test is generally characterized as having a high sensitivity and low specificity. Used in a population of non adult-vaccinated cattle, the sensitivity and specificity the CARD test was determined to be 99.0% and 30.6% respectively, when evaluated based on bacteriologic culture and isolation of *B. abortus* as the definitive test (Huber and Nicoletti, 1986). Agreement between the CARD test and the serum tube agglutination test (STAT)[3] under field conditions is reportedly high (O'Reilly and Cunningham, 1971; Prior et al., 1975). The CARD test was used in this study in addition to the BRT, allowing for the identification of brucellosis-positive animals in the non-milking portion of the cattle herd.

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[3] The serum tube agglutination test (STAT) is used to test for the presence of antibodies in serial dilutions of the subject's serum produced in response to brucellosis infection. The highest serum dilution showing 50% or more agglutination is recorded as the end-point Alton, G., Jones, J., 1967, Laboratory Techniques in Brucellosis. World Health Organization, Geneva.

Serum samples were tested using the protocol outlined in the Brucellosis Card Test Kit instructions. One drop (0.03ml) of serum and one drop (0.03ml) of *Brucella* antigen (USDA Rose-Bengal stained, *B. abortus* strain 1119-3) were placed in an individual well on the standard card, mixed with an individual wooden stick and then rocked for 4 minutes. The contents of the wells were examined in good light for signs of agglutination and results were recorded in the field notebook as "positive agglutination", "weak agglutination", "very weak agglutination" or "absent agglutination"[4].

Brucellosis card test (CARD) positive serum samples were stored and submitted to the Mongolian State Central Veterinary Diagnostic and Sanitary Laboratory for further testing. Serum from CARD-negative animals was discarded. The serum from the CARD-positive animals, including samples with a "very weak" positive agglutination reaction, was retested in the laboratory using the Mongolian Complement Fixation Test (CFT). The CFT is accepted and often used as a confirmatory test for bovine brucellosis (OIE, 2002a). The CFT is a serologic test designed to determine the *Brucella* titer of the serum under test. The CFT utilizes *Brucella* antigen, complement and sheep red blood cells to measure the degree of hemolysis. The titer of the serum under test is read as the highest dilution showing 50% or less hemolysis. In cattle a titer of 1:40 indicates an infected animal and one of 1:20 is suspicious (Alton and Jones, 1967).

The sensitivity and specificity of the CFT, at a test titer > 40, in a population of non adult-vaccinated cattle was 64.8% and 81.4%, respectively (Huber and Nicoletti, 1986). The best relationship is seen between the CARD test and CFT when the level of infection in the population under test is high (Timbs et al., 1978).

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[4] Positive agglutination = complete clumping of antigen; Weak agglutination = fine particles observed with some clumping; Very weak agglutination = very fine particles present with no clumping; Absent agglutination = No visible agglutination

Bacteriology:

Milk can samples from positive herds were saved after testing, refrigerated and eventually frozen. The samples were submitted to the Central Research Laboratory at the National Medical University of Mongolia in Ulaanbaatar for bacteriologic exam. The equipment and facilities necessary for *Brucella* culture were, however, unavailable and no culture results were obtained.

G. Case Definitions

Sampled herds were classified as brucellosis-seropositive if **either** a BRT-positive result was detected in a mixed raw bulk milk sample **or** at least one individual animal was classified as a reactor to the CARD test. An individual was classified as a reactor if their serum had a "positive" or "weak" agglutination reaction on the CARD test. An animal with a serum sample exhibiting "very weak" or "absent" agglutination on the CARD test was considered negative.

H. Herd Management Survey

A 3-page, structured livestock management questionnaire was conducted as an in-person interview with an adult member of the herder household involved in livestock rearing and self-identified as the "head of household" (See translation of "Livestock Management" questionnaire in Appendix B). The interview was conducted in the Mongolian language by a bilingual (Mongolian and English) native-Mongolian speaker. Questions included requests for information on livestock numbers, herding practices, and a history of vaccination and veterinary interventions. The herders' awareness of the characteristics of livestock brucellosis was assessed using two open-ended questions. Interview responses were recorded in Mongolian and later translated into English.

I. Head of Household Survey

A 2-page questionnaire was conducted as an in-person interview with the available male or female individual self-identified as the "head of the herder household". The interview was conducted in Mongolian by a bilingual (Mongolian and English) native Mongolian speaker. The questions were designed to characterize herder contact with livestock and the practices surrounding the preparation and use of livestock products. Additionally the interviewees were asked to indicate the presence or absence of the occurrence of a list of 17 non-specific clinical signs and symptoms of disease within the past 12 months. Specific information on the subjects' previous brucellosis diagnosis, testing and treatment was also collected. The final questions assessed the degree of herder awareness of the zoonotic nature of livestock brucellosis and the modes of disease transmission (See translation of "Human Health/Livestock Contact/Product Consumption" questionnaire in Appendix C).

2.3 DATA ANALYSIS

A. Herd-Level Data

Data from the BRT and CARD test results were transferred from field notebooks to a computer database. The test results were entered in a Microsoft Excel spreadsheet database (Microsoft, 2000) along with the herd identifier, the number of cows contributing to the mixed milk samples and information on the age and breed of individual animals. The prevalence of brucellosis-seropositive herds in the 6-soum study area was calculated as the proportion of test positive herds among those herds tested. The percent prevalence and 95% confidence intervals were adjusted to account for the stratified sample using the final sampling weights. **Equation 2** (Lohr, 1999) was used to determine the overall proportion of brucellosis-positive herds in the region and the variance of this stratified sample was calculated using **Equation 3** (Lohr, 1999).

Equation 2

$$\text{Estimated Prevalence} = \sum_{h=1}^{H} N_h/N\ (P_h)$$

Equation 3

$$\text{Estimated Variance} = \sum_{h=1}^{H} (1-n_h/N)\ (N_h/N)^2\ [P_h\ (1-P_h)/\ n_h - 1]$$

H=strata (6); h=stratum; N=total herder households in sampling frame; N_h=total herder households in stratum; P_h=proportion of positive herds per stratum; n_h=number of herder households sampled per stratum.

B. Individual Animal-Level Data

The number of cattle with a positive CARD test in the herd divided by the total number of cattle tested in the herd was used to approximate the prevalence of brucellosis-seropositive cattle in each herd. The overall individual cattle brucellosis-seroprevalence level in the study area was calculated as the total number of cattle with a positive CARD test divided by the total number of cattle tested.

C. Concordance Measurement

The "interrater" reliability of the BRT and CARD test as methods for determining cattle herd brucellosis status was measured by calculating the Kappa statistic, a measure of concordance between the BRT and CARD classification of the cattle herds.

D. Questionnaire Data

Data from the "livestock management" and "human health/livestock interaction/product consumption" questionnaires were entered into Microsoft Excel spreadsheets (Microsoft Office 2000). Imported Excel spreadsheet data were analyzed using the SAS software program (SAS version 8.2, Cary N.C.: SAS Institute, Inc. 1999-2001). Cattle abortion rates, calving rates and neonatal mortality rates were computed and entered as new variables. The individual animal data was used to calculate the within herd prevalence of brucellosis CARD reactors and the average age of cows in the herd. Descriptive statistics were computed for herd characteristics and management factors, human /livestock interaction, livestock product consumption habits and human health status reporting.

2.4 RESULTS

A. General Characteristics of Livestock Herds

Herd Composition:

Yaks (*Bos grunniens*) were the predominant milk producing species in the study region. All of the herder households raised cattle and milked at least one cow. The mean size of cattle herds in the study was 20 with a mean of 7.7 milking cows per herd. Fifty-five percent of the 100 heads of herder households interviewed reported that they herded exclusively the yak species of cattle. The majority of the remaining cattle herds were composed of crossbred (*Bos grunniens* and *Bos taurus)* animals or both species. Ninety percent of the individual cattle selected for testing were *Bos grunniens* or *Bos grunniens* crosses. The term "cattle" in this thesis refers to the bovine livestock in the region that were predominantly the yak or *Bos grunniens* species.

The majority (70%) of livestock herds included in this study were mixed herds of cattle, sheep, goats and horses. The total number of livestock could only be calculated for approximately 60% of the herds due to errors in data recording. The mean number of livestock per herd was 95, with a median of 67, and a range of 3-390. The mean number of cattle per herd was 20, with a median of 17, and a range of 1-70. The mean number of sheep was 65 and the mean number of goats was 26. Data on the total number of horses was not collected. Five percent of the herds included a few camels in their composition. Of the 61 herds for which we had total livestock number data, 80% were composed of fewer than 150 head of livestock, the Mongolian government's official definition of poverty.

Livestock Management:

All of the herder households included in this study were nomadic, moving their livestock and felt dwellings, or gers, an average of 4 times per year. Groups of herder households often migrate together and generally graze their livestock on shared pasture. In addition, 75% of herders reported the mixing or their livestock with other herds at water sources, 75% mixed with other herds in sheltered winter areas and 73% of herds mixed with other livestock overnight.

All of the herder households included in the study milked cattle. Only 6% of the herders also milked sheep and 13% also milked goats. Sixty-eight percent of the herders reported that their livestock had received veterinary services within the past 12 months. When asked specifically about brucellosis vaccination, 4 herders reported that their cattle had been vaccinated against brucellosis. Two herders reported that the vaccination had occurred in the last year.

Livestock Health and Production Parameters:

Abortions were observed in cattle in 21% of the herds, in sheep in 12% of the herds and in goats in 25% of the herds. Neonatal calf mortality was reported in 84% of herds and the average calf mortality per herd was 10%. Horses with draining sores in the vicinity of the withers were only reported in 1 herd.

Herders reported that the average age of a first calf heifer was 3 or 4 years. The mean age of lactating cattle per herd was 7 years. The calculated mean calving rate per herd in the sampled population was approximately 80%.

Livestock Brucellosis Knowledge:

Typical symptoms of livestock brucellosis (abortions and decreased milk production) could be described by 15% of the herders interviewed. Only 10% indicated that they knew that livestock brucellosis could be prevented with vaccination. The routes of disease transmission were identified by less than 10% of the individuals interviewed.

B. Prevalence and Distribution of Brucellosis-Seropositive Cattle Herds

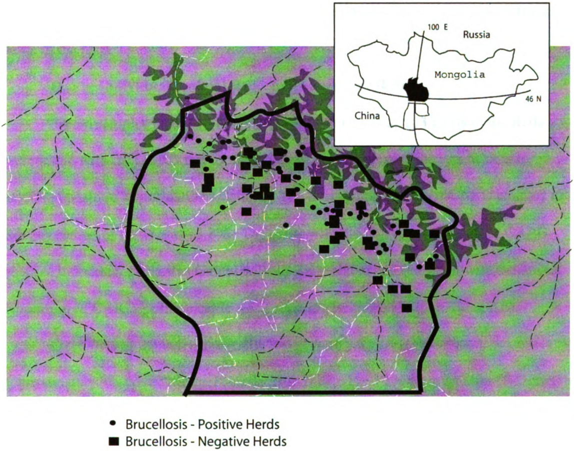
One hundred and four of the 105 herder households selected for sampling were located. The exact location of the herds ranged from N 46°33'13.2", E 101°26'46.7" to N 47°23'37.7", E 098°25'29.9", a point to point distance of approximately 250 km. Only one head of household refused to enroll their livestock herd in the study or respond to the questionnaires. Bulk milk samples were collected from 102 of the 103 herds participating in the study and results were obtained from 98 milk samples tested with the brucellosis milk ring test (BRT).[5] Forty of the 98 milk samples tested were positive. The proportion of BRT-positive herds, adjusted for stratification, was 41.2% (95% C.I. +/- 9.8%). The soum (district) with the highest proportion of BRT-positive herds was Gurvanbulag with a prevalence of 53.8% (7/12). The soums (districts) with the lowest proportion of BRT-positive herds were Zag and Erdentsogt, both with a prevalence of 30% (3/10 and 6/20), (See Table 2.3).

Blood samples were collected from 513 cattle representing 101 different herds. Serum from a sub-sample of cattle from each herd was tested with the CARD serum agglutination test and an additional 14 herds were identified as brucellosis-positive with this method. Forty herds had at least one animal classified as CARD-test positive. The proportion of cattle herds with at least one CARD-positive bovine, adjusted for stratification, was 39.2% (95% C.I. +/- 9.3%). The proportion of cattle herds classified as brucellosis-seropositive after testing in series with the CARD followed by the Mongolian CFT was 22.7% (95% C.I. +/- 8.1%). The proportion of cattle herds classified as brucellosis-positive by both the BRT and CARD test was 24.3 (95% C.I. +/- 8.1%). When either a positive CARD test or a positive BRT sample was used to classify a herd as positive, the adjusted estimate of brucellosis-seropositive herds in the study region was 53.0% (95% C.I. +/- 9.5%).

[5] Four of the milk samples appeared grossly abnormal at the time of testing. The milk samples had curdled during the storage period and test results were not considered valid.

A summary of the test results and the proportion of brucellosis-seropositive herds are presented in Table 2.4. The brucellosis-seropositive herds were distributed across the study area (See Figure 2. 2) with no obvious geographical clustering of positive herds.

Figure 2.2. Map of the Khangai Mountain Region of Bayankhongor Aimag (Province), Mongolia. Labeled Points Indicate the Location (Global Positioning System) and Brucellosis Status (squares = positive herds; circles = negative herds) of Each Cattle Herd Selected for Testing.



C. Concordance of Herd-Level Brucellosis Testing

The use of the BRT to test bulk milk samples and the CARD test to detect individual reactor cattle resulted in a similar estimation of the prevalence of brucellosis-seropositive herds, 41.2% and 39.2% respectively, in the study area. The kappa statistic, which quantifies the degree of concordance between the BRT and CARD test, was 0.45 (See Table 2.5). The individual cattle tested with the CARD method and those that contributed to the mixed milk sample were not always the same animals. The CARD test, performed on serum, had the potential to detect brucellosis-positive non-milking cattle in the herd.

D. Individual-Animal Brucellosis-Seroprevalence

Serum samples from 513 individual cattle from 101 of 103 herds in the study were tested with the CARD test for brucellosis. Seventy-one of the serum samples exhibited positive agglutination reactions. The overall prevalence of CARD reactors in this population of cattle was 13.8%. The soum with the highest proportion of CARD-positive individual cattle was Galuut with 19.2% reactors and the soum with the lowest proportion of CARD-positive cattle was Erdenstogt with 10.8% (See Table 2.6).

Over 90% of the individual cattle tested were yaks (*Bos grunniens*), approximately 3% were "Mongol" cattle (*Bos Taurus*) and 2% were hainaks (F-1 cross of *Bos grunniens* and *Bos taurus*). Yaks, "Mongol" cattle and hainaks were all represented in the CARD-positive population of cattle. The mean age of the cattle tested was 6.67 years and the median age was 6.00 years.

The CARD test results on individual cattle were used to estimate the "within herd" prevalence of brucellosis-seropositive cattle in the herds. The gross "within herd" seroprevalence levels ranged from 0 to 100% with a mean of 13% and 95% C.I. (9.0%, 17.0%). The mean within herd prevalence among the herds with at least one CARD-positive animal was 33.3%.

E. Human-Livestock Interaction

Data was collected on the nature of contact between livestock and humans in each herder household by interviewing the male or female head of the household and administering a questionnaire (See Appendix C). Information was gathered from 101 (all but 3) herder households. The average age of the interviewed herders (heads of herder households) was 42, median 41, with a range of 17-81. The interviewed herders had received an average of 5.7 years of formal education, median 8 years, with a range of 0 to 12 years. A slightly higher proportion of the herders interviewed were female (52.75%).

Herder Occupation:

The majority (92%) of interviewees identified "livestock herding" as their primary occupation or profession. Six percent of the individuals identified other occupations including 3 veterinary technicians and a human health worker. None of the interviewed individuals were involved in commercial livestock slaughter or trade.

Livestock Contact:

As expected, all members of the herder household participate in livestock rearing. As mentioned before, 75% of these herds were composed of multiple species of livestock. The presence of cattle was recorded in the largest number of herds (100%) followed by sheep, goats and camels. Home slaughter of livestock for personal consumption and hand milking of individual animals is common practice among

Mongolian herders. Of the 100 individuals interviewed, 84% reported participation in cattle slaughter, 87% in sheep, 80% in goat and 3% in camel slaughter. Ninety-six percent of the individuals interviewed reported hand-milking cattle, 24% reported hand-milking goats and 16% reported hand-milking sheep. Specific questions addressing herder assistance of livestock during parturition or dystocia were not asked, however, 39% of herders reported that weak neonates (primarily lambs and kids) were brought into the family's living quarters for observation and extra care.

Meat/Milk Consumption:

Mongolian livestock herders generally consume the meat and milk products of the livestock they raise. One hundred percent (100/100) of the heads of herder households interviewed reported consuming beef, 93% (93/100) reported consuming mutton, 75% (75/100) reported consuming goat meat and 3% (3/100) reported consuming camel meat. Traditionally meat is thoroughly cooked, either by boiling or roasting. One respondent reported consumption of raw meat of camel, cattle, sheep and goat origin. The consumption of raw milk was reported by 15% (15/100) of respondents. Twelve percent (12/100) reported consuming raw cow or yak milk, 3% (3/100) raw goat milk and 3% (3/100) raw sheep milk. Thirty-three percent (33/100) of respondents reported making dairy products with raw milk.

F. Human Health Status and Brucellosis Awareness

Human Health Status:

Twenty-seven percent (26/97) of interviewed heads of herder households reported a history of brucellosis diagnosis. Twelve percent of the interviewed herders (approximately ½ with a history of brucellosis) had been diagnosed within the previous

12 months. Eight percent of the individuals reported that they had been tested for brucellosis with a blood test and 5% reported skin testing. None of the interviewed individuals reported having been treated for brucellosis either as outpatients or in hospitals or clinics. None of the individuals reported that they had been rechecked for brucellosis after initial diagnosis and 1 person reported experiencing a relapse of disease (See Table 2.7).

In addition to the series of questions on brucellosis diagnosis and testing, the heads of herder households were asked to indicate which of the 17 clinical signs and symptoms on the list provided, they had experienced within the previous 12 months. (See questionnaire in Appendix C). The most common symptom experienced was joint pain at 18%. The other symptoms experienced were headache (16%), lower back pain (12%), stiff joints (11%), swollen joints (10%), muscle pain (9%), extreme weakness (9%), dizziness (9%), bone pain (9%), abdominal distension (6%), difficulty sleeping (4%), night sweats (2%), fever (2%), enlarged lymph nodes (2%), abdominal pain (1%) and 0% experiencing chills and depression. When a number of these non-specific signs were combined into a group characterizing acute brucellosis (chills, headache, fever or night sweats) and a group characterizing chronic brucellosis (joint pain, stiff joint, swollen joint or lower back pain), the prevalence of acute brucellosis-like symptoms was calculated as 16% (16/100) and the prevalence of chronic brucellosis-like symptoms in this interviewed population was calculated as 20% (20/100), (See Table 2.8).

