

Seroprevalence survey of brucellosis among rural people in Mongolia

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

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

Results: A total of 2856 randomly selected rural people aged four to 90 years were enrolled in the study. The seroprevalence of *Brucella* spp. was 11.1% (95% confidence interval [CI]: 10.0–12.1), ranging between 2.3% and 22.6% in the eight provinces; 39.2% ($n = 609$) of nomadic camps had at least one seropositive participant. Risk factors associated with brucellosis seropositivity were being older than 45 years (adjusted odds ratio [AOR] = 6.9, 95% CI = 5.1–8.7) and being a veterinarian (AOR = 2.8, 95% CI = 1.5–5.0).

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

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

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

Mongolia has the second highest incidence of human brucellosis worldwide; another seven republics of the former Soviet Union are included in the

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

In 2011, a national brucellosis serosurvey was conducted that sampled 168 027 head of livestock from 11 528 nomadic camps (two to more than four herder families that share the same pasture and water source) of 337 districts of 21 provinces.⁶ Twenty-one provinces, 57.3% of all districts and 8.0% of all nomadic camps

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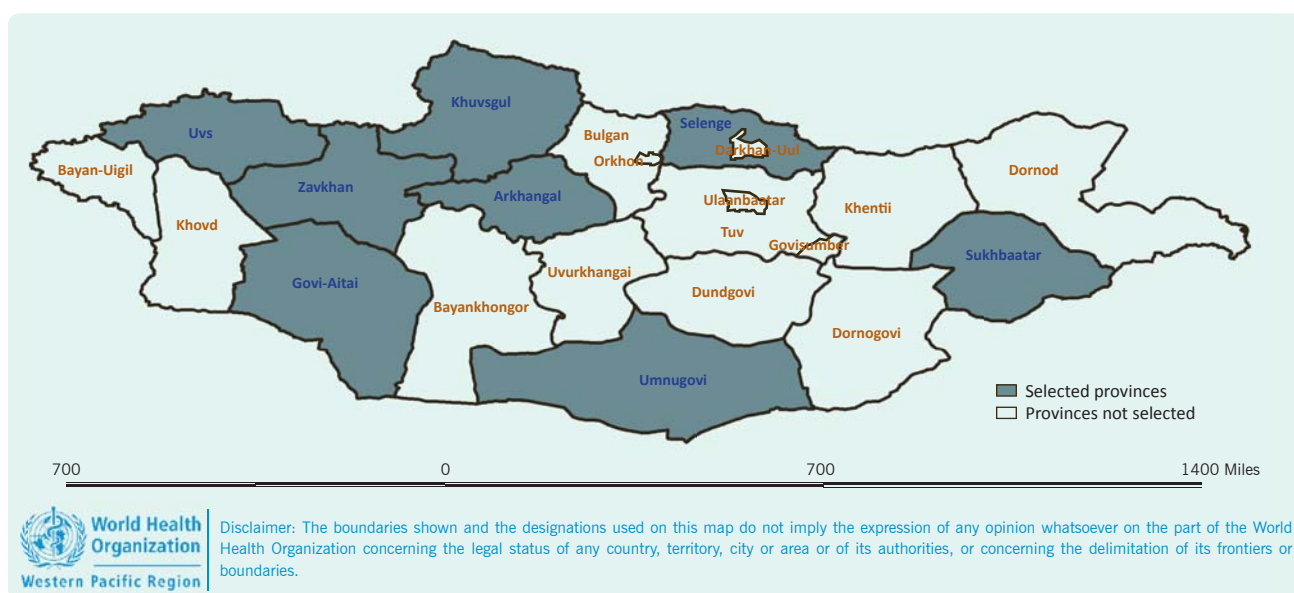
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Figure 1. Map of Mongolia by province highlighting provinces where the study was conducted



had seropositive livestock including camels, cattle, sheep and goats. Livestock seroprevalence was found in 0.7% of camels, 1.8% of cattle, 0.7% of sheep and 0.5% of goats using parallel interpretations of Rose Bengal Tests (RBT), complement fixation tests and competitive-enzyme-linked immunoabsorbent assay (ELISA).⁶

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

METHODS

Study design and population

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

The cluster sample size calculation as described elsewhere⁷ assumed a human brucellosis seroprevalence among Mongolian rural people of 20%.⁸ In addition, the number of clusters and number of individuals per

cluster was optimized according to the feasibility and the available budget.