<u>Knowledge of Prevention and Transmission</u>:

Twenty-five percent of the heads of herder households interviewed understood that brucellosis was a zoonotic disease and that infected livestock were a risk to human health. Seven percent of the herders interviewed identified both livestock product consumption and contact with aborted material as a means of brucellosis transmission to humans. An additional 18% identified either consumption of contaminated livestock products (meat and milk) or direct contact with aborted material as the means of transmission of disease from livestock to humans.

Eighteen percent of the heads of herder households correctly identified ways to prevent brucellosis occurrence in humans. Overall 12% identified either livestock vaccination/prevention or sanitary measures (cooking/boiling meat and milk and use of protective clothing). Six percent of interviewees identified both livestock brucellosis prevention and the institution of sanitary measures.

2.6 DISCUSSION

A. Brucellosis Prevalence and Distribution

The adjusted estimated prevalence of Brucellosis Ring Test (BRT)-positive cattle herds in the Khangai region of Bayankhongor, Mongolia, was 41.3% (95% C.I.; +/- 9.8%). The addition of herds detected as brucellosis-positive with the CARD test brings the herd prevalence in the region to over 50%, demonstrating a significant burden of disease in this population. Recent herd level data from Mongolia is unavailable for comparison, however, the estimated prevalence of 41.3% found in the study area exceeds or was similar to the herd prevalence reported in countries with no systemic brucellosis control program and similar systems of nomadic or extensive livestock management (Salman et al., 1984). Recent reports include a study of cattle brucellosis in Eritrea reporting a 36.6% prevalence of seropositive cattle herds (Omer et al., 2001), a study in

Syria reporting a prevalence of 17.48% (Darwish and Benkirane, 2001), and a study of cattle herds belonging to nomadic pastoralists in Chad reporting a brucellosis-seropositive herd prevalence of 64% (Schelling et al., 2003).

The gross individual animal sero-prevalence of 13.8% was higher than expected. The 1999, nationwide livestock brucellosis surveillance report produced by the State Veterinary and Animal Breeding Department of the Mongolian Ministry of Food and Agriculture, reported the sero-prevalence in cattle in Bayankhongor Aimag (Province) as 5.4% (114 positive/2,129 tested), and 1.3% nationwide. When the official data are evaluated for adult male and female cattle separately, the reported prevalence in male cattle in Bayankhongor is 2.1% (23 positive/1,098 tested) and 8.8% (91 positive/1,031 tested) for female cattle. The individual animal brucellosis-seroprevalence calculated in this study was based on the testing of female cattle only. Although a higher (13.8% vs. 8.8%) estimate of brucellosis-seroprevalence was found in this study than that reported for Bayankhongor as a whole, together the study data and the official reports indicate that significant levels of brucellosis infection are present in Bayankhongor's cattle population.

Another explanation for the discrepancy between the official report of brucellosis prevalence in Bayankhongor Aimag (Province) and that found in the study area is the possible non-uniform distribution of brucellosis-seropositive livestock. The 6 strata (5 soums and 1 bagh) selected for inclusion in this study represent a region for which there was anecdotal evidence of significant levels of bovine brucellosis. It is possible that the officially reported Bayankhongor Aimag-level prevalence is lower due to the sampling from both the study region and the potentially lower prevalence southern 2/3 of the province. Little information is available on the sampling or testing methods used in the national brucellosis survey. Most Mongolian laboratories use a version of the highly sensitive serum agglutination test on bovine serum. Sampling methods are generally unsystematic and do not allow one to describe or evaluate data on the geographical distribution of disease.

Cattle herds in this study were classified as brucellosis-seropositive based either on the brucellosis milk ring test (BRT) or the USDA serum agglutination CARD test. When the herds are classified with the BRT and CARD test independently, the adjusted estimated herd prevalence is very similar (BRT = 41.2 % +/- 9.8% and CARD = 39.2 % +/- 9.3%). In both cases the herd is classified based on the testing of a subset of individuals in the herd. Milking adult female cattle contributed to the mixed sample tested with the BRT and approximately ½ the female cattle > 6 months of age were included in the group of individuals tested with the CARD test. The discordance between the BRT and CARD test results was due in part to the fact that the individual cattle tested with the CARD method (not necessarily milking) and those that contributed to the mixed milk sample (only milking cows) were different.

Classification of a herd as seropositive if either the BRT or an individual CARD test were positive involved the use of the greatest amount of information (number of cattle tested) to make the decision on herd status. The adjusted seroprevalence estimate of herds classified as positive based on the BRT or CARD test was 53.0%.

Misclassification of cattle herds as brucellosis-seropositive is also a concern in this study due to the relatively low specificity of both the BRT and CARD tests (Huber and Nicoletti, 1986; Nielsen et al., 1996; Vanzini et al., 2001). To increase the specificity of our testing system we used a parallel testing strategy whereby both BRT and CARD test results had to be positive to establish a more "conservative" adjusted estimated herd prevalence of 24.3%.

Serial testing systems are also often used in brucellosis monitoring programs to confirm screening tests and improve the specificity of the testing system (Emmerzaal et al., 2002). The complement fixation test (CFT) is assumed to be 100% specific in non-vaccination populations (Dohoo et al., 1986) and was used to confirm the positive results of the CARD test on individual serum samples in our study. The brucellosis classification of cattle herds based on serial (CARD + CFT) testing results in the adjusted estimated herd prevalence of 22.8%.

None of the serologic tests used in this study can distinguish between brucellosis antibodies produced due to an infection and those generated after *Br. abortus* strain-19 vaccination (Omer et al., 2001). Although 4 of the herders surveyed reported that their cattle had been vaccinated for brucellosis in the past, we received assurance from the Bayankhongor Aimag (Province) veterinarians that no brucellosis vaccines (*Br. abortus* Strain-19 or *Br. melitensis* Rev-1) had been administered or distributed in Bayankhongor since 1988. Due to the centralized system of veterinary service delivery, the state monopoly on vaccine production and strictly enforced vaccine importation bans, it is most likely that the practice reported by the Aimag veterinarians is correct. Local soum (district) veterinarians were also interviewed about brucellosis vaccination practices and they confirmed that brucellosis vaccination had not been performed in Bayankhongor since at least 1990.

With the potential for false positive reactions due to the presence of vaccine-induced antibodies eliminated, confidence in the specificity of our screening tests is increased. False-positive results in the CFT and CARD test can also occur as a result of cross-reactive antibodies induced by a variety of Gram-negative bacteria (Emmerzaal et al., 2002), however, the cattle screened in this study appeared healthy and there was no reason to believe that widespread infection with pathogenic Gram-negative bacteria was present in the study population. The specificity of the BRT can be reduced if samples are grossly abnormal (curdled) or if the sample contains colostrum (OIE, 2002a). None of the cattle tested in this study were in early lactation (no colostrum) and abnormal milk samples were discarded and not subjected to testing. Calls have been made to review the use of the BRT as a screening test for brucellosis due to the increasing size of dairy herds in some regions of the world (Vanzini et al., 2001). Concerns surrounding the reduced sensitivity of the BRT applied to bulk tank milk samples to which large (>100) numbers of cows have contributed, are not applicable in this study. The number of cows contributing to a milk sample in this study was always less than 30 and the test was used on a "milk can" scale as it was originally intended (Alton and Jones, 1967).

B. Livestock Management

The general system of livestock management is similar across the Khangai mountain region of Bayankhongor Aimag (Province). People extensively manage mixed herds of livestock as mobile pastoralists. Although very little mixing of livestock occurs at sale yards or in markets, herders do graze their livestock in communally owned pastures. Veterinary service delivery, in terms of coverage and interventions, is relatively uniform and livestock are raised primarily for family consumption. The occurrence of the yak as the primary milking animal in the region and the relative similarity of livestock management systems across the study area allowed us to estimate the brucellosis seroprevalence for the region as a whole. Differences do, however, exist from herd to herd in terms of production parameters and abortion reporting that will allow us to compare characteristics of herds with BRT-positive milk or CARD-positive animals to those herds classified as brucellosis-seronegative. These differences will be further analyzed with the use of univariate and multivariable statistical techniques reported in chapter 3.

C. Human/Livestock Interaction and Human Health

The heads of herder households interviewed represented a normal distribution of individuals in terms of age and educational level with an equal number of males and females surveyed. As livestock herders, all of the household members have regular and repeated contact with livestock. Potentially high-risk interactions with livestock include home slaughter of multiple livestock species and hand milking of cattle, sheep and goats. Generally adults are involved in livestock slaughter with men involved in the killing, skinning and deboning of the animals and women in the cleaning and preparing the gastrointestinal tract and internal organs. Both adults (primarily women) and children are observed milking cattle, sheep and goats. The questionnaire did not include specific information on human exposure to livestock placentas, uterine fluid or aborted material but weak neonates, especially kids and lambs, were brought into the dwellings of 39% of the herders interviewed.

Of greater concern in this population was the high percent of interviewees who reported consuming raw cow's milk (12%) and preparing dairy products with raw milk (33%). It is probable that the habits of the entire household would reflect the actions of the head of household in terms of raw milk consumption and that all members of the household would consume dairy products produced from the raw milk. Although milk is routinely boiled immediately after collection these responses indicate that there is the potential for a significant level of human brucellosis exposure through the consumption of raw milk and dairy products in this population.

Of further concern was the high number, 26/97 or 27%, of interviewees who reported that they had a history of brucellosis diagnosis. Fourteen of 20 individuals with a history of brucellosis diagnosis (54%) who responded to the follow-up question about the timing of their brucellosis diagnosis were diagnosed within the previous 12 months. The fact that laboratory testing was only reported to have been performed on a subset of the individuals who had been diagnosed (7 of 26 or 27%) calls into question the accuracy of the diagnosis. In addition, none of the individuals reported receiving any treatment for brucellosis.

As an alternative to "brucellosis history", questioning on the occurrence of non-specific signs of illness was employed and results indicated that a portion of the interviewees had experienced the clinical signs often associated with acute (16%) and chronic (20%) brucellosis within the previous 12 months. The variables characterizing livestock/human interaction, education level, brucellosis knowledge, and livestock herd brucellosis status are compared in individuals who did and did not report a history of brucellosis diagnosis or clinical signs and symptoms associated with brucellosis in chapter 4 and analyzed with univariate and multivariable statistical techniques.

D. Herder Knowledge of Brucellosis

Less than 25% of the herders interviewed could identify the clinical signs and symptoms associated with livestock brucellosis or means of livestock brucellosis prevention and transmission. A smaller percent were aware of the risk of brucellosis to human health and ways to prevent zoonotic transmission. Due to this demonstration of a lack of brucellosis knowledge, educational pamphlets describing brucellosis as a zoonotic disease and means of disease prevention, were created and distributed to all of the herders in the study area (both those selected and those not selected for inclusion in the study) through their local veterinarians.

2.5 CONCLUSION

The prevalence of brucellosis-seropositive cattle herds in the Khangai mountain region of Bayankhongor, Mongolia, is undoubtedly associated with livestock production losses and probably associated with the occurrence of clinical signs and symptoms suggestive of brucellosis reported by the humans in the region. Whether the more conservative estimate of herd prevalence based on serial serum testing (22.8%) and BRT and CARD agreement (24.3%), or the less conservative estimates of prevalence based on the BRT (41.3%), CARD (39.2%) and the combination of BRT and CARD test information (53%), the data support concerns about the re-emergence of brucellosis in Mongolia (Ebright et al., 2003; Roth et al., 2003; WHO, 2001) and its classification as a major public health issue.

Although this study represents data from only a small portion of Mongolia's livestock population there is no reason to believe that the occurrence of brucellosis in cattle is restricted to this study region. Historical publications (Jezek et al., 1972; Jezek et al., 1974; Otgon, 1968; Pinigin et al., 1968b; Tserendash, 1980, 1984; Tserendash and Shumilov, 1970) and more recent reports (OIE, 2002b) indicate that brucellosis is found throughout Mongolia. Additionally, the individual animal data collected in this study supports official reports that highlight Bayankhongor as a high prevalence Aimag (Province), but also indicate that the official reports may be underestimating the prevalence of brucellosis in Bayankhongor and possibly the nation as a whole.

Since the elimination of Mongolia's systematic brucellosis control and surveillance program in the late 1980's, efforts have been made to recreate and fund a new brucellosis control program in Mongolia. The World Health Organization (WHO) provided technical support for the preparation of a National Brucellosis Control Plan in 1999, that called for the mass vaccination of ruminants and was due to begin in 2000 (Mikolon, 2000; WHO, 2000a; Zinsstag et al., submitted 2004). Despite the lack of international funding for the program and significant set backs posed by the combination of drought and severe snowstorms in 1999 through 2002 (UNDP, 2001), the Mongolian government has pledged to follow a brucellosis control strategy that consists of a 10-year vaccination program designed to vaccinate of all adult bovine, caprine and ovine herds in the country twice within 6 years (Zinsstag et al., submitted 2004). The 10-year vaccination plan was due to be launched in Bayankhongor Aimag (Province) 1-2 years after the completion of this cross-sectional study. The data collected in this study will provide important baseline information for the evaluation of the success of the vaccination program in the Khangai mountain region of Bayankhongor. The identification of risk factors for both livestock and human brucellosis, discussed in chapters 3 and 4, will contribute to the understanding of the epidemiology of brucellosis in Mongolia today and enhance the effectiveness of current and future brucellosis control and public health education programs.

Table 2.1. The 5,062 Herder Household Sampling Frame in the Khangai Mountain Region of Bayankhongor Aimag (Province), Mongolia, and the Relative Proportion of Herder Households from Each Stratum.

Stratum (Soum/Bagh)	No. Herder Households	Relative Proportion (%)
Gurvanbulag	755	14.9
Zag	562	11.1
Jargalant	1183	23.4
Galuut	1375	27.2
Erdentsogt	989	19.5
Shargaljuut	198	3.9
TOTAL	**5,062**	

Table 2.2. The Number and Percent of Herder Households Sampled in Each Stratum and the Overall Proportion of Participation.

Stratum (Soum/Bagh)	No. Herder Households	No. Sampled	Proportion (Herds/Stratum) Sampled (%)
Gurvanbulag	755	15	2.0
Zag	562	12	2.1
Jargalant	1183	22	1.9
Galuut	1375	27	2.0
Erdentsogt	989	20	2.0
Shargaljuut	198	7	3.5
TOTAL	**5062**	**103/105 = 98% Participation Proportion**	

Table 2.3. The Prevalence of Brucellosis Ring Test (BRT)-Positive Cattle Herds in East Stratum and the Adjusted Overall Estimated Prevalence and 95% Confidence Intervals of Brucellosis-Seropositive Cattle Herds in the Khangai Mountain Region of Bayankhongor Aimag (Province), Mongolia.

Stratum (Soum/Bagh)	**No. Herds Tested**	**No. Positive Herds**	**Prevalence (%)**	**95% C.I.**
Gurvanbulag	12	7	58.3	
Zag	10	3	30.0	
Jargalant	22	9	40.9	
Galuut	27	12	44.4	
Erdentsogt	20	6	30.0	
Shargaljuut	7	3	42.9	
TOTAL	**98**	**40**	**41.2%**	**±9.8%**

Table 2.4. A Comparison of the Adjusted Estimates of the Prevalence of Brucellosis-Seropositive Cattle Herds in the Khangai Mountain Region of Bayankhongor Aimag (Province), Mongolia, Based on the Results of the USDA Brucellosis Ring Test (BRT), the USDA Brucellosis Card Test (CARD), the CARD and BRT (Both Positive), the CARD and the Mongolian Complement Fixation Test (CFT) in Series and the CARD or BRT (Either Positive).

Test	No. +/Total Herds	Prevalence of Positive Herds	95% C. I.
BRT+	40/98	**41.2 %**	(31.4, 51.0)
CARD+	39/101	**39.2 %**	(29.9, 48.4)
CARD+ and BRT+	25/96	**26.5 %**	(17.8, 35.1)
CARD + and CFT+	23/101	**22.7 %**	(14.6, 30.8)
CARD+ or BRT+	54/103	**53.0 %**	(43.5, 62.6)

Table 2.5. The Degree of Concordance (Kappa Statistic) Between the Brucellosis Ring Test (BRT) and the Brucellosis Card Test (CARD) as a Herd-Level Brucellosis Testing Method.

	CARD Positive	CARD Negative	Total
BRT Positive	25	14	39
BRT Negative	11	46	57
Total	36	60	96
		Kappa = 0.45; 95% C.I. (0.27, 0.64)	

Table 2.6. Individual-Animal Brucellosis-Seroprevalence in Cattle Tested in the Khangai Mountain Region of Bayankhongor Aimag (Province), Mongolia, with the USDA Brucellosis Card Test (CARD) and confirmed with the Mongolian Complement Fixation Test (CFT).

Stratum (Soum/Bagh)	**Number Tested**	**Number Positive CARD**	**(CFT)**	**Proportion Positive CARD**	**(CFT)**
Gurvanbulag	85	12	(8)	14.1%	(9.4%)
Zag	49	6	(2)	12.2%	(4.1%)
Jargalant	118	13	(7)	11.0%	(5.9%)
Galuut	130	25	(14)	19.2%	(10.8%)
Erdentsogt	93	10	(2)	10.8%	(2.2%)
Shargaljuut	38	5	(3)	13.2%	(7.9%)
TOTAL	**513**	**71**	**(36)**	**13.8%**	**(6.2%)**

Table 2.7. The Reported History of Brucellosis Diagnosis, Testing and Treatment in the Surveyed Heads of Herder Households in the Khangai Mountain Region of Bayankhongor Aimag (Province), Mongolia.

Reported History of Brucellosis Diagnosis	**Reported History of Brucellosis Diagnosis Within Previous 12 months**		**Reported History of Brucellosis Testing**		**Reported History of Brucellosis Treatment**
Overall	Overall	Diagnosed	Overall	Diagnosed	Overall
26/97 (26.8%)	14/97 (14.4%)	14/26 (53.8%)	11/97 (11.3%)	7/26 (26.9%)	0/0 (0.0%)

Table 2.8. Reported Occurrence of Clinical Signs and Symptoms Suggestive of Acute, Chronic, and General Brucellosis in the 100 Surveyed Heads of Herder Households in the Khangai Mountain Region of Bayankhongor, Mongolia.

	Acute	**Chronic**	**General (Acute + Chronic)**
	Chills, Fever, Headache, or Night Sweats	Joint Pain, Stiff Joint(s), Swollen Joint(s), or Lower Back Pain	Chills, Fever, Headache, Night Sweats, Joint Pain, Stiff Joint(s), Swollen Joint(s), or Lower Back Pain
Prevalence	**16%**	**20%**	**24%**

Chapter 3

MANAGEMENT-RELATED RISK FACTORS FOR BRUCELLOSIS IN CATTLE/YAK HERDS IN THE KHANGAI MOUNTAIN REGION OF BAYANKHONGOR AIMAG (PROVINCE), MONGOLIA

3.0 STRUCTURED ABSTRACT

Objective: This component of the study was undertaken to determine the specific management practices associated with increased risk of brucellosis-seropositive status of cattle (predominantly yak) herds in the Khangai mountain region of Bayankhongor Aimag (Province), Mongolia. The primary objective was to examine livestock management factors within the broad categories of grazing and watering, migration frequency, herd species composition, contact with outside herds, veterinary care, age structure, and size of herd for associations with cattle herd brucellosis status. The secondary objective was to determine whether herd brucellosis seropositivity affected calving or neonatal mortality rates on the herd level or was associated with the prevalence or occurrence of livestock abortions.

Design: Cross-sectional study

Sample Population: A stratified random sample (n=105) of the 5,062 livestock herds in 5 contiguous soums (districts) and 1 bagh (sub-district) of the Khangai mountain region of Bayankhongor Aimag (Province), Mongolia.

Procedure: A livestock management questionnaire was administered as an in-person interview to the head of each selected herder household. Cattle herd brucellosis-seroprevalence data was based on testing with the Brucellosis Milk Ring Test (BRT) and the Brucellosis Card Test (CARD). Livestock management data and reports of disease occurrence (abortions and neonatal deaths) were collected from the questionnaire. Descriptive statistics were generated and the data were analyzed with univariate and multi-variable methods. Attempts were made to identify herd-level and individual-level risk factors for brucellosis-seropositivity and assess the association between brucellosis-seropositivity and cattle health and production parameters.

Results: The stratification-adjusted prevalence of brucellosis-seropositive cattle herds in the study region was 53% (95% C.I. +/- 9.5%) based on having either/or a positive BRT or CARD test. The adjusted prevalence based on using the BRT and CARD test in parallel was 24.3% (95% C.I. +/- 8.1%). A significant association was found between larger cattle herd size and an increased risk of brucellosis seropositivity. Brucellosis herd seropositive status was associated with an increased number and prevalence of cattle abortions.

Conclusions: Bovine brucellosis is an important livestock disease in the Khangai mountain region of Bayankhongor Aimag (Province), Mongolia, and the findings of this study support the concerns surrounding the re-emergence of brucellosis in Mongolia. The reproductive losses (bovine abortion and infertility) affect both the supply of replacement stock and milk production, which in turn significantly impact the food security and economic viability of herder households. The human consequences of the circulation of brucellosis in livestock are a concern given the zoonotic nature of this disease.

3.1 INTRODUCTION

Brucellosis is an infectious disease of livestock and is one of the most widespread zoonotic diseases in the world (Corbel, 1989). The worldwide distribution of endemic brucellosis includes Mongolia, a sparsely populated country (1.5 persons/km^2) in Central Asia where livestock herding is the backbone of the county's economy and an important part of its cultural heritage (Mongolia, 2001c; OIE, 2002b). Major efforts have been undertaken around the world to control brucellosis due the significant economic losses associated with the occurrence of the disease in domestic livestock and the often debilitating disease associated with human infection (Halling and Boyle, 2002; Ragan, 2002; Reddy, 2003). Clinical signs of brucellosis in domestic livestock include late term abortions, decreased milk production and lowered fertility in both males and females (Drost and Thomas, 1996).

Particular biovars of the species of *Brucella melitensis* are associated with pathogenicity in different domestic animals. The disease in cattle is most commonly caused by the biovar *abortus* (Reddy, 2003). The vast majority of human infection is the result of direct or indirect transmission of the disease from infected livestock through the ingestion of contaminated milk or dairy products or through direct contact with aborted material or uterine fluid from infected animals (Nicoletti, 1989).

Historically, Mongolia has had a high incidence of brucellosis in its human population (Otgon 1968; Jezek Rusinko et al. 1972; Jezek, Rusinko et al. 1974) as a consequence of the high prevalence of the disease in domestic livestock (Beulig et al., 1969; Jezek et al., 1972; Jezek et al., 1974; Otgon, 1968; Pinigin et al., 1968a; Pinigin et al., 1968b; Tserendash, 1980, 1984; Tserendash and Shumilov, 1970; Zherikova et al., 1972). Brucellosis prevention, through livestock vaccination, began in Mongolia in the 1960's (Corbel, 1989; Tserendash, 1980). A second prevention program, lead by the World Health Organization (WHO), began in the mid-1970's and focused on the vaccination of small ruminants (Corbel, 1989). The former Soviet Union continued to support a brucellosis livestock vaccination program in Mongolia (*Brucellosis abortus* strain-19 vaccine in cattle and *Br. melitensis* Rev-1 vaccine primarily in small ruminants) through the late 1980's.

In the later half of the 20th century, the risk factors associated with livestock brucellosis in Mongolia included the establishment of state dairy farms and the more intensive management of cattle and small ruminants (Otgon, 1968). In addition the movement of cattle across the country was associated with the regional spread of livestock brucellosis (Denes, 1997; Otgon, 1968).

Initially the brucellosis control programs were deemed a success by Mongolian scientists (Tserendash, 1980) and by WHO officials who reported that the annual incidence of the disease in humans dropped to less than 1 case/10,000 in the early 1980's (Mikolon, 2000; Roth et al., 2003). Despite evidence in the early 1980's of a significant reduction in the incidence of brucellosis in Mongolia, concerns have been raised recently about the re-emergence of the disease in the country (Ebright et al., 2003; Joshi, 1991; Roth et al., 2003; WHO, 2000b, 2001, 2003). Brucellosis surveillance in Mongolia from 1990, until 2001, has consisted of unsystematic sampling of individual livestock and sporadic herd level testing (Roth et al., 2003). These data do not allow one to quantify the regional prevalence of disease or confirm the re-emergence of brucellosis, however, they do demonstrate that livestock brucellosis continues to persist in Mongolia.

The persistence and possible re-emergence of brucellosis as a major public health problem in Mongolia has been blamed on the collapse of the veterinary service delivery system and public health infrastructure in Mongolia which began in the late 1980's (Ebright et al., 2003; Mikolon, 2000). The Mongolian veterinary system in the 1990's reflects the changes that were experienced by the country as a whole as Mongolia transitioned from a centrally planned economy and socialist system of government to a democratic system with a market economy (Ebright et al., 2003; Rossabi, 1999). The combination of the abrupt loss of economic aid from the former Soviet Union, the privatization of livestock in 1990, and the privatization of veterinary service delivery in 1999, meant the end of large scale, government subsidized, livestock health campaigns including comprehensive livestock vaccination programs and systematic surveillance for brucellosis (Mikolon, 2000).

Recently collected data on the prevalence, incidence and distribution of brucellosis in Mongolia's livestock and human population is largely unavailable and no published reports of the risk factors associated with disease exist. Official government reports provide some information on disease occurrence but the sampling methods employed are unknown and little insight into the level and distribution of disease can be gained. The aim of this study was, therefore, to systematically collect data on the prevalence of brucellosis in livestock in Mongolia and examine livestock management practices used in the region. In addition an effort was made to quantify the production level of the herd and examine the data for associations between cattle herd brucellosis status and the clinical signs and symptoms often associated with bovine brucellosis. The decision was made to focus on the Khangai mountain region of Bayankhongor Aimag (Province), where brucellosis was reported to occur.

3.2 HYPOTHESES

1) There are specific management practices associated with increased risk of brucellosis-seropositive status of cattle herds in the Khangai mountain region of Bayankhongor Aimag (Province), Mongolia, within the broad categories of: grazing and watering, migration frequency, herd species composition, contact with outside herds, level of veterinary care, age structure and size of herd.

2) Herd brucellosis-seropositivity is a risk factor for the occurrence of abortions, a reduction in calving rate, and increased rates of neonatal mortality.

3.3 MATERIALS AND METHODS

A. Study Design and Herd Selection

A stratified random sample (n=104) of the 5,062 herds in the 5 contiguous Khangai soums (districts) of Gurvanbulag, Zag, Galuut, Jargalant, Erdenstogt, and the bagh (sub-district) of Shargaljuut were selected for sampling. The study area, sample size calculation and randomized herd selection methods are described in chapter 2 (Section 2.3).

A cross-sectional survey was used to determine the association between the prevalence of brucellosis-seropositive herds in the Khangai mountain region of Bayankhongor Aimag (Province) and livestock management practices as described below.

B. Data Collection Team and Herd Location

The data collection team consisted of two veterinarians, a physician, a driver, and a volunteer data recorder. The data collection team met with local officials and the soum (county) veterinarian in each soum center. The selected herder households were located with the assistance of the soum veterinarian and local officials. The soum veterinarian accompanied the data collection team on all herd visits and introduced the team to the herder household members. The Mongolian bilingual (English and Mongolian) collection team member explained the project and informed the herders that their participation in the study was voluntary. All team members were available for questions. Data collection proceeded only if consent was obtained.

C. Brucellosis Testing of Cattle Herds

The brucellosis status of the selected cattle herds was determined by using serological tests on milk and serum samples obtained from each herd. The milk samples were tested for the presence of *Brucella* antibodies using standard *B. abortus* strain 1119-3 BRT antigen provided by the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), National Veterinary Services Laboratories (NVSL), Ames, Iowa, USA.[6] Serum samples were collected from approximately ½ of the individual female cattle > 6 months of age in each herd and tested with the USDA brucellosis card test kit (CARD)[7]. The milk and serum samples were

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[6] The Brucellosis Milk Ring Test (BRT) is an internationally recognized method for detecting brucellosis in a herd of cattle. Blue haematoxylin-stained *Brucella abortus* antigen is used to detect *Brucella* antibodies in the milk of affected cows Alton, G., Jones, J., 1967, Laboratory Techniques in Brucellosis. World Health Organization, Geneva..

[7] The Brucellosis Card Test Kit (CARD) with Rose-Bengal stained, buffered *Brucella abortus* strain 1119-3 antigen is produced by the USDA, APHIS, NVSL in Ames, Iowa, USA. The CARD test is a modified serum agglutination test that detects the presence of antibodies against *Brucella* and was originally developed in the United States to meet the need for a rapid field test for the serological diagnosis of brucellosis in the ranch or stockyard setting Prior, M.G., Niilo, L., Reeker, W.H., 1975, Use of the

collected and tested using standard international protocols (OIE, 2002a) as described in chapter 2 (section 2.3). Serum from the CARD-positive individuals was submitted to the Mongolian State Central Veterinary Diagnostic and Sanitary Laboratory for confirmatory testing with the Complement Fixation Test (CFT)[8].

D. Case Definitions

Two primary case definitions of a brucellosis-positive herd were used in this component of the study. A cattle herd was classified as "Positive" if **either** a BRT-positive result was recorded for the mixed raw milk sample **or** at least one individual animal was classified as a reactor to the CARD test. A herd was classified as "Both Positive" if the BRT **and** CARD tests, were positive. These two case definitions of a brucellosis-seropositive herd were used in the univariate and multi-variable data analyses described below (section 3.4). The individual animal sero-prevalence data were used to create two alternative case definitions. A herd was classified as "> 1 CARD-positive", if more than 1 individual in the herd was identified as a CARD-test reactor. A herd was classified as "CFT-confirmed CARD-positive" if an individual in a herd tested with the CARD and CFT in series was positive on both tests. These two secondary case definitions were used as alternative outcome variables in the multi-variable logistic regression analyses described below (section 3.4).

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brucellosis card test for screening cattle in Saskatchewan. Canadian Journal of Comparative Medicine 39, 107-109.

[8] The complement fixation test is a serologic test designed to determine the *Brucella* titer of the serum under test. The CFT utilizes *Brucella* antigen, complement and sheep red blood cells to measure the degree of hemolysis. The titer of the serum under test is read as the highest dilution showing 50% hemolysis. In cattle a titer of 1:40 indicates an infected animal and one of 1:20 is suspicious, indicating a need to retest in 2 weeks Alton, G., Jones, J., 1967, Laboratory Techniques in Brucellosis. World Health Organization, Geneva..

E. Collection of Data Relating to Possible Risk Factors for Brucellosis-Seropositive Cattle Herds

A 3-page questionnaire was conducted as an in-person interview with an adult member of the herder household involved in livestock rearing. The interview was conducted by a bilingual (Mongolian and English) native Mongolian speaker. (See translation of questionnaire in Appendix B). The in-person interview and collection of bovine milk and serum samples for brucellosis testing were performed during the same herd visit. The livestock management data collected included: herd composition (species/numbers of livestock and young/year), herding practices (number of migrations/year, mixing with other livestock at water points, winter and night shelters), a history of veterinary care (brucellosis vaccination and other interventions) and livestock milking practices. In addition data were collected on the occurrence and timing of livestock abortions, male infertility and neonatal mortality. The final section of the questionnaire assessed herder awareness of characteristics of livestock brucellosis. Interview responses were recorded in Mongolian and later translated into English.

3.4 DATA ANALYSIS

A. Univariate Analysis of Herd and Individual-Level Data

Data from the livestock management questionnaires and BRT, CARD and CFT test results were entered into Microsoft Excel spreadsheets (Microsoft Office 2000). Data were subsequently analyzed using the SAS software program (SAS version 8.2, Cary, N.C.: SAS Institute, Inc. 1999-2001). Descriptive statistics were computed for the risk factors and dependent variables of interest. Cattle abortion rates, calving rates and neonatal mortality rates were computed for each herd. Multiple herd mixing variables (water, shelter, night) were collapsed into 1 variable, "mix". A poverty variable was created based on the number of livestock per herder household. If the herder household owned less than 150 head of livestock they were classified as "poor".

Individual animal data (age, sex, breed and CARD test results) were transferred from field data sheets and entered into Microsoft Excel spreadsheets (Microsoft Office 2000). Descriptive statistics were computed for the individual animal level risk factors. The individual animal data were used to calculate the within herd prevalence of brucellosis reactors and the average age of cows in the herd. These two calculated herd-level characteristics were incorporated into the primary data set as new herd-level variables.

The t-test (PROC TTEST) was used to test for significant associations between herd brucellosis status and continuous risk factors such as herd size, number of individual species and numbers of migrations per year. The Wilcoxon nonparametric test (PROC NPAR1WAY) was used in addition to the t-test for those continuous risk factors with a skewed distribution. Pearson's X^2 test (PROC FREQ; "TABLES/CHISQ") was used to test for significant associations between herd brucellosis status and categorical risk factors such as mixing with other herds, the occurrence of livestock abortions, and herd location. The X^2 test for trend (PROC FREQ; "TABLES/CHISQ"; MH) was computed when the categorical risk factors represented ordinal levels. The t-test and Pearson's X^2 test were also used to test for significant associations between the individual animal brucellosis status continuous and categorical risk factors such as age and breed.

B. Multi-Variable Analysis of Herd-Level Data

The original complete herd-level data set contained 44 variables. A subset of the original variables were considered for inclusion in the full logistic regression model. Variables excluded from the multi-variable logistic regression analysis included those variables with a large proportion of missing responses (>20% missing); redundant variables; and those deemed not to be biologically relevant to the occurrence of brucellosis. Eleven risk factors or disease indicators were examined in the full logistic regression model. Soum (county) was entered as a class variable. No variables were forced into the model and no interactions were included. Backward elimination was used to model the relationship between cattle herd brucellosis status (classified by "BRT and CARD-positive", by "BRT or CARD-positive", by ">1 CARD-positive" and by "CFT-confirmed CARD-positive") and livestock management practices. Observations with missing data were removed during the modeling procedure.

3.4 RESULTS

A. Description of Sample Population

The head of the herder household volunteered to be interviewed for the livestock management questionnaire portion of this study in 103, of the 104 herds (99%), contacted. Information on cattle herd brucellosis status was available for the same 103 herds. The total number of cattle herds found to be seropositive for brucellosis based on either the Brucellosis Milk Ring Test (BRT) or the Brucellosis Card Test (CARD) was 54 of 103, an adjusted prevalence of 53% (95% C.I. +/- 9.5%). The number of cattle herds found to be seropositive on both the BRT and CARD test was 25 of 96, an adjusted cattle herd prevalence of 26.5% (95% C.I. +/- 8.7%).

The completeness of data collected in each of the interview sessions varied. The variables with the greatest amount of missing data included the following: reported abortion in ewes (44% missing), reported abortion in does (37% missing), number of reproductive-age female cattle (35% missing), number of goats (27% missing), number of sheep (26% missing), number of cattle (22% missing), and total number of calves (21%). The data for the remaining variables covered in the livestock management survey were 80% to 100% complete. The primary explanations for the missing data were incomplete interviews due to time constraints and recording errors. The degree of missing data on livestock numbers also affected the completeness of some of the calculated variables including calving rate, calf survival, and cattle abortion rate.

B. Descriptive and Univariate Analyses of Herd Management Risk Factors

The data on total number of cattle (mean = 20.26), total cows or reproductive-age females (mean = 10.93), and total milking cows (mean = 7.71) indicate the presence of an association between herd size and brucellosis seropositivity in this population. The mean number of both milking cows and adult females was statistically significantly higher at the $p<0.05$ level in the cattle herds classified as brucellosis-seropositive by both or either the BRT and CARD tests (See Table 3.1). The same trend was observed in the total number of cattle but the difference in means was not significant at the $p=0.05$ level, (See Table 3.1). The data on cattle herd size were also categorized and analyzed using the X^2 test for trend. A significant ($p=0.0013$) trend of increasing risk associated with increasing herd size was found (See Table 3.2). An association was not found between the total number of livestock (cattle, sheep and goats) and cattle herd brucellosis status.

All of the herders interviewed reported that they milked cattle. Neither the presence or the number of sheep, nor the presence or the number of goats in the herd was significantly associated with cattle brucellosis seropositivity. Milking goats appeared to be a protective factor for brucellosis in cattle herds (0/12 herds that milked goats were sero-negative, compared to 22/54 that did not milk goats), however, the association was not found when cattle herds were classified as brucellosis-seropositive based on either a positive BRT or CARD test (See Table 3.2).

No statistically significant (p-value <= 0.05) associations were found between cattle herd brucellosis seropositivity and any other of the livestock management or herd characteristics examined, including the degree of mixing with other livestock, number of migrations per year, level of veterinary service, reported use of brucellosis vaccine, breed of cattle, the socio-economic status of the herder household or the level of knowledge of livestock brucellosis symptoms and prevention (See Table 3.1 and 3.2).

No association between individual animal brucellosis sero-status and cattle breed, pure yak (*Bos Grunniens*) or yak crossed with "Mongol" cattle (*Bos Taurus*), was found. (See Table 3.3). The mean age of CARD-positive individual cattle was significantly higher (p=0.0253) than the mean age of CARD-negative individuals (See Table 3.3). The X^2 test for trend, however, did not show a significant increasing risk of CARD-positive brucellosis status with increasing age class (See Table 3.3). In addition "average cow age" as a herd-level risk factor was not associated with cattle herd brucellosis seropositivity.