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

Data collection

Study questionnaire

All study participants were interviewed using a questionnaire which included demographics, risk factors and clinical symptoms for brucellosis. The questionnaire was pre-tested during the 2010 study in Sukhbaatar and Zavkhan⁷ and revised for the extended study to improve understanding of questions and to eliminate overly-sensitive questions.

Blood sample collection and handling

Venous blood was taken with 5 ml Vacutainer® tubes. The blood samples were centrifuged in 3000 rounds per minute for five minutes. Separated 1.5 ml tubes of serum were kept in a cool box and transported to the provincial laboratories for storage and cooling before shipment

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

Province	Number of districts surveyed	Number of participants	Seropositives	% of positivity*	95% confidence interval
Khuvsugul	4	400	46	11.5	8.72–14.2
Umnugovi	4	400	49	12.3	9.64–14.9
Govi-Altai	4	398	30	7.5	4.17–10.8
Selenge	4	391	60	15.3	12.9–17.6
Arkhangai	4	400	9	2.3	0.45–9.15
Uvs	3	293	17	5.8	1.27–10.3
Sukhbaatar	4	318	72	22.6	20.5–24.6
Zavkhan	4	256	33	12.9	9.7–16.1
Total	31	2856	316	11.1	10.0–12.1

* Based on parallel interpretation of the RBT and ELISA test.

to the serological laboratory of the National Center for Communicable Diseases in Ulaanbaatar where they were tested for brucellosis.

Serological test

Sera were tested with the RBT for detection of antibodies to *Brucella abortus/melitensis* from Tulip Diagnostic Ltd (Bambolim, India). Positive sera were re-tested with the RBT using 1/2 to 1/32 serum dilutions,⁹ and with enzyme immunoassay for the qualitative determination of IgG class antibodies against *Brucella* from the NovaTec Immundiagnostica GmbH (Dietzenbach – 63128 Germany). The ELISA test was performed according to manufacturer's instruction.

Data entry and statistical analysis

All data were double-entered in Access 2007, compared in Epi Info™ 3.5 to correct entry errors and analysed using STATA 10.1. Study participants who tested positive by either ELISA or RBT were considered seropositive for the statistical analysis.

To assess the association between risk factors and human brucellosis seropositivity we used Pearson χ^2 or Fisher's exact tests for explanatory variables such as demographics, behaviour-related risk factors and reported clinical symptoms. We also conducted univariate logistic regression using the binary serological outcome with the *xtgee* command and random effect on the nomadic camp level. A multivariate logistic regression model (with random effect at the nomadic camp) using backward stepwise selection and a removal level for covariates at $P = 0.10$ based on the likelihood-

ratio test was then constructed. Variables with p values less than 0.05 in the univariate analysis were included in the multivariate model.

To determine the proportion of the general population seroconverting each year due to brucellosis exposure, the seroprevalence data were divided by the duration of seropositivity, assumed to be 10.9 years.¹⁰ Using a conservative estimate of 20% of seroconversions representing true clinical cases (note that among all seropositives detected, 58.5% had at least two symptoms and 31.5% had at least three symptoms at time of interview), these proportions were multiplied by 0.3 and converted to rates per 100 000 for the general population.

RESULTS

There were 2856 study participants from 609 nomadic camps from 31 districts in the eight selected provinces between four and 90 years of age (median 38 years). This included 2260 (79.1%) herders, 142 (5.0%) students, 96 (3.4%) office workers, 70 (2.5%) workers, 37 (1.3%) retired people, 20 (0.7%) veterinarians, 18 (0.6%) entrepreneurs, 16 (0.6%) unemployed adults, 13 (0.5%) children under six years, and 184 (6.4%) other residents.

Seroprevalence

The seroprevalence of *Brucella* spp. among participants was 11.1% (95% CI: 10.0–12.1) ranging from 2.3% to 22.6% in the eight provinces (Table