C. Descriptive and Univariate Analyses of Disease Indicators

A statistically significant association was found between cattle herd brucellosis-seropositivity (positive BRT and CARD) and reported cattle abortion (p=0.0017), number of cattle abortions (p=0.0429) and percent of cattle abortions in a herd (p=0.0077), (See Table 3.4 and 3.5). The same trend was seen in those herds classified as brucellosis-seropositive by either or the BRT or CARD test but the associations were not significant at the p=0.05 level, (See Table 3.4 and 3.5). No association was found between cattle herd brucellosis seropositivity and reported abortions in goats but a significant association was found between CARD and BRT-positive cattle herds and reported abortions in sheep (p=0.0398). The only herd with a horse with draining sores over the withers was from a brucellosis-seropositive cattle herd.

No significant associations were found between cattle herd brucellosis seropositivity and the reporting of bovine neonatal deaths. No significant differences in the mean calving rates, calf mortality or survival was seen between brucellosis-seropositive and negative herds, although the calculated calving rate was lower in brucellosis-seropositive herds, (See Table 3.5).

D. Multi-Variable Analysis of Management Risk Factors and Disease Indicators Associated with Herd Brucellosis-Seropositive Status

The full multi-variable logistic regression model was based on 68 observations (66% of cattle herds) and included 11 variables, (See Table 3.6). The number of observations was reduced from the original 103 herds due to missing data on herd size. The overall prevalence of brucellosis-seropositive herds in the 68 herds included in the logistic regression model was 22% for herds classified as positive if both the "BRT and CARD" test were positive and 54% for herds classified as positive if either the "BRT or

CARD" test were positive. The brucellosis herd sero-prevalence calculated for the 68 herds included in the logistic regression model was the proportional to that calculated for the 103 herds in the original sample; 22% vs. 23% for the "BRT and CARD", and 54% vs. 53% for the "BRT or CARD", in the 68 and 103-herd sample, respectively.

The final reduced model (Table 3.7) included 2 variables; "number of milking cows" and "observed bovine abortion". An increasing number of milking cows was associated with an increased risk of cattle herd brucellosis-seropositive status. The odds of a herd being classified as brucellosis-seropositive according to the "BRT and CARD" and "BRT or CARD" definition was increased by 0.24 and 0.13 times, respectively, for every 1 cow increase in herd size. The observation of bovine abortion in the herd in the previous year was associated with an increased risk of cattle herd brucellosis-seropositive status in herds classified by the "BRT and CARD" and the "BRT or CARD" testing methods. The increase in odds of classification as brucellosis-seropositive by the "BRT and CARD" in herds in which bovine abortion had occurred was 25 (95% C.I.; 4.5 – 139.5) compared with herds reporting no bovine abortion. The increase in odds of brucellosis-seropositive classification by "BRT or CARD" was 4.4 (95% C.I.; 1.2 – 16.3) compared to herds that reported observing no bovine abortions in the previous year.

The full logistic regression model was also run with the two secondary case definitions, ">1 CARD-positive" and "CFT-confirmed CARD-positive" as the outcome variable. The final reduced logistic regression model with the two secondary case definitions as the outcome variable also identified "the occurrence of bovine abortion" and an "increasing number of milking cows" as risk factors for brucellosis-seropositive herd status. The odds ratios and 95% confidence intervals are presented in Table 3.7.

3.5 DISCUSSION

A. Distribution of Brucellosis-Seropositive Cattle Herds

Serologic testing of cattle herds in the Khangai mountain region of Bayankhongor Aimag (Province), Mongolia, revealed a high prevalence of brucellosis-seropositive herds (53.0% based on having either/or a positive BRT or CARD test and 26.5% based on using the BRT and CARD test in series) in the region as well as a high prevalence of individual reactors to the brucellosis card test (13.8%). This level of disease is similar to the prevalence described in countries with no systemic brucellosis control programs and similar systems of nomadic or extensive livestock management, including Mexico (Salman et al., 1984), Eritrea (Omer et al., 2001), Syria (Darwish and Benkirane, 2001), Jordan (Al-Talafhah et al., 2003) and Chad (Schelling et al., 2003).

Brucellosis-seropositive cattle herds were detected throughout the study region, in each of the soums (districts) and bagh (sub-district) tested. A visual inspection of the mapped points indicating the location of positive and negatives herds (See Figure 2.2) did not reveal any degree of clustering, and herd location (soum) was not determined to be a significant risk factor for either cattle herd or individual animal brucellosis seropositivity.

B. Livestock Management Risk Factors for Cattle Herd Brucellosis Seropositivity

Livestock management practices were largely consistent across the study region. Yaks (*Bos grunniens*) were the primary livestock species in the Khangai mountain region of Bayankhongor Aimag (Province,) yet almost all of the herds were composed of mixed species. Herders migrated seasonally (approximately 4 times per year) in search of pasture for their livestock, and all of the land in the region was commonly owned. The uniformly extensive management system, and the largely unrestricted contact between herds, resulted in the identification of very few differences from herd to herd.

The univariate analyses of livestock management practices examined revealed significant associations between herd disease status and variables relating to cattle herd size, especially the total number of adult female cattle and the total number of milking cows. The mean number of total adult female cattle and total milking cows was significantly larger in the seropositive herds. Increasing cattle herd size has often been found to be associated with an increased risk of brucellosis infection (Nicoletti, 1980a) which can be explained by the increase in cattle density that is often associated with larger herds and leads to an increase in cattle to cattle transmission, the inability to identify and remove infected cattle and the greater likelihood of the addition of replacement cattle from outside sources.

The multi-variable logistic regression model confirmed the trends seen in the univariate analyses. With the exception of increasing herd size, none of the other risk factors examined (presence of sheep or goats, mixing of herds, brucellosis vaccination, veterinary care, herder knowledge of livestock brucellosis, herd location or migration frequency) emerged as significant risk factors in the modeling of disease in this population.

The association between increasing cow herd size and the risk of brucellosis-seropositive status was stronger (larger odds ratios and smaller p-values) when the herd was classified as positive using the more conservative, and arguably more specific, case definition of "both BRT and CARD positive" vs. the less conservative case definition of "either BRT or CARD positive". The association is likely weakened when the less conservative and less specific case definition is used, due to misclassification bias resulting from false positive tests.

C. Cattle Brucellosis Disease Indicators

Bacteriologic culture and *Brucella* isolation and identification was unavailable in Mongolia at the time this study was conducted. To compensate for the inability to culture-confirm *Brucella* infection in the seropositive herds, data were collected on the prevalence of clinical signs (abortion, decreased milk production, orchitis, male infertility, and neonatal weakness or death) associated with brucellosis in cattle (Reddy, 2003). None of the herders interviewed had observed or suspected any degree of male infertility within their livestock herds, however, no systematic qualitative or quantitative assessment of male fertility was practiced.

Signs of female infertility in the form of observed late term abortions were reported frequently for cattle, sheep and goats. All of the cattle abortions were reported to have occurred in January, February or March, the time period that corresponds to the final 100 days of gestation in this seasonally and naturally bred population. Univariate analyses revealed a strong association between the occurrence, number and prevalence of abortions and brucellosis-seropositive cattle herds. The calving rate was lower in the seropositive cattle herds though the difference in the mean calving rate in the brucellosis positive and negative herds was not significant.

The multi-variable logistic regression model revealed that the occurrence of cattle abortion is highly associated with brucellosis positive herd status with an adjusted odds ratio of 25.0 (95% C.I., 4.5 – 139.5) and 4.4 (95% C.I., 1.2 – 16.3) in the "BRT and CARD" and "BRT or CARD" classified herds, respectively. A similarly strong association was seen when the more specific case definitions for a positive herd were used. Odds ratios of 14.8 (95% C.I., 2.7-81.8) and 10.2 (95% C.I., 2.5 – 42.0) for the ">1 CARD-positive" and "CFT-confirmed CARD-positive", respectively, were strongly supportive of a causal relationship between bovine abortion and brucellosis in these herds.

Although other causes of abortion in this population could not be ruled out, brucellosis is thought to be the main infectious cause of abortions in cattle in this region of Mongolia (Mondongadis, 2000). It is possible that some of the late term abortions noted were a consequence of nutritional deficits related to a severe winter, or trauma associated with migration from winter to spring pastures, however, the strong association of reported abortion and herd brucellosis-seropositive status points to *Brucella* infection as an important cause of bovine abortion in this population. A strong association between reported cattle abortions and herd brucellosis-seropositive status has been reported in other population based studies of cattle brucellosis (McDermott and Arimi, 2002) (Schelling et al., 2003). Although brucellosis infection could not be confirmed with bacteriologic culture in this study, it is clear that the seroprevalence data is associated with the clinical signs and morbidity we would expect with *Brucella* infection in cattle.

We were unable to confirm the strain of *Brucella* circulating in cattle of the Khangai mountain region of Bayankhongor Aimag (Province), Mongolia, within the scope of this study, however, data on the occurrence of abortion in sheep and goats in the sampled herds points toward the *abortus* biotype. A significant association was seen between reported abortions in sheep and the seropositive status of the cattle herd, but not between the reported occurrence of abortion in goats and the seropositive status of the cattle in the herds sampled. *B. abortus* is reported to have a low pathogenicity for sheep and goats although it has been associated with a number of outbreaks in sheep (Nicoletti, 1980b). *B. abortus* infection in goats has only rarely been recorded (Corbel, 1989). The fact that the same herds experienced abortions in their sheep and cattle but not their goats supports the assumption that the observed abortions in cattle were due to *B. abortus* infection.

D. Sero-Prevalence and Risk Factors Associated with Disease in Individual Cattle

This study provided the opportunity to collect data on the prevalence of brucellosis-seropositive individuals in the herds selected for testing. The overall prevalence was CARD test-positive individuals was 13.8% (71/513). The prevalence of CARD-positive individuals that were also complement fixation test (CFT)-positive was 6.2%. Although there were very few non-yak bovines in the sample, cattle breed did not appear to be a risk factor for disease, as has been shown in other studies of brucellosis in cattle (Crawford et al., 1990). The prevalence of seropositive animals was greatest in the 2 to 4-year age range (27.3%) and the lowest in those less than 2 (4.5%). This corresponds to previous findings that suggest that young cattle are less susceptible to brucellosis infection than older sexually mature cattle (Crawford et al., 1990).

E. Limitations

The lack of bacteriological confirmation of the serological findings in this study is unfortunate, however, this population of cattle did not have a history of brucellosis vaccination thereby eliminating the primary cause of false-positive results associated with the testing methods used.

The stratified random sampling technique used to select cattle herds for testing in this study allows us to extrapolate our findings on the prevalence of brucellosis-seropositive herds, and the risk factors and clinical signs associated with the disease, to the entire Khangai mountain region of Bayankhongor Aimag (Province), Mongolia. The external validity of our findings beyond this region, however, is limited. Other mountainous regions of the country where the yak is the primary livestock species, and where the cattle density is similar, would most likely experience a similar level of bovine brucellosis. The data collected in this study support the presence of a high prevalence of brucellosis infection in the region. The past implementation of national brucellosis control programs in Mongolia lead us to believe that livestock brucellosis is an important disease across the country. It is also recognized that the prevalence of cattle brucellosis and the risk factors associated with disease transmission and persistence, will vary from one ecological zone to another where the composition of livestock herds, cattle density and general livestock management practices differ.

The cross-sectional nature of this study allows us only to describe the disease burden in a snap shot in time. The patterns described reflect associations only, and do not provide the data necessary to make firm inferences on temporal associations or cause and effect relationships. The study was, however, conducted in the late summer in an attempt to reduce the limitations of data obtained in a cross-sectional study. In this seasonally bred population of cattle, the late summer represents the post-calving period when one would expect the clinical signs and symptoms associated with bovine brucellosis to be most apparent and the livestock management practices that would limit or promote the spread of disease to have the most impact.

The limited herd-to-herd variability made the identification of herd-level risk factors for brucellosis seropositivity difficult. It is possible that the livestock management risk factors and the indicators of disease that were identified in this study were associated not with bovine brucellosis-seropositive status but another unidentified factor. This possibility is unlikely given that the associations identified are biologically plausible and consistent with the results of others. The data collected indicate the need for a broader survey of livestock brucellosis in Mongolia and provide an interesting basis for further research.

3.6 CONCLUSION

Bovine brucellosis is an important livestock disease in the Khangai mountain region of Bayankhongor Aimag (Province), Mongolia. The direct losses due to bovine abortion and impaired fertility are clear. These reproductive losses affect both the supply of replacement stock and milk production, which in turn significantly impact the food security and economic viability of herder households. The human consequences of the circulation of brucellosis in livestock are a concern and the findings of a further investigation in this area will be presented in the following chapter (chapter 4). On a national level, the presence of brucellosis in livestock impacts access to markets for both live animals and livestock products. The national health program is undoubtedly burdened, and human productivity is likely hampered by the zoonotic transmission and incidence of human brucellosis infection in Mongolia.

The findings of this study indicate that concerns surrounding the re-emergence of brucellosis in Mongolia (Ebright et al., 2003; Roth et al., 2003; WHO, 2001; Zinsstag et al., submitted 2004) are well founded. The national brucellosis control program, currently underway in Mongolia, will need to be evaluated as to its effectiveness in reducing and eventually limiting the spread of brucellosis in Mongolia. Greater attention should be paid to areas with higher herd prevalence, larger herds or an increased density of livestock, as indicated both in this study of a population of cattle in Mongolia, and historically in regions across the globe.

Table 3.1. Univariable Analyses of Continuous Livestock Management Related Risk Factors for Herd Brucellosis Status of Herds in the Khangai mountain region of Bayankhongor Aimag (Province), Mongolia, Tested in 2000, using the BRT and CARD test [N=103 (BRT or CARD); N=96 (BRT and CARD); Positive Herds=54 (BRT or CARD); Positive Herds=25 (BRT and CARD)]*

Risk Factor	Herd Brucellosis Status (BRT and CARD)	n	Mean	S.D.	[t]	p	Herd Brucellosis Status (BRT or CARD)	n	Mean	S.D.	[t]	p
Total Cattle	Positive	23	23.65	15.18	1.32*	0.1932	Positive	45	22.53	16.00	1.51*	0.1340
	Negative	51	18.69	14.42			Negative	35	17.34	14.56		
Total Cows (Adult)	Positive	16	16.19	9.23	3.62	0.0006	Positive	35	13.11	7.68	2.82*	0.0065
	Negative	49	9.35	5.48			Negative	32	8.53	5.54		
Total Cows (Milking)	Positive	24	10.38	6.86	2.89	0.0048	Positive	52	9.10	5.76	2.90	0.0046
	Negative	70	6.95	4.22			Negative	48	6.20	4.10		
Cow Age (Average)	Positive	25	7.72	2.57	1.57*	0.1250	Positive	53	7.10	2.22	0.22*	0.8234
	Negative	71	6.84	1.90			Negative	48	7.00	1.93		
Livestock (Total)	Positive	18	94.28	90.72	0.15*	0.8780	Positive	35	92.34	96.57	0.28*	0.7786
	Negative	39	98.49	105.36			Negative	26	99.62	101.42		
Migrations (# per year)	Positive	22	3.75	1.40	0.45	0.6555	Positive	50	4.13	2.65	0.41*	0.6839
	Negative	64	4.05	2.99			Negative	43	3.88	3.10		

*Indicates the use of the Satterthwaite method to generate the t-value when variances are not equal

Table 3.1. Continued

Risk Factor	Herd Brucellosis Status (BRT and CARD)	n	Mean	S.D.	[t]	p	Herd Brucellosis Status (BRT or CARD)	n	Mean	S.D.	[t]	p
No. Sheep	Positive	20	49.40	68.54	0.53	0.5992	Positive	43	75.56	217.62	0.64	0.5243
	Negative	51	73.77	201.1			Negative	33	50.52	63.16		
No. Goats	Positive	20	21.05	18.34	0.96	0.3418	Positive	42	25.88	37.30	0.01	0.9938
	Negative	50	29.42	37.21			Negative	33	25.94	24.84		

Table 3.2. Univariate Analysis of Herd Management Related Categorical Risk Factors for Cattle Herd Brucellosis Status of Herds in the Khangai Mountain Region of Bayankhongor Aimag (Province), Mongolia, Tested in 2000, Using the BRT and CARD test [N=103 (BRT or CARD); N=96 (BRT and CARD); Positive Herds=54 (BRT or CARD); Positive Herds=25 (BRT and CARD)].*

Risk Factor	**Frequency by Herd Brucellosis Status CARD and BRT**		X^2	**p-value**	**Frequency by Herd Brucellosis Status CARD or BRT**		X^2	**p-value**
	Pos.	**Neg.**			**Pos.**	**Neg.**		
Horse Sores	0	0	--	--	1	0	0.94	1.0000
Sheep Present	17	47	0.03	0.8694	38	32	0.30	0.5823
Goats Present	17	44	0.29	0.5902	36	31	0.13	0.7177
Milk Cattle	22	66	--	--	50	45	--	--
Milk Sheep	0	6	2.15	0.3300*	3	3	0.02	1.0000*
Milk Goats	0	12	4.63	0.0326*	6	6	0.04	0.8451
Mix w/Other Livestock	15	51	0.62	0.5467*	36	33	0.13	0.7174
Veterinary Service	15	46	0.42	0.5182	34	32	0.37	0.5464
Brucellosis Vaccination	1	2	0.15	0.5683*	1	3	1.23	0.3463*

* Indicates the use of the 2-sided probability from the Fisher's exact test when 25% of the cells have counts less than 5.

Table 3.2. Continued

Risk Factor	Frequency by Herd Brucellosis Status CARD and BRT Pos.	Neg.	X^2	p-value	Frequency by Herd Brucellosis Status CARD or BRT Pos.	Neg.	X^2	p-value
Breed-Mixed (*Bos grunniens* and *Bos taurus*)	5	10	0.49	0.5273*	12	5	2.69	0.1008
Poor Households (<150 Livestock)	15	31	0.12	1.0000*	28	21	0.01	0.9404
Herders Identified Symptoms of Brucellosis	6	6	4.25	0.0687*	7	6	0.00	1.0000*
Herders Understood Brucellosis Prevention	3	6	0.32	0.6879*	5	4	0.01	1.0000*

X^2 Test for Trend

Herd Size	Pos.	Neg.	Mantel-Haenszel X^2	p-value	Pos.	Neg.	Mantel-Haenszel X^2	p-value
1-5 cows	1	15	10.29	0.0013	5	11	8.95	0.0028
6-10 cows	2	14			8	10		
11-15 cows	4	9			8	5		
16-42 cows	6	4			9	1		

* Indicates the use of the 2-sided probability from the Fisher's exact test when 25% of the cells have counts less than 5.

Table 3.3. Univariate Analysis (X^2-Test) of Cattle Breed, Cattle Age and Cattle Age Class (X^2 Test for Trend) as Risk Factors for Individual Bovine brucellosis Sero-Positivity in a Population of Cattle in the Khangai Mountain Region of Bayankhongor Aimag (Province), Mongolia, Tested in 2000, Using the CARD Test (N= 516; Positive Cattle=71)

Breed	**Pos.**	**Neg.**	**Mantel-Haenszel X^2**	**p-value**
Pure (*Bos grunniens*)	69	424	0.5202	0.7559*
Cross (*Bos taurus*)	2	21		

	n	**Mean Age**	**S.D.**	**[t]**	**p-value**
Positive	71	6.15	2.76	2.28	0.0253
Negative	438	6.97	3.01		

Age Class	**Pos.**	**Neg.**	**Mantel-Haenszel X^2**	**p-value**
(1-2 years)	1	22	2.95	0.0858
(>2-4 years)	21	77		
(>4-6 years)	23	112		
(> 6 years)	26	227		

Table 3.4. Univariate Analysis of Disease Indicators for Cattle Herd Brucellosis Status of Herds in the Khangai Mountain Region of Bayankhongor Aimag (Province), Mongolia, Tested in 2000, Using the BRT and CARD Test [N=103 (BRT or CARD); N=96 (BRT and CARD); Positive Herds=54 (BRT or CARD); Positive Herds=25 (BRT and CARD)].*

Risk Factor	Frequency by Herd Brucellosis Status CARD and BRT		X^2	p-value	Frequency by Herd Brucellosis Status CARD or BRT		X^2	p-value
Cattle Abortions	9	7	11.67	0.0017*	13	5	3.03	0.0819
Goat Abortions	3	11	0.49	0.6725*	7	9	0.02	0.8847
Sheep Abortions	3	3	6.24	0.0398*	5	2	1.71	0.2458*
Neonatal Deaths	6	14	0.66	0.5399*	13	9	1.28	0.2580

* Indicates the use of the 2-sided probability from the Fisher's exact test when 25% of the cells have counts less than 5.

Table 3.5. Univariable Analyses of Continuous Disease Indicators for Herd Brucellosis Status of Herds in the Khangai Mountain Region of Bayankhongor Aimag (Province), Mongolia, Tested in 2000, using the BRT and CARD test [N=103 (BRT or CARD); N=96 (BRT and CARD); Positive Herds=54 (BRT or CARD); Positive Herds=25 (BRT and CARD)]*

Risk Factor	Herd Brucellosis Status (BRT and CARD)	n	Mean	S.D.	[t]	p	Herd Brucellosis Status (BRT or CARD)	n	Mean	S.D.	[t]	p
No. Cattle Abortions	Positive	19	1.42	2.41	2.06	0.0429	Positive	47	0.95	2.07	1.14	0.2583
	Negative	61	0.47	1.52			Negative	40	0.50	1.49		
% Cattle Abortions	Positive	13	0.13	0.21	2.77	0.0077	Positive	31	0.06	0.15	1.25	0.2155
	Negative	43	0.02	0.08			Negative	27	0.02	0.10		
% Calf Survival	Positive	17	0.93	0.12	0.33	0.7393	Positive	38	0.88	0.23	1.08*	0.2826
	Negative	58	0.91	0.21			Negative	41	0.93	0.17		
% Calf Death	Positive	11	0.04	0.08	0.58	0.5670	Positive	26	0.04	0.08	0.97	0.3372
	Negative	40	0.07	0.16			Negative	27	0.08	0.18		
Calving Rate	Positive	13	0.75	0.21	0.78*	0.4438	Positive	32	0.77	0.21	0.77*	0.6382
	Negative	47	0.80	0.21			Negative	30	0.80	0.22		

*Indicates the use of the Satterthwaite method to generate the t-value when variances are not equal

Table 3.6. Risk Factors in the Full Logistic Regression Model of Livestock Management and Disease-Related Risk Factors for Cattle Herd Brucellosis Sero-Positive Status in the Khangai Mountain Region of Bayankhongor Aimag (Province), Mongolia, Tested in 2000, using the BRT, CARD and CFT test [N=103 (BRT or CARD); N=96 (BRT and CARD); Positive Herds=54 (BRT or CARD); Positive Herds=25 (BRT and CARD)].

Categorical Risk Factors	**Frequency by Herd Brucellosis status (CARD and BRT)**		**X^2**	**p-value**	**Frequency by Herd Brucellosis status (CARD or BRT)**		**X^2**	**p-value**
	Pos.	**Neg.**			**Pos.**	**Neg.**		
Reported Cattle Abortion	9	7	11.67	0.0017*	13	5	3.03	0.0819
Sheep Present	17	47	0.03	0.8694	38	32	0.30	0.5823
Goats Present	17	44	0.29	0.5902	36	31	0.13	0.7177
Mix w/Other Livestock	15	51	0.62	0.5467*	36	33	0.13	0.7174
Veterinary Service	15	46	0.42	0.5182	34	32	0.37	0.5464
Brucellosis Vaccination	1	2	0.15	0.5683*	1	3	1.23	0.3463*
Identified Symptoms	6	6	4.25	0.0687*	7	6	0.00	1.0000*
Understood Prevention	3	6	0.32	0.6879*	5	4	0.01	1.0000*

* Indicates the use of the 2-sided probability from the Fisher's exact test when 25% of the cells have counts less than 5.

Table 3.6. Continued

Class Variable		Herd Brucellosis Status (BRT and CARD)		Herd Brucellosis Status (BRT or CARD)	
Soum	**Coding**	**Test**	**Wald X^2 p-value**	**Test**	**Wald X^2 p-value**
Gurvanbulag	1	1 vs 6	0.9906	1 vs 6	0.9340
Zag	2	2 vs 6	0.4378	2 vs 6	0.9844
Jargalant	3	3 vs 6	0.7607	3 vs 6	0.7318
Galuut	4	4 vs 6	0.8756	4 vs 6	0.4849
Erdentsogt	5	5 vs 6	0.5943	5 vs 6	0.9148
Shargaljuut	6				

Continuous Risk Factors	Herd Brucellosis Status (BRT and CARD)	n	Mean	S.D.	[t]	p	Herd Brucellosis Status (BRT or CARD)	n	Mean	S.D.	[t]	p
Migrations	Positive	22	3.75	1.40	0.45	0.6555	Positive	50	4.13	2.65	0.41*	0.6839
(# per year)	Negative	64	4.05	2.99			Negative	43	3.88	3.10		
Total Cows	Positive	24	10.38	6.86	2.89	0.0048	Positive	52	9.10	5.76	2.90	0.0046
(Milking)	Negative	70	6.95	4.22			Negative	48	6.20	4.10		

*Indicates the use of the Satterthwaite method to generate the t-value when variances are not equal

Table 3.7. Final Reduced Logistic Regression Model of Livestock Management and Disease-Related Risk Factors for Cattle Herd Brucellosis Sero-Positive Status in the Khangai Mountain Region of Bayankhongor Aimag (Province), Mongolia, Tested in 2000, with the USDA Brucellosis Milk Ring and Card Test.

	Herd Brucellosis Status (BRT and CARD)			**Herd Brucellosis Status (BRT or CARD)**		
Risk Factor	**Wald X^2 p-value**	**Odds Ratio**	**95% Confidence Interval (Wald)**	**Wald X^2 p-value**	**Odds Ratio**	**95% Confidence Interval (Wald)**
Reported Cattle Abortion	0.0005	13.61	3.13 – 59.20	0.0394	3.56	1.06 – 11.92
No. Milking Cattle (Unit Increase = 1)	0.0029	1.20	1.06 – 1.35	0.0281	1.15	1.03 – 1.27

	Herd Brucellosis Status (>1 BRT-positive)			**Herd Brucellosis Status (CFT-confirmed BRT-positive)**		
Risk Factor	**Wald X^2 p-value**	**Odds Ratio**	**95% Confidence Interval (Wald)**	**Wald X^2 p-value**	**Odds Ratio**	**95% Confidence Interval (Wald)**
Reported Cattle Abortion	0.0014	16.42	2.94 – 91.71	0.0019	8.09	2.16 – 30.32
No. Milking Cattle (Unit Increase = 1)	0.0091	1.20	1.05 – 1.37	0.0205	1.14	1.02 – 1.26

Chapter 4

THE OCCURRENCE OF CLINICAL SIGNS SUGGESTIVE OF HUMAN BRUCELLOSIS AND ASSOCIATED RISK FACTORS IN LIVESTOCK HERDERS IN THE KHANGAI MOUNTAIN REGION OF BAYANKHONGOR AIMAG (PROVINCE), MONGOLIA

4.0 STRUCTURED ABSTRACT

Objective: This component of the study was undertaken to 1) record the occurrence of clinical signs and symptoms suggestive of human brucellosis in livestock herders in the Khangai mountain region of Bayankhongor Aimag (Province), Mongolia, and 2) identify the risk factors associated with brucellosis-like disease occurrence in the Bayankhongor herder population. In addition, attempts were made to compare the prevalence of brucellosis in livestock (cattle) and the occurrence of brucellosis-like disease in humans.

Design: Cross-sectional study

Sample Population: The male or female head of household of a stratified random sample of livestock herder households in 5 contiguous soums (district) and 1 bagh (sub-district) of the Khangai mountain region of Bayankhongor Aimag (Province), Mongolia.

Procedure: A questionnaire was administered as an in-person interview to the head of each herder household. The questionnaire collected information on the history of diagnosis and treatment of brucellosis in the herders as well as the occurrence of non-specific clinical signs and symptoms experienced within the previous 12-month period.

Information on the consumption of meat and dairy products and contact with livestock was collected. Descriptive statistics were generated and the data were analyzed with univariate and multi-variable methods.

Results: Twenty-six 100 herders interviewed reported a history of brucellosis diagnosis, and 14 reported that they had received a diagnosis of brucellosis within the previous 12 months. Similarly, 20 of the 100 individuals interviewed reported experiencing clinical signs suggestive of chronic brucellosis, and 16 reported experiencing clinical signs suggestive of acute brucellosis within the previous 12 months. Thirty-three percent of herders reported preparing dairy products from raw milk, 12% reported drinking raw cow's milk and 30% reported bringing weak neonates (lambs, calves and kids) into their living quarters for extra care. All three of these behaviors were determined to be significant risk factors for the occurrence of clinical signs suggestive of brucellosis.

Conclusions: Data on the occurrence of brucellosis-like symptoms in the herder population indicates that the disease has a significant impact on the health and well being of the human herder population. Consumption of raw milk, and close contact with weak, newborn livestock, were identified as risk factors for the occurrence of clinical signs suggestive of brucellosis in this herder population.

Data from this study support the Mongolian government's classification of brucellosis as a priority public health concern. Because brucellosis is a zoonotic disease, the focus of Mongolia's planned national brucellosis control program is livestock vaccination. The program is designed to control the disease in livestock and thereby reduce and eventually eliminate the source of human *Brucella* infections.

4.1 INTRODUCTION

Brucellosis is an infectious disease of livestock and one of the most widespread zoonotic diseases in the world (Corbel, 1989). The worldwide distribution of endemic brucellosis includes Mongolia, a sparsely populated country (1.5 persons/km^2) in Central Asia where livestock herding is the backbone of the county's economy and an important part of its cultural heritage (Mongolia, 2001c; OIE, 2002b). Major efforts have been undertaken around the world to control brucellosis due to the significant economic losses associated with the occurrence of the disease in domestic livestock and the often debilitating disease associated with human infection (Halling and Boyle, 2002; Ragan, 2002; Reddy, 2003). Health officials in Mongolia today have highlighted brucellosis, along with hepatitis (B & C) and tuberculosis, as one of their top three infectious disease public health priorities (Ebright et al., 2003; Mikolon, 2000).

Brucella infections in humans are almost exclusively of animal origin and human to human transmission of brucellosis is extremely rare (Nicoletti, 1989). Humans are infected either by direct or indirect contact with infected animals or animal products or by the ingestion of infected milk or dairy products (Slack, 2004b). In endemic areas of the world the majority of cases occur in people who have occupational contact with livestock such as livestock herders, dairy farmers, butchers, abattoire workers or veterinarians or in children if they live in close proximity with domestic animals (Slack, 2004b). The biovars of *Brucella melitensis* that are most commonly associated with brucellosis in humans in decreasing order of occurrence are *B. melitensis* (primary hosts sheep, goats, and camels), *B. abortus* (primary host cattle and camels), *B. suis* (primary host swine), and *B. canis* (primary host dogs) (Black, 2004b; Reddy, 2003; Slack, 2004b).

Brucellosis in humans is often associated with a number of nonspecific symptoms including fever ("undulent fever"), night sweats, anorexia, fatigue, headache, myalgia, malaise and depression (Benenson, 1995; Black, 2004b; Young, 1989). Brucellosis is a systemic infection and can involve any organ system and potentially cause osteoarticular, gastrointestinal, pulmonary, neurologic and cardiovascular complications (Black, 2004b). The incubation period is variable (weeks to months) and the onset of disease can be acute or insideous. Untreated brucellosis in humans can lead to chronic localized infections often manifesting as joint pain, joint effusion and debilitating arthritis (Benenson, 1995; Young, 1989).

Historically, Mongolia has had a high incidence of brucellosis in its human population (Otgon 1968; Jezek Rusinko et al. 1972; Jezek, Rusinko et al. 1974) as a consequence of the high prevalence of the disease in domestic livestock (Beulig et al., 1969; Jezek et al., 1972; Jezek et al., 1974; Otgon, 1968; Pinigin et al., 1968a; Pinigin et al., 1968b; Tserendash, 1980, 1984; Tserendash and Shumilov, 1970; Zherikova et al., 1972). Brucellosis prevention, through livestock vaccination, began in Mongolia in the 1960's (Corbel, 1989; Tserendash, 1980). A second prevention program, lead by the World Health Organization (WHO), began in the mid-1970's and focused on the vaccination of small ruminants (Corbel, 1989). The former Soviet Union continued to support a brucellosis livestock vaccination program in Mongolia (*Brucellosis abortus* strain-19 vaccine in cattle and *Br. melitensis* Rev-1 vaccine primarily in small ruminants) through the late 1980's.

Initially the brucellosis control programs were deemed a success by Mongolian scientists (Tserendash, 1980) and by WHO officials who reported that the annual incidence of the disease in humans dropped to less than 1 case/10,000 in the early 1980's (Mikolon, 2000; Roth et al., 2003). Despite evidence in the early 1980's of a significant reduction in the incidence of brucellosis in Mongolia, concerns have been raised recently about the re-emergence of the disease in the country (Ebright et al., 2003; Joshi, 1991; Roth et al., 2003; WHO, 2000b, 2001, 2003). Brucellosis surveillance in the human population of Mongolia since 1990, has consisted primarily of the unsystematic sampling and screening of high risk populations (herders and slaughterhouse workers) and reports on the number of hospitalized cases (Mikolon, 2000; Mongolia, 2001d; Roth et al., 2003). Both occupational hazards, through direct contact with infected livestock, (Roth et al., 2003) and the consumption of unpasturized milk and dairy products have been identified as modes of disease transmission from livestock to humans in Mongolia (Mikolon, 2000).

The persistence and possible re-emergence of brucellosis as a major public health problem in Mongolia has been blamed on the collapse of the veterinary service delivery system and public health infrastructure in Mongolia which began in the late 1980's (Ebright et al., 2003; Mikolon, 2000). The Mongolian veterinary system in the 1990's reflects the changes that were experienced by the country as a whole as Mongolia transitioned from a centrally planned economy and socialist system of government to a democratic system with a market economy (Ebright et al., 2003; Rossabi, 1999). The combination of the abrupt loss of economic aid from the former Soviet Union, the privatization of livestock in 1990, and the privatization of veterinary service delivery in 1999, meant the end of large scale, government subsidized, livestock health campaigns including comprehensive livestock vaccination programs and systematic surveillance for brucellosis (Mikolon, 2000).

Recently collected data on the prevalence, incidence and distribution of brucellosis in Mongolia's livestock and human population is largely unavailable and no published reports of the risk factors associated with disease exist. In this study data on the possible burden of brucellosis disease in livestock herders were assessed alongside a study of the prevalence of brucellosis in cattle in the Khangai mountain region of Bayankhongor Aimag (Province), Mongolia. By collecting information on the occurrence of clinical signs and symptoms suggestive of brucellosis disease in the human population, and information on the history of brucellosis diagnosis, attempts were made to identify risk factors associated with brucellosis-like disease in the livestock herders of the region.

4.2 HYPOTHESES

1) Clinical signs and symptoms suggestive of brucellosis disease in humans occur in livestock herders in the Khangai mountain region of Bayankhongor Aimag (Province), Mongolia, where livestock brucellosis is endemic.

2) Consumption of unpasturized dairy products and direct contact with livestock are associated with an increased risk of the occurrence of brucellosis-like clinical signs and symptoms in livestock herders.

4.3 MATERIALS AND METHODS

A. Study Design

A cross-sectional survey was used to determine the prevalence of clinical signs and symptoms suggestive of brucellosis, the prevalence of a history of brucellosis diagnosis, and the patterns of livestock product consumption and livestock contact, in a population of nomadic herders in the Khangai mountain region of Bayankhongor Aimag (Province), Mongolia. The unit of interest in this portion of the study was an individual herder in each herder household. One individual, identified as the head of the selected herder household, was interviewed to collect the information on their individual experience of symptoms suggestive of brucellosis and their practice of potential risk factors associated with the occurrence of brucellosis-like disease.

B. Herder Household Selection

A sample (n=104) of the 5,062 herder households located in the 5 contiguous soums (districts) of Gurvanbulag, Zag, Galuut, Jargalant, Erdenstogt, and the sub-district of Shargaljuut, in the Khangai mountain region of Bayankhongor Aimag (Province), Mongolia, were selected using a stratified random sampling technique. The sampling frame, sample size calculation and herder household identification methods were identical to those used for the selection of herder households for cattle/yak brucellosis testing. The methods were summarized previously in Section 2.3.

C. Data Collection Team and Herd Location

The same data collection team who conducted the livestock brucellosis testing and livestock management questionnaire was responsible for this portion of the study. Livestock herder households enrolled in this portion of the study were the same as those enrolled in the livestock brucellosis portion of the study. The same methods for locating herder households and obtaining and recording informed consent were employed. See Section 3.3.

D. Head of Herder Household Interviews: Health Status and Livestock Contact/Product Consumption Data

A 2-page questionnaire was conducted as an in-person interview with the male or female head of the selected herder households. The questionnaire (Appendix C) was adapted from a one used previously for a brucellosis survey in eastern Mongolia. The interview was conducted in Mongolian by a bilingual (Mongolian and English), native Mongolian speaker. The in-person interview was conducted during the herd visit as described in chapters 2 and 3, often with the same individual who participated in the livestock management survey.

Basic demographic information (age, sex, education level) was collected at the beginning of the interview and the questionnaire was coded to correspond with the livestock herd identification number. The first part of the questionnaire focused on recording the nature of herder contact with livestock through both specific occupational and routine activities in the herd. Patterns of livestock product consumption were also recorded, including practices related to the preparation and consumption of both meat and milk products (See Appendix C).

The second part of the questionnaire was designed to collect information on the occurrence of non-specific clinical signs and symptoms of disease in the herder population that could then be further categorized as typical of "acute" or "chronic" brucellosis. Data on the history of brucellosis diagnosis was also collected and herder knowledge of the routes of brucellosis transmission from livestock to humans and the means of brucellosis prevention were assessed. Interview responses were recorded in Mongolian and later translated into English.

E. Case Definitions

The responses recorded in the "non-specific clinical signs and symptoms" section of the questionnaire were used to identify herders who, in the previous 12 months, had experienced clinical signs and symptoms suggestive of brucellosis disease. A case definition of "acute" brucellosis was created based on the reporting of one or more of the signs usually experienced at the onset of brucellosis infection, including chills, headache, fever or night sweats (Benenson, 1995; Black, 2004b). A case definition of "chronic" brucellosis was created based on the reporting of one or more of the signs usually experienced with the osteoarticular complications associated with chronic localized brucellosis infections, including joint pain, stiff joints, swollen joints or lower back pain (Benenson, 1995; Black, 2004b). A third case definition of "general brucellosis" was also identified that included individuals who reported clinical signs and symptoms typical of both, or either, "acute" or "chronic" brucellosis.

Information on the history of brucellosis diagnosis in this population of herders was also collected and used as an alternative "case definition" in the analyses, described below (Section 4.4).

4.4 DATA ANALYSIS

A. Univariate Analysis of Risk Factors Associated with Clinical Signs Suggestive of Brucellosis and Reported Brucellosis Diagnosis in Livestock Herders

Data from the human health status/livestock contact questionnaire were entered into Microsoft Excel spreadsheets (Microsoft Office 2000). Data files containing information on the cattle herd brucellosis status, collected as described in chapter 2, were merged with the Excel file containing the human questionnaire data.[9] Imported Excel spreadsheet data were analyzed using the SAS software program (SAS version 8.2, Cary N.C.: SAS Institute, Inc. 1999-2001). Descriptive statistics were computed for the risk factors and dependent variables of interest. Datasets were created within SAS that corresponded to the disease classification and case definitions described in Section 4.3, E.

The t-test (PROC TTEST) was used to test for significant associations between the occurrence of brucellosis-like clinical signs and symptoms and the history of brucellosis diagnosis in humans and the continuous variables of age and years of education. Pearson's X^2 test (PROC FREQ; "TABLES/CHISQ") was used to test for significant associations between the occurrence of brucellosis-like clinical signs and symptoms, history of brucellosis diagnosis in humans and potential categorical risk factors for disease. An initial investigation of the presence of confounding by gender was investigated by performing a gender-stratified analysis of the categorical data and computing the Mantel-Haenszel X^2 test for stratified tables (PROC FREQ; "TABLES/CHISQ"; MH).

B. Multi-Variable Analysis of Risk Factors for the Occurrence of Clinical Signs Suggestive of Brucellosis in Livestock Herders

The complete human disease data set contained 76 variables. A subset of the original variables were considered for inclusion in the full logistic regression model. Variables excluded from the multi-variable logistic regression analysis included those variables with a large proportion of missing responses (>20% missing); redundant

[9]Serologic tests (BRT, CARD, and CFT) were used to test the milk and serum of cattle in the selected herds. Six classifications of a "positive" herd were created based on the positive results of 1 or a series of serologic tests. Generally the "BRT or CARD-Positive" classification was the most sensitive but least specific classification and the ">1 CARD-Positive" and "CFT-Confirmed CARD-Positive" the less sensitive but the most specific classification.

variables; and those deemed not to be biologically relevant to the occurrence of brucellosis.

Sixteen independent variables were included in the full logistic regression model. Soum (district) and gender were entered as class variables. Biologically relevant interaction variables were included in the full model. Backward elimination was used to model the relationship between potential risk factors and confounders (livestock product consumption practices, livestock handling practices, age, and gender) and the occurrence of brucellosis-like clinical signs and symptoms (general, acute, and chronic) in the human population. A significance level of 0.05 was used for removal of effects from the model. An assessment of confounding was preformed using non-statistical means. If the removal of a covariate resulted in more than a 10% change in the point estimate of the remaining covariates the removed covariate was added back to the model. Observations with missing values for the response or explanatory variables were removed during the modeling procedure.

4.5 RESULTS

A. Description of Sample Population

Participation and Demographics:

The human health and risk factor questionnaire was administered to an individual identified as the head of the herder household in 101, of the 104 herds (97%), targeted for inclusion in the study. The three herds for which we were not able to collect questionnaire information were not significantly different from other herds in the study. The reasons for our inability to collect questionnaire information from the three herds included one herder who refused to enroll the herd in any aspect of the study, one herder who refused to participate in the questionnaire due to lack of time, and a data recording error that prevented us from matching the data from the completed questionnaire to the appropriate cattle herd.

The mean age of the interviewed herders was 42, (median 41, range 17-81). The interviewed herders had received an average (mean) of 5.7 years of formal education (median 8, range 0 to 12). Just over half of the herders interviewed were female (52.75%).

Herder Occupation:

The majority (92%) of interviewees identified "livestock herding" as their primary occupation or "profession". Six percent of the individuals identified other occupations, including 3 veterinary technicians and a human health worker. None of the interviewed individuals were involved in commercial livestock slaughter or trade.

Livestock Contact:

Traditionally all members of the herder household participate in livestock rearing. As detailed in chapter 2, 75% of these herds were composed of multiple species of livestock. The presence of cattle was recorded in the largest number of herds (100%) followed by sheep, goats and camels. Home slaughter of livestock for personal consumption and hand milking of individual animals is common practice among Mongolian herders. Of the 100 individuals interviewed, 84% reported participation in cattle slaughter, 87% in sheep, 80% in goat and 3% in camel slaughter. Ninety-six percent of the individuals interviewed reported hand milking cattle, 24% reported hand-milking goats and 21% reported hand-milking sheep. Specific questions addressing herder assistance of livestock during parturition or dystocia were not asked, however, 30% of herders reported that weak neonates (primarily lambs and kids) were brought into the family's living quarters for observation and extra care.

Meat/Milk Consumption in Herder Households:

Mongolian livestock herders generally consume the meat and milk products of the livestock they raise. One hundred percent of the heads of herder households interviewed reported consuming beef, 93% reported consuming mutton, 75% reported consuming goat meat and 3% reported consuming camel meat. Traditionally meat is thoroughly cooked, either by boiling or roasting. One respondent reported consumption of raw meat of camel, cattle, sheep and goat origin. The consumption of raw milk was reported by 15% of respondents. Twelve percent reported consuming raw cow or yak milk, 3% raw goat milk and 3% raw sheep milk. Thirty-three percent of respondents reported preparing dairy products with raw milk.

Knowledge of Brucellosis Prevention and Transmission:

Twenty-five percent of the heads of herder households interviewed understood that brucellosis was a zoonotic disease and that infected livestock were a risk to human health. Seven percent of the herders interviewed identified both livestock product consumption and contact with aborted material as a means of brucellosis transmission to humans. An additional 18% identified either consumption of contaminated livestock products (meat and milk) or direct contact with aborted material as the means of transmission of disease from livestock to humans.

Eighteen percent of the heads of herder households correctly identified ways to prevent brucellosis occurrence in humans. Overall 12% identified either livestock vaccination/prevention or sanitary measures (cooking/boiling meat and milk and use of protective clothing). Six percent of interviewees identified both livestock brucellosis prevention and the institution of sanitary measures.

Prevalence of Brucellosis Diagnosis, Testing and Treatment History:

Twenty-seven percent (26/97) of interviewed heads of herder households reported a history of brucellosis diagnosis. Twelve percent of the interviewed herders (approximately ½ with a history of brucellosis) had been diagnosed within the previous 12 months. Eight percent of all of the individuals survey reported that they had ever been tested for brucellosis with a blood test and 5% reported skin testing. None of the interviewed individuals, including those with a history of brucellosis diagnosis, reported having been treated for brucellosis either as outpatients or in hospitals or clinics. None of the individuals reported that they had been rechecked for brucellosis after initial diagnosis and 1 person reported experiencing a relapse of disease.

Prevalence of Reported Non-Specific Clinical Signs and Symptoms:

The surveyed individuals indicated which of a list of non-specific clinical signs and symptoms they had experienced within the previous 12 months. (See questionnaire in appendix C). The most common symptom experienced was joint pain at 18%. The other symptoms experienced were headache (16%), lower back pain (12%), stiff joints (11%), swollen joints (10%), muscle pain (9%), extreme weakness (9%), dizziness (9%), bone pain (9%), abdominal distension (6%), difficulty sleeping (4%), night sweats (2%), fever

(2%), enlarged lymph nodes (2%), abdominal pain (1%) and 0% experiencing chills and depression (See Table 4.1).

The prevalence of individuals experiencing one or more symptoms typical of brucellosis (chills, fever, headache, night sweats, joint pain, stiff joints, swollen joints or lower back pain) was 24% (24/100). When the list of typical brucellosis-like symptoms was further divided into those more characteristic of acute disease brucellosis (chills, fever, headache, or night sweats) and those more typical of chronic infection (joint pain, stiff joints, swollen joints or lower back pain) the prevalence of acute brucellosis-like symptoms was 16% and the prevalence of chronic brucellosis-like symptoms was 20%.

B. Univariate Analyses: History of Brucellosis Diagnosis

Association with Brucellosis-Like Symptoms:

No significant associations were found between the history ("recent" or "ever") of brucellosis diagnosis and the reporting of clinical signs and symptoms typical of either acute or chronic brucellosis (See Table 4.2). Additionally no significant associations were found between the history of brucellosis diagnosis and any of the individual clinical signs and symptoms reported by the surveyed individuals.

Association with Brucellosis Status of Cattle Herd:

No significant associations were found between the reported diagnosis of brucellosis in livestock herders and the brucellosis status of their cattle herd by any of the testing methods employed (See Table 4.3).

Association with Livestock Product Consumption and Herding Practices:

No significant associations were found between the history of brucellosis diagnosis and any livestock product consumption or herding practices with the exception of "bringing neonates into living quarters" ($X^2 = 6.71$; Fisher's p-value = 0.0108).

Age and Gender:

There were no associations between either age or gender and the reported diagnosis of brucellosis ("ever" or "recent") in the herder population.

Association with Knowledge of Brucellosis Prevention and Transmission:

There was no association found between knowledge of brucellosis prevention or routes of transmission and the reported history of brucellosis diagnosis ("ever" or "recent") in the herder population.

C. Univariate Analysis: Reporting of Clinical Signs and Symptoms Suggestive of Acute and Chronic Brucellosis

Association with Brucellosis Status of Cattle Herd:

Significant associations were found between the reporting of typical signs of "chronic" brucellosis and the "BRT and CARD-Positive" classification of cattle ($X^2 = 4.91$; p-value = 0.0267) and the "CARD-Positive" classification of cattle in the same herd ($X^2 = 4.77$; p-value = 0.0290). Though not significant at the .05 level, a strong association was also found between the reporting of typical signs of chronic brucellosis and the ">1 CARD-Positive" classification of cattle in the same herd.

No significant associations were found at the 0.05 level between the reporting of typical signs of "acute" brucellosis and the brucellosis positive status of cattle in the same herd, however, associations nearing significance were found between typical signs of "acute" brucellosis in humans and the "BRT and CARD-Positive" ($X^2 = 3.27$; p-value =

0.0707) and the ">1 CARD-Positive" ($X^2 = 3.12$; p-value = 0.0775) classification of their cattle herds (See Table 4.4).

Association with Livestock Product Consumption and Herding Practices:

The practice of drinking raw milk and producing dairy products from raw milk were highly associated ($X^2 = 7.56$-20.06; p-values = 0.0060 - <0.0001) with the occurrence of both "acute" and "chronic" brucellosis-like symptoms in the herder population (See Table 4.5). The practice of milking, slaughtering or eating meat from camels, cattle, sheep or goats was not associated with either acute or chronic brucellosis-like symptoms in the herder population (See Table 4.5). Keeping neonates (primarily kids and lambs) in the family's living quarters was associated with the reported occurrence of both "acute" brucellosis-like symptoms ($X^2 = 4.62$; p-value = 0.0317) and "chronic" brucellosis-like symptoms ($X^2 = 11.03$; p-value = 0.0014) in the herder population. All of the herders in this population but 1 reported that they herded yaks (*Bos grunniens*), cattle (*Bos taurus*) or hybrid crosses of the two species. Herders' reports of both "acute" and "chronic" brucellosis-like symptoms were positively associated with the practice of herding strictly *Bos taurus* cattle whereas the practice of herding strictly *Bos grunniens* cattle was positively associated with the absence of brucellosis-like symptoms in the interviewed herders (See Table 4.5).

Age and Gender:

The mean age of herders reporting the occurrence of signs and symptoms suggestive of "acute" and/or "chronic" brucellosis was slightly older but not significantly different from the mean age of those who experienced no clinical signs and symptoms (See Table 4.6). Being female was associated with the reporting of the occurrence of "acute" brucellosis-like symptoms ($X^2 = 3.89$; p-value = 0.0495). There was no difference in the odds of men and women having a history of brucellosis diagnosis, or experiencing chronic or general brucellosis-like symptoms (See Table 4.7). Males were more likely to report their participation in cattle slaughter ($X^2 = 6.33$; p-value = 0.0119).

Occupation:

Individuals who identified themselves as animal health workers were more likely to report the occurrence of clinical signs and symptoms of "acute" and "chronic" brucellosis, though the association did not reach statistical significance; "acute" brucellosis ($X^2 = 5.91$; p-value = 0.0658) and "chronic" brucellosis ($X^2 = 4.21$; p-value = 0.1011), (See Table 4.5).

Knowledge of Brucellosis Prevention and Transmission:

No associations were found between herders' knowledge of brucellosis transmission (either or both direct and indirect routes) and their reporting of "acute" or "chronic" brucellosis-like clinical signs and symptoms. Similarly, no associations were found between herders' knowledge of brucellosis prevention (either or both livestock disease control and persona/food safety) and their reporting of acute or chronic brucellosis-like clinical signs and symptoms (See Table 4.8).

An additional analysis was performed to determine whether or not there was an association with herder understanding of brucellosis transmission and prevention and the livestock product consumption and herding practices found to be associated with the occurrence of brucellosis-like symptoms in this population. The consumption of raw milk (all species), the consumption of raw cow's milk, preparing dairy products with raw milk, bringing neonates into the living quarters and gender (being female) were all examined. No significant associations were found (See Table 4.9).

B. Multi-Variable Analysis of Livestock Product Consumption and Livestock Herding Practices and the Occurrence of Brucellosis-Like Symptoms in the Human Herder Population

General Brucellosis-Like Symptoms:

The full multi-variable logistic regression model of the occurrence of brucellosis-like symptoms, the occurrence of "acute" brucellosis-like symptoms and the occurrence of "chronic" brucellosis-like symptoms in the human population was based on 60 observations (61% of interviewed heads of herder households, and 59% of all herds) and included 16 variables. A relatively high prevalence of missing data on age (24%) led to observations being removed from the model. There were 20/60 (33%) individuals reporting "general" brucellosis-like symptoms, 14/60 (23%) reporting "acute" brucellosis-like symptoms, and 16/60 (27%) reporting "chronic" brucellosis-like symptoms in this sample.

The final reduced model for "general" brucellosis-like symptoms included 2 risk factors; "preparation of dairy products from raw milk" (OR=16.25; 95% C.I.=2.83-93.38) and "neonates in living quarters" (OR=10.60; 95% C.I.=1.92-58.40). Age was a significant variable but not a significant confounder. A 1-year increase in age represented an 8.8% increase in risk of the occurrence of "general" brucellosis-like symptoms (See Table 4.10).

The final reduced model for "acute" brucellosis-like symptoms (Table 4.11) included 2 risk factors; "preparation of dairy products from raw milk" (OR=9.33; 95% C.I.=2.22-39.31) and "drinking raw cow's milk" (OR=6.52; 95% C.I.=1.24-34.40).

Gender was a significant confounder. Being female was associated with a 5 fold increase in the odds of the occurrence of clinical signs suggestive of "acute" brucellosis (OR=5.3; 95% C.I.=1.03-27.32), (See Table 4.11).

The final reduced model for "chronic" brucellosis-like symptoms included 3 risk factors; "drinking raw cow's milk" (OR=6.52; 95% C.I.=1.24-34.40), "neonates in living quarters" (OR=10.60; 95% C.I.=1.92-58.40), and "herding strictly *Bos taurus* cattle" (OR=5.64; 95% C.I.=1.00-31.80). Age was a significant variable but not a confounder. A 1-year increase in age represented a 5.4% increase in risk of the occurrence of "chronic" brucellosis-like symptoms (See Table 4.12).

4.5 DISCUSSION

A. Hypotheses

Hypothesis 1, that clinical signs and symptoms suggestive of brucellosis occur in the population of livestock herders in the Khangai mountain region of Bayankhongor Aimag (Province), Mongolia, where livestock brucellosis is endemic; and **hypothesis 2,** that human behaviors related to the consumption of unpasturized dairy products, and direct contact with livestock are associated with an increased risk of the occurrence of brucellosis-like clinical signs and symptoms in livestock herders in the Khangai region of Bayankhongor Aimag (Province), Mongolia; were both found to be true and confirmed in this cross-sectional study.

B. Prevalence of Reported Symptoms Suggestive of Brucellosis and History of Brucellosis Diagnosis in Herder Population

The prevalence of brucellosis-like symptoms (24/100; 24%) and the reported history of brucellosis diagnosis (26/100; 26%) in this population of livestock herders from the Khangai mountain region of Bayankhongor Aimag (Province), Mongolia, is high. Studies of brucellosis sero-prevalence among livestock herders in other regions of the world with endemic livestock brucellosis report sero-prevalence of 3.8% (Schelling et al., 2003). Although the brucellosis disease prevalence data collected in this study are not laboratory-confirmed, the prevalence recorded in this Bayankhongor Aimag (Province) herder population is similar to the figure of 25% brucellosis sero-prevalence in Mongolia's herder population reported by A. Mikolon (Mikolon, 2000) and in the *Mongolian Health Sector Review* cited by Roth, et al, in which 16% of herders and livestock workers were classified as infected with brucellosis (Roth et al., 2003).

Although the figure of brucellosis prevalence in this population of herders that is based on reported diagnosis and the reported occurrence of brucellosis-like clinical signs and symptoms is similar, the correlation one would expect between these two means of classification does not exist. There is no association between individuals who reported that they had a history of either "ever" or "recent" brucellosis diagnosis, and their report of the occurrence of clinical signs and symptoms associated with human brucellosis disease. Further investigation revealed that none of the individuals who reported a history of brucellosis diagnosis had ever been treated for the disease. The individuals were questioned about this in more than one way as they were asked to identify what they were treated with, whether or not they had been hospitalized, treated as an outpatient or in a community clinic. The medical background of the individual making the diagnosis of brucellosis in this instance is unknown. Additionally only a fraction of the herders surveyed reported that they had been tested with a blood or skin test for brucellosis. The uncertainty of the source of brucellosis diagnosis in these individuals and the lack of correlation with a history of clinical signs of disease, led us to suspect that this case definition (self-reported "ever diagnosed" with brucellosis) was prone to serious misclassification error.

Interestingly, when the "history of brucellosis" disease classification was used to test for associations (univariate and multi-variable) with potential risk factors for disease (livestock product consumption, livestock herding practices, gender and age) no significant associations were found with the exception of the practice of bringing weak neonates (primarily lambs and kids) into the living quarters for extra care and attention. This risk factor also proved to be significant when alternative case definitions for brucellosis were used, and will be discussed below in terms of its potential role as a significant "high-risk" human behavior for the acquisition of brucellosis.

C. Cattle Herd Brucellosis Status and Human Brucellosis-Like Disease

Although the presence of brucellosis in livestock in itself does not guarantee the transmission of disease to the humans who tend the livestock and consume its products, one would suspect that the chance of zoonotic transmission would be higher in those herder households in which there was evidence of the presence of brucellosis in livestock, and especially those herder households in which there was evidence of more than a single case of livestock brucellosis. This was found to be true in this population of herders.

Cattle herds meeting the more stringent definition of a brucellosis seropositive herd (more than 1 positive test, or more than 1 individual with a positive brucellosis test) were found to be significantly associated with the reporting of the occurrence of "chronic" brucellosis-like symptoms in the interviewed head of herder household. Relationships nearing significance were found between brucellosis-positive classified cattle herds and the report of "acute" brucellosis-like clinical signs in the head of the herder households.

No significant associations were found between cattle herd brucellosis status and the reported history of brucellosis diagnosis in the head of the herder household.

Livestock Herding Practices as Risk Factors for the Occurrence of Brucellosis-Like Symptoms in Herders:

The livestock herding practice most consistently associated with the reported occurrence of brucellosis-like symptoms in interviewed herders was the practice of bringing weak neonates (primarily lambs and kids) into the family's living quarters for extra care and attention (O.R. of 15-20). Interviewed herders were not asked questions about their contact with aborted livestock fetuses and placentas, or practices associated with assisting livestock species during the birthing process. Direct human contact (through breaks in the skin or conjunctiva) with uterine fluid, aborted fetuses and especially placentas of brucellosis infected livestock, have been identified as significant modes of zoonotic transmission of brucellosis (Benenson, 1995; Reddy, 2003). The practice of brining weak neonates into the living quarters characterizes the close involvement of the livestock herders in the birthing process and the close contact they and their family have with neonates potentially coated with brucellosis containing uterine and amniotic fluid. The subset of herder households that follow this practice are therefore at increased risk of contracting brucellosis through direct contact with their infected livestock.

The practice of hand-milking livestock or involvement in the slaughter and butchering process does not appear to be associated with an increased risk of experiencing clinical signs and symptoms characteristic of acute or chronic brucellosis in this population. The practice of herding strictly yaks (*Bos grunniens*) appears to be somewhat protective while herders who reported a strictly *Bos taurus* composition of their cattle herd were more likely to experience brucellosis-like symptoms. This relationship held true especially in the multivariable model of chronic brucellosis-like symptoms. The history of cattle production in Mongolia may explain this pattern. As previous authors have mentioned, the regional spread of brucellosis in Mongolia in the 1950's – 1970's was linked to the establishment of intensive state owned dairy cooperatives, often with imported cattle (Denes, 1997; Otgon, 1968). When livestock were privatized in Mongolia in 1990, the state farms were dismantled and the cattle distributed to Mongolian herders. Herders who received and maintained *Bos taurus* cattle from the state farms may have a more persistent problem with brucellosis in their herds today than do herders whose herds are composed of indigenous cattle and yaks that have been raised extensively and at lower densities for decades.

<u>Livestock Product Consumption Practices as Risk Factors for the Occurrence of Brucellosis-Like Symptoms in Herders:</u>

The consumption of raw cow's milk and the use of raw milk in the preparation of dairy products were both identified as significant risk factors for the occurrence of brucellosis-like symptoms in this populations of livestock herders (O.R. range = 6-30). Slight variations in the estimation of the odds ratios were found in the multi-variable models when the definition of disease was changed from "general", to "acute", to "chronic" brucellosis-like symptoms. The strongest association with brucellosis-like symptoms was seen with the practice of consuming of raw cow's milk followed by the practice of preparing dairy products from raw milk. The identification of these two livestock product consumption risk factors is consistent with the knowledge that unpasturized milk and diary products are potential sources of infection for humans (Benenson, 1995; Reddy, 2003).

Occupation:

Livestock herders in general represent a high-risk population for brucellosis infection in countries where the disease is endemic in livestock (McDermott and Arimi, 2002). The majority of individuals interviewed in this study identified livestock herding as their primary occupation. Within this population of herders there were no individuals who identified an alternative occupation considered a hazard for brucellosis infection with the exception of the three individuals who identified themselves as livestock health workers. Two of these three individuals reported the occurrence of clinical signs and symptoms consistent with both "acute" and "chronic" brucellosis infection. Although the numbers are small, we can speculate that livestock health workers are subjected to an increased risk of brucellosis infection within this herder population due to their increased opportunities for contact with infected livestock.

Gender and Age:

Age is a significant variable but it does not confound the relationship between the identified behavioral risk factors for brucellosis (drinking raw milk, preparing dairy products from raw milk and bringing neonates into the living quarters) and the occurrence of "general" and "chronic" brucellosis-like symptoms in this population of livestock herders. Brucellosis is an infectious disease with chronic sequelae so the slight increase in the odds of symptom occurrence with age is expected. Older individuals have had a longer period of time in which to become exposed to the infectious agent. It is also likely that there is a general increase in the prevalence of many of the non-specific symptoms that are consistent with both brucellosis and many other conditions in older individuals.

Being female was associated with a significantly higher odds (OR = 5.31; 95% C.I. = 1.03-27.32) of reporting clinical signs as symptoms consistent with "acute" brucellosis infection. One would not expect to see any association between gender and brucellosis infection since males and females are equally susceptible to the disease. However, in some cases there is a division of labor that falls along gender lines in livestock herding practices that may lead to differences in exposure to infection. In this population of herders the only identified herding or livestock consumption product consumption practice that was associated with gender was cattle slaughter. Significantly more men (X2 = 6.33; p-value = 0.0119) reported that they slaughtered cattle, though this did not prove to be a significant risk factor for disease in the reduced logistic regression model.

The higher probability of reporting signs typical of "acute" brucellosis among women in this herder population may be due to a gender specific role in an aspect of livestock herding not captured in the study survey. As mentioned previously, specific questions surrounding the herders' involvement in obstetric procedures and the handling of aborted material and livestock placentas were not asked. Personal observations indicate that women and girls generally take care of female stock nearing parturition and newborn stock. Men and boys spend time grazing adult livestock in the surrounding pastures. This study was performed in July and August, approximately 1-2 months after peak calving, lambing and kidding season. The higher prevalence of women reporting clinical signs associated with "acute" brucellosis may be related to their more frequent exposure to infected (aborting) livestock.

D. Limitations

A significant amount of data in this portion of this study was collected using a questionnaire that was conducted as an in-person interview. Individuals were asked to recall the occurrence of a list of clinical signs and symptoms within the past 12 months and also asked to report on their typical practices and habits surrounding livestock herding and livestock product consumption. Although we can expect that individuals will remember the occurrence of signs of illness over a 12-month period, it is still a significant time and the data may be susceptible to recall bias. We have no reason to believe that the questions concerning livestock herding practices or livestock product consumption would have led individuals to hesitate to give us accurate answers but the normal variation in practice from day to day and season to season may have led to some misclassification bias with regards to those who did and did not participate in the potentially risky practices. These points represent the limitations of a cross-sectional study using self-reported data. Since the data is collected at one point in time, making seasonal trends or variation over time hard to capture.

One of the major limitations of this study was that confirmatory laboratory testing of individuals for brucellosis infection was not available. The information collected on the occurrence of clinical signs and symptoms was subject to misclassification bias due to the non-specific nature of many of the clinical signs associated with both "acute" and "chronic" brucellosis. Despite this drawback to our method, the case definitions based on clinical signs and symptoms seemed much more consistent with disease than did the case definition based on the report of a previous or recent diagnosis of brucellosis.

Only the heads of households were interviewed and asked to report the occurrence of their clinical signs and symptoms over the 12-month period. We were able to interview roughly equal numbers of men and women but the majority of the individuals were middle-aged adults and no data on the occurrence of brucellosis-like symptoms in infants, children or very young adults were collected. The burden of disease in children, found to be significant in previous studies (Jezek et al., 1972; Jezek et al., 1974), was not assessed.

4.6 CONCLUSION

Bovine brucellosis is an important livestock disease in the Khangai mountain region of Bayankhongor aimag, Mongolia, and the data on the occurrence of brucellosis-like symptoms in the herder population indicates that the disease also has a significant impact on the health and well being of the human herder population. The practices identified as risk factors for disease in this region of the world are not different from those that have been identified in other areas where livestock brucellosis is endemic. Raw milk, consumed directly or used in the preparation of dairy products, will continue to be a source of infection for humans as long as brucellosis is circulating in the livestock population. Educational campaigns will have some effect on herder understanding of the risks associated with their livestock production consumption habits but, as was seen when examining these data, knowledge of brucellosis transmission routes has little effect on the practices that enhance its transmission.

The second major risk factor for human disease identified in this study was a practice closely associated with the routine care of newborn livestock. In a population where livestock herding represents both cultural and economic survival, individuals will be resistant to giving up a practice, like bringing weak livestock into warm and sheltered human living quarters, which are perceived to increase the productivity of their flocks. The only way to reduce the burden of human disease associated with brucellosis infection is to reduce, and eventually eliminate, the disease in livestock.

Influenced by both national and international concerns surrounding the re-emergence of brucellosis in Mongolia (Ebright et al., 2003; Roth et al., 2003; WHO, 2001; Zinsstag et al., submitted 2004), the Mongolian government has identified brucellosis as a priority economic and public health concern. A national brucellosis control program is currently underway in Mongolia. Data from this study contributes to the evidence that brucellosis control in livestock is an important step in not only improving the productivity of the livestock sector, but also in enhancing the health and well being of all Mongolians.

Table 4.1. Prevalence of Clinical Signs and Symptoms of General Illness Experienced Within the Previous 12 Months and Reported by the 100 Individuals Surveyed in the Khangai Mountain Region of Bayankhongor Aimag (Province), Mongolia, in August 2000.

Symptom	Yes	No	Period (12 months) Prevalence
Joint Pain	18	82	18%
Headache	16	84	16%
Lower Back Pain	12	88	12%
Stiff Joint(s)	11	89	11%
Swollen Joint(s)	10	90	10%
Extreme Weakness	9	91	9%
Muscle Pain	9	91	9%
Bone Pain	9	91	9%
Dizziness	7	93	7%
Abdominal Distention	6	94	6%
Difficulty Sleeping	4	96	4%
Enlarged Lymph Nodes	2	98	2%
Fever	2	98	2%
Night Sweats	2	98	2%
Abdominal Pain	1	99	1%
Chills	0	100	0%
Depression	0	100	0%

Table 4.2. Univariate Analysis of Reported Brucellosis Diagnosis in Humans and the Reported Occurrence of Clinical Signs and Symptoms Typical of Acute and Chronic Brucellosis in the 100 Individuals Surveyed in the Khangai Mountain Region of Bayankhongor Aimag (Province), Mongolia, in August, 2000. N=100; 16% "Yes" for Typical Acute Brucellosis; 20% "Yes" for Typical Chronic Brucellosis.

Brucellosis Diagnosis	**Symptoms Typical of Acute Brucellosis**		$\mathbf{X^2}$	**p-value**	**Symptoms Typical of Chronic Brucellosis**		$\mathbf{X^2}$	**p-value**
	Yes	**No**			**Yes**	**No**		
Brucellosis Diagnosis (Ever Diagnosed)	2	24	1.64	0.3412*	4	22	0.40	0.7732*
Brucellosis Diagnosis (Within 12 months)	0	14	2.99	0.1174*	2	12	0.29	0.7305*

* Indicates the use of the 2-sided probability from the Fisher's exact test when 25% of the cells have counts less than 5.

Table 4.3. Univariate Analysis of Cattle Herd Brucellosis Status and Reported Brucellosis Diagnosis in the 100 Individuals Surveyed in the Khangai Mountain Region of Bayankhongor Aimag (Province), Mongolia, in August, 2000. N=100; 26.80 "Yes" for "Ever" Diagnosed with Brucellosis; 14.43% "Yes" for "Recent-w/in 12 months" Diagnosis with Brucellosis.

Cattle Herd Brucellosis Status	"Ever" Diagnosed with Brucellosis		X^2	p-value	"Recent" (w/in 12 mo.) Brucellosis Diagnosis		X^2	p-value
	Yes	No			Yes	No		
BRT and CARD Positive	3	18	2.15	0.1700*	2	19	0.54	0.7248*
BRT or CARD Positive	13	36	0.00	0.9510	8	41	0.29	0.5918
CARD Positive	9	26	0.01	0.9190	4	31	0.24	0.7620*
BRT Positive	7	28	1.47	0.2255	6	29	0.16	0.6870
>1 CARD Positive	2	13	1.55	0.3394*	2	13	0.00	0.9656*
CFT-Confirmed CARD Positive	6	14	0.09	0.7664	4	16	0.56	0.4810*

* Indicates the use of the 2-sided probability from the Fisher's exact test when 25% of the cells have counts less than 5.

Table 4.4. Univariate Analysis of Cattle Herd Brucellosis Status and the Occurrence of Clinical Signs and Symptoms Typical of Acute and Chronic Brucellosis in the 100 Herders Surveyed in the Khangai Mountain Region of Bayankhongor Aimag (Province), Mongolia, in August, 2000. N=100; 16% "Yes" for Typical Acute Brucellosis; 20% "Yes" for Typical Chronic Brucellosis.

Cattle Herd Brucellosis Status	Symptoms Typical of Acute Brucellosis		X^2	p-value	Symptoms Typical of Chronic Brucellosis		X^2	p-value
	Yes	No			Yes	No		
BRT and CARD Positive	6	18	3.27	0.0707	8	16	4.91	0.0267
BRT or CARD Positive	9	43	0.14	0.7104	13	39	1.69	0.1932
CARD Positive	9	29	2.46	0.1168	12	26	4.77	0.0290
BRT Positive	6	32	0.24	0.6259	9	29	1.44	0.2294
>1 CARD Positive	5	11	3.12	0.0775	6	10	3.44	0.0637
CFT-Confirmed CARD Positive	2	20	1.09	0.5124*	5	17	0.10	0.7592

* Indicates the use of the 2-sided probability from the Fisher's exact test when 25% of the cells have counts less than 5.

Table 4.5. Univariate Analysis of Potential Human Behavior and Occupation-Related Risk Factors and the Occurrence of Clinical Signs and Symptoms Typical of Acute and Chronic Brucellosis in the 100 Individuals Surveyed in the Khangai Mountain Region of Bayankhongor Aimag (Province), Mongolia, in August, 2000. N=100; 16% "Yes" for Typical Acute Brucellosis; 20% "Yes" for Typical Chronic Brucellosis.

Risk Factor	Symptoms Typical of Acute Brucellosis		X^2	p-value	Symptoms Typical of Chronic Brucellosis		X^2	p-value
	Yes	No			Yes	No		
Neonates in Ger	9	21	4.62	0.0317	12	18	11.03	0.0014*
Slaughter Camels	0	3	0.59	1.0000*	0	3	0.77	1.0000*
Slaughter Cattle	12	72	1.15	0.2806*	18	66	0.67	0.5162*
Slaughter Goats	11	69	1.51	0.2196	15	65	0.39	0.5320
Slaughter Sheep	12	74	2.36	0.2162	17	69	0.08	0.7224*
Slaughter Livestock (Not Species Specific)	13	76	0.43	0.4528*	18	71	0.03	1.0000*
Eat Camel Meat	2	1	5.91	0.0658*	1	2	0.34	0.4919*
Eat Cattle Meat	16	84	----	----	20	80	----	----
Eat Goat Meat	12	63	0.00	1.0000	14	61	0.33	0.5637

Table 4.5. Continued

Eat Sheep Meat	15	78	0.02	1.0000*	18	75	0.35	0.6246*
Milk Camels	0	0	----	----	0	0	----	----
Milk Cattle (*Bos taurus*)	4	12	1.15	0.2806*	5	11	1.51	0.2196
Milk Goats	5	19	0.55	0.4588	3	21	1.11	0.3874*
Milk Sheep	5	16	1.21	0.2721	3	18	0.54	0.5545*
Milk Yaks (*Bos grunniens*)	14	75	0.04	0.8343	18	71	0.03	1.000*
Drink Raw Camel's Milk	0	0	-----	------	0	0	----	----
Drink Raw Cow's Milk (*Bos taurus* and *Bos grunniens*)	6	6	11.73	0.0006	7	5	12.52	0.0004
Drink Raw Goat's Milk	3	0	16.24	0.0035*	3	0	12.37	0.0071*
Drink Raw Sheep's Milk	3	0	16.24	0.0035*	3	0	12.37	0.0071*
Drink Raw Milk (Not Species Specific)	6	9	7.56	0.0060	8	7	12.25	0.0005
Prepare Dairy Products From Raw Milk	13	20	20.06	<.0001	15	18	19.95	<.0001

Table 4.5. Continued

Herd Camels	2	3	2.26	0.1799*	2	3	1.32	0.2605*
Herd Cattle (*Bos taurus*)	13	32	10.11	0.0015	13	32	4.04	0.0444
Herd Goats	9	51	0.11	0.7383	13	47	0.26	0.6098
Herd Sheep	13	59	0.81	0.3686	16	56	0.79	0.3754
Herd Yaks (*Bos grunniens*)	7	64	6.87	0.0088	10	61	5.35	0.0213
Herd Livestock (Not Species Specific)	15	77	0.08	1:0000*	18	74	0.14	0.6586*
Commercial Livestock Slaughter	0	0	----	----	0	0	----	----
Livestock Product Trade	0	0	----	----	0	0	----	----
Veterinarian (Animal Health Assistant)	2	1	5.91	0.0658*	2	1	4.21	0.1011*
"Other" Occupation	1	5	0.00	1.0000*	1	5	0.04	0.8332

* Indicates the use of the 2-sided probability from the Fisher's exact test when 25% of the cells have counts less than 5.

Table 4.6. Univariate Analysis (T-Test) of the Association Between Herder Age and the Occurrence of Clinical Signs and Symptoms Typical of Acute and Chronic Brucellosis in the 100 Individuals Surveyed in the Khangai Mountain Region of Bayankhongor Aimag (Province), Mongolia, in August, 2000.

		n	**Mean Age**	**S.D.**	**[t]**	**p-value**
Acute	Yes:	16	46.88	15.38		
	No:	62	40.95	14.35	1.45	0.1508
Chronic	Yes:	11	46.27	15.49		
	No:	67	41.49	14.66	1.00	0.3193

Table 4.7. Univariate Analysis of the Association Between Gender and the Occurrence of Clinical Signs and Symptoms Typical of Acute and Chronic Brucellosis in the 100 Individuals Surveyed in the Khangai Mountain Region of Bayankhongor Aimag (Province), Mongolia, in August, 2000.

Risk Factor	**Symptoms Typical of Acute Brucellosis**		X^2	**p-value**	**Symptoms Typical of Chronic Brucellosis**		X^2	**p-value**
	Yes	**No**			**Yes**	**No**		
Gender:								
(Female)	12	36			11	37		
(Male)	4	39	3.86	0.0495	9	34	0.05	0.8193

Table 4.8. Univariate Analysis of Interviewed Individuals' Understanding of the Routes of Transmission and Ways to Prevent Brucellosis and the Occurrence of Clinical Signs and Symptoms Typical of Acute and Chronic Brucellosis in the 100 Individuals Surveyed in the Khangai Mountain Region of Bayankhongor Aimag (Province), Mongolia, in August, 2000. N=100; 16% "Yes" for Typical Acute Brucellosis; 20% "Yes" for Typical Chronic Brucellosis.

Brucellosis Understanding	**Symptoms Typical of Acute Brucellosis**		X^2	**p-value**	**Symptoms Typical of Chronic Brucellosis**		X^2	**p-value**
	Yes	**No**			**Yes**	**No**		
Transmission 1 (Identified Direct and Indirect Routes)	2	5	0.89	0.3108*	3	4	2.46	0.1408*
Transmission 2 (Identified Direct or Indirect Routes)	6	19	1.59	0.2077	7	18	1.33	0.2482*
Prevention 1 (Identified Livestock Disease Control and Personal/Food Safety)	2	4	1.43	0.2446*	2	4	0.71	0.5967*
Prevention 2 (Identified Livestock Disease Control or Personal/Food Safety)	4	14	0.63	0.4796*	3	15	0.15	1.0000*

* Indicates the use of the 2-sided probability from the Fisher's exact test when 25% of the cells have counts less than 5.

Table 4.9. Association of Herder Knowledge of Routes of Brucellosis Transmission and Brucellosis Prevention and the Practice of Behaviors or Risk Factors Associated with Clinical Signs of Brucellosis Infection in Surveyed Individuals in the Khangai Mountain Region of Bayankhongor, Mongolia, Interviewed in August of 2000.

Risk Factor Practiced	**Transmission Route Identified**		**X^2**	**p-value**	**Prevention Method Identified**		**X^2**	**p-value**
	Yes	**No**			**Yes**	**No**		
Neonates in Ger	12	18	2.41	0.1207	7	23	0.05	0.8320
Drink Raw Cow's Milk (*Bos taurus* and *Bos grunniens*)	2	10	0.51	0.7250*	1	11	0.86	0.6884*
Drink Raw Milk (Not Species Specific)	4	11	0.03	1.0000*	2	13	0.26	1.0000*
Prepare Dairy Products From Raw Milk	11	22	1.82	0.1768	5	28	0.27	0.6028
Sex (Female=Yes)	11	37	1.06	0.3036	9	39	0.07	0.7944

* Indicates the use of the 2-sided probability from the Fisher's exact test when 25% of the cells have counts less than 5.

Table 4.10. Final Reduced Logistic Regression Model of Risk Factors for Typical Signs of General (Acute and/or Chronic) Brucellosis Reported in Livestock Herders Interviewed in the Khangai Mountain Region of Bayankhongor Aimag (Province), Mongolia, in 2000.

Risk Factor	**Wald X^2 p-value**	**Odds Ratio**	**95% Confidence Interval (Wald)**
Age (Unit=1 year)	0.0114	1.09	1.20 – 1.16
Age (Unit=5 years)	0.0114	1.52	1.10 – 2.11
Neonates Housed In Ger	0.0018	16.25	2.83 – 93.38
Consuming Raw Cow's Milk	0.0067	10.60	1.92 – 58.40

Table 4.11. Final Reduced Logistic Regression Model of Risk Factors for Typical Signs of Acute Brucellosis Reported in Livestock Herders Interviewed in the Khangai Mountain Region of Bayankhongor Aimag (Province), Mongolia, in 2000.

Risk Factor	Wald X^2 p-value	Odds Ratio	95% Confidence Interval (Wald)
Sex (Female)	0.0456	5.31	1.03 – 27.32
Consuming Raw Cow's Milk	0.0271	6.52	1.24 – 34.40
Preparing Dairy Products with Raw Milk	0.0023	9.33	2.22 – 39.31

Table 4.12. Final Reduced Logistic Regression Model of Risk Factors for Typical Signs of Chronic Brucellosis Reported in Livestock Herders Interviewed in the Khangai Mountain Region of Bayankhongor Aimag (Province), Mongolia, in 2000.

Risk Factor	Wald X^2 p-value	Odds Ratio	95% Confidence Interval (Wald)
Age (Unit=1 year)	0.0646	1.054	1.00 – 1.12
Age (Unit=5 years)	0.0646	1.30	0.98 – 1.72
Neonates Housed In Ger	0.0012	20.06	3.29 – 122.44
Drink Raw Cow's Milk	0.0071	31.17	2.55 – 381.05
Herd Strictly *Bos taurus* Cattle	0.0500	5.64	1.00 – 31.80

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Summary

These studies have described the prevalence of brucellosis sero-positive cattle herds in the Khangai mountain region of Bayankhongor aimag (province) Mongolia. Stratified random sampling methods were used to select herder households for inclusion in the studies. Livestock management risk factors for brucellosis sero-positive herd status were identified, and clinical signs associated with the disease in livestock were described. The prevalence of brucellosis-like clinical signs and symptoms in the humans from the same herder households in the region was described. Livestock contact and livestock production consumption practices associated with human brucellosis-like infection were identified. Univariate and multi-variable logistic regression methods were used to identify the risk factors for both livestock and human infection, and to model brucellosis disease in this population.

The high prevalence of brucellosis sero-positive cattle herds recorded in this study suggests that brucellosis is an important re-emerging disease in Mongolia. The prevalence of brucellosis sero-positive cattle herds in the Khangai mountain region of Bayankhongor aimag (province), Mongolia, was similar to historical accounts of brucellosis in Mongolian livestock before any control measures were instituted in the 1960's. Cattle in over 50% (54/103) of herds were recorded as brucellosis sero-positive based on the results of either the Brucellosis Ring Test (BRT) performed on milk, or the Brucellosis Card Test (CARD), an agglutination test performed on serum. Twenty-four percent (23/101) of the cattle herds were classified as positive by both tests.

Evidence of brucellosis infection (antibody presence) in the cattle herds of the Bayankhongor region was associated with significant reductions in herd productivity. The proportion and number of bovine abortions was higher in brucellosis sero-positive herds and the report of bovine abortion was significantly associated with brucellosis sero-positive status (OR = 25.00; 95% C.I. 4.49 – 139.55). None of the specific livestock management factors examined in the study were associated with an increased risk of brucellosis infection, however, increasing cattle herd size was associated with an increased risk of brucellosis sero-positive status (5 unit increase; OR = 2.90; 95% C.I. = 1.41-5.94). Larger cattle herd size is likely associated with a more mobile cattle herd and a higher probability of contact with brucellosis infected cattle in the population.

The investigation of brucellosis prevalence in the human herder population mirrored the levels of infection detected in the cattle. Although laboratory testing was not available for confirmation, we identified approximately ¼ of the herders interviewed who had experienced clinical signs and symptoms associated with brucellosis in the previous 12 months. The occurrence of brucellosis-like disease in humans was associated with the detection of the disease in their livestock, as would be expected with this zoonotic disease in a system where livestock herders are primarily consuming milk and meat from their own livestock. Additionally, those individuals who consumed raw dairy products, or dairy products prepared with raw milk, were significantly more likely to experience brucellosis-like symptoms.

The practice of housing weak newborn livestock in the family's living quarters was associated with an increased risk of experiencing brucellosis-like symptoms. This practice most likely characterizes the close contact that often occurs between herders and the livestock they care for. The weak neonates, vs. health ones, may themselves be more likely to be contaminated with infected uterine fluids from their dams. This livestock management practice, that is perceived to improve the productivity of the herd by increasing lamb/kid/calf survival, may be a difficult practice to change.

Interestingly, being female was associated with a significantly higher probability (OR = 5.31; 95% C.I. = 1.03-27.32) of reporting clinical signs and symptoms consistent with "acute" brucellosis infection. One would not expect to see any gender bias in brucellosis infection since males and females are equally susceptible to the disease. However, in some cases there is a division of labor that falls along gender lines in livestock herding practices, or different habits of livestock product consumption between men and women, that leads to differences in exposure to infection. A specific behavior, with significant gender bias that would explain the increased odds of women experiencing clinical signs suggestive of brucellosis was not identified in this study, however, it is the author's observation that women and girls are more likely than men to take care of livestock nearing parturition and the offspring they produce.

Conclusions

- The prevalence of brucellosis sero-positive cattle herds in the Khangai mountain region of Mongolia was high. The recorded prevalence of 25% -50% indicates active circulation of infection in this population and evidence of the re-emergence of brucellosis in this region of Mongolia.
- Significant production losses in the form of bovine abortion and decreased calving rates were associated with brucellosis infection in this population.

- Only increasing cattle herd size was identified as a risk factor for brucellosis sero-positive cattle herd status in this region of Mongolia where livestock management practices are relatively homogeneous.
- The prevalence of clinical signs suggestive of brucellosis in the human herder population in this region of Mongolia mirrored the brucellosis sero-prevalence data collected from their cattle. Approximately 25% of the individuals surveyed had experienced clinical signs consistent with brucellosis disease in the previous 12 months.
- Risk factors for the occurrence of clinical signs suggestive of brucellosis in this population included the consumption of raw milk, preparing dairy products from raw milk, and the practice of housing weak neonates, primarily kids and lambs, in the family's living quarters.
- Increasing age in humans was associated with a higher probability of reporting clinical signs consistent with "chronic" brucellosis. Gender (being female) was associated with an increased odds of the occurrence of "acute" brucellosis-like symptoms. This gender difference may be related to a gender-based division of labor in the care of cattle nearing parturition and their newborn offspring.

Recommendations

1. Increase Brucellosis Surveillance in Mongolia

This study revealed evidence of a higher than expected prevalence of brucellosis in the Khangai mountain region of Bayankhongor aimag (province), Mongolia. Both the prevalence of sero-positive livestock herds, and the prevalence of humans reporting the occurrence of clinical signs and symptoms consistent with brucellosis, were higher than would be expected based on the nationally reported levels of brucellosis in Mongolia.

The stratified random sampling methods for herd selection used in this study could be applied to the testing of herds in other regions of Mongolia. The random technique allows for the extrapolation of results across the sampled region with a calculated precision. The technique poses significant advantages over the quota sampling methods currently employed in Mongolia.

The hospital case-based reporting of human brucellosis disease should be expanded to include population-based studies of both the "high risk" herder population and the semi-settled and urban population in Mongolia.

It is also essential that systematic surveillance for brucellosis in livestock and humans be incorporated into any control programs so that the effectiveness of brucellosis control measures can be evaluated.

2. Implement a Livestock Brucellosis Vaccination Program

The zoonotic nature of brucellosis dictates that the efforts of eliminating this economically devastating, and public health impacting disease be focused on controlling and eliminating the disease in livestock. The herder population in Mongolia has very few means of protecting themselves from brucellosis. Pre-sale testing of livestock is not available, and food products produced by their livestock are rarely subject to any regulatory inspections. Although educational campaigns may be effective in changing livestock product consumption behaviors (boiling milk and cooking meat) livestock herders can do little to protect themselves from the risk of direct contact with infected livestock.

As other authors have pointed out (Roth et al., 2003; Zinsstag et al., submitted 2004) brucellosis control in Mongolia should be a priority of multiple sectors including human health, agriculture and the economy. The focus should be the elimination of brucellosis in livestock with recognition of the benefits this will have on livestock production, human health and the overall agricultural economy. This study and others indicate that the current prevalence of livestock brucellosis in Mongolia is too high to institute a "test and slaughter" program, but re-instituting livestock immunization for brucellosis should be seriously considered.

Subsequent to the completion of this study, the Veterinary Services Department of the Mongolian Ministry of Food and Agriculture, with some assistance from the World Health Organization (WHO), designed a 10-year plan for the control of livestock brucellosis in Mongolia. The plan involves 2 years of mass immunization of livestock (cattle with *B. abortus* Strain-19, and small ruminants with Rev-1) followed by an 8-year period of immunization of all young female stock. It is recommended that this plan be implemented in its entirety, with frequent assessment of its effectiveness in reducing brucellosis infection rates in Mongolia. The presence of vaccine related antibodies in the livestock population once this control program is implemented will make disease surveillance a challenge, but methods are available in Mongolia to distinguish between vaccine and wild-type *Brucella* reactions. Funding from both the National Government of Mongolia and international donors should be made available for the implementation of a comprehensive brucellosis control program in Mongolia.

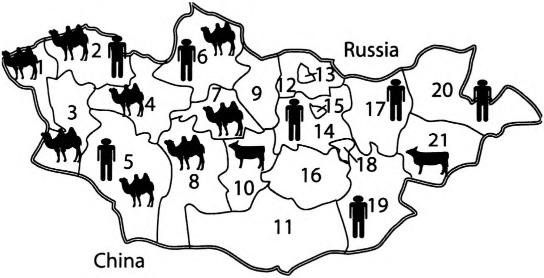
3. Further Research: *Brucella* Identification and Longitudinal Data

Culturing and identifying the *Brucella* species/strain circulating in this population of livestock and humans would enhance our understanding of the epidemiology of brucellosis in the Khangai mountain region of Bayankhongor aimag (province) and provide updated information on the strains of *Brucella* that occur in Mongolia. A longitudinal study involving livestock herders and the animals (sheep, goats, horses, camels, dogs, and cattle) that compose their herds, would also allow us to assess interspecies transmission of *Brucella*, calculate disease incidence rates, assess seasonal trends in disease transmission, and possibly identify risk factors for disease not apparent in the cross sectional study performed in July and August of 2000, described in this thesis.

APPENDIX A

Figure 1.0. Map of the 19 Aimags (Provinces) and 2 Cities (Ulaanbaatar and Darkhan-Uul) of Mongolia. Inset: Mongolia in Central Asia.

Mongolia



Key: List of Aimags (Provinces) and Cities (Ulaanbaatar and Darkhan-Uul)

8. Bayan-Olgi
9. Uvs
10. Khovd
11. Zavkhan
12. Govi-Altai
13. Khovsgol
14. Arkhangai

8. Bayankhongor
9. Bulgan
10. Ovorkhangai
11. Omnogovi
12. Selenge
13. Darkhan-Uul
14. Tuv

15. Ulaanbaatar (Capital)
16. Dundgovi
17. Khentii
18. Govisumber
19. Dornogovi
20. Dornod
21. Sukhbaatar

APPENDIX B

LIVESTOCK MANAGEMENT QUESTIONNAIRE
Khangai Mountain Region of Bayankhongor Aimag (Province), Mongolia

Date of interview:
Soum (district):
Bag (sub-district):

Please list how many and what type of livestock you have:

SHEEP
GOATS
CATTLE
CAMELS
HORSES
YAKS

List the number of adult female animals that gave birth to live babies this year:

SHEEP
GOATS
CATTLE
CAMELS

Where do your animals give birth?

This year, how many baby animals born alive died during the first week of life?

LAMBS
KIDS
CALVES
CAMEL CALVES
FOALS

List the number of adult females that aborted or gave birth to dead babies this year:

SHEEP
GOATS
CATTLE
CAMELS

What months did the abortion(s) occur?

How did you dispose of the aborted fetus and placenta? In open space, etc.

Did you consult a veterinarian about the abortions?
If yes, how much money did they charge?

When was the last time your animals were vaccinated?
For what disease(s) were they vaccinated?

Have your animals ever been vaccinated for brucellosis?
If yes, when was the last time?

How much money did the veterinarian charge to vaccinate for brucellosis?

Do any male animals in your herd have orchitis or lumpy testicles?

Are any of your breeding males infertile?

Which animals do you milk (circle):

SHEEP GOATS CATTLE CAMELS HORSES YAKS

On average, how many weeks or months are your animals in lactation per year?

SHEEP
GOATS
CATTLE
CAMELS
HORSES
YAKS

How many times do you move your herd per year?

Do neighboring herds mix with your animals while grazing?
- In the overnight area?
- In the watering area?
- In the winter shelter?

What do you think, what symptoms do animals with brucellosis have?

____

____

What can be done to prevent brucellosis in animals?

____

____

APPENDIX C

HUMAN HEALTH AND LIVESTOCK CONTACT/PRODUCT CONSUMPTION QUESTIONNAIRE
Khangai Mountain Region of Bayankhongor Aimag (Province), Mongolia

Date of interview:
Soum (district): Sex: MALE FEMALE
Bag (sub-district): Age:

Occupation:

Highest level of education completed:

Have you worked with livestock? What kind of work?
1. veterinary service
2. commercial livestock slaughter
3. hide and wool trade
4. herding livestock
5. other

Do you herd the following livestock? (circle)

SHEEP GOATS CATTLE CAMELS YAKS

Do you keep baby animals inside the ger? YES NO

Do you personally milk the following livestock? (circle)

SHEEP GOATS CATTLE CAMLES YAKS

Do you drink raw (unheated or unpasteurized) milk? YES NO

If yes, milk from (circle) SHEEP GOATS CATTLE CAMLES

Do you prepare dairy products from raw milk? YES NO

Do you personally slaughter the following livestock? (circle)

SHEEP GOATS CATTLE CAMELS

Which animals do you use for meat? (circle)

SHEEP GOATS CATTLE CAMELS

Have you consumed raw or undercooked meat of the following types? (circle)

SHEEP GOAT CATTLE CAMEL

Do you handle cattle dung with your hands? YES NO

Do you have any horses with open draining sores on their withers or top of their head?
YES NO

How do you sever the umbilical cord of animals?

Have you ever been diagnosed with the disease brucellosis?
If yes, when?

How have you been diagnosed?

a. Have you ever had a skin test for brucellosis?
b. Have you ever had a serological (blood) test for brucellosis?

Which of the following symptoms, if any, have you had this year? (circle)

FEVER CHILLS HEADACHES NIGHT SWEATS JOINT PAIN

SWOLLEN JOINT(S) STIFF JOINT(S) BONE PAIN MUSCLE PAIN

LOWER BACK PAIN EXTREME WEAKNESS DIZZINESS

DEPRESSION ABDOMINAL PAIN ABDOMINAL DISTENTION

ENLARGED LYMPH NODES DIFFICULTY SLEEPING

What kind of treatment did you receive for the disease? If possible, list medications.

____

How long were you treated?

a. Were you treated in a hospital/clinic? YES NO
b. Were you treated outside a hospital/clinic? YES NO

Did you experience any relapse of illness after treatment? YES NO

Did you see a doctor (medical officer) at the end of your treatment? YES NO

How do you think people get brucellosis?

____

____

What do you think can be done to prevent brucellosis infection?

____

____

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LITERATURE CITED

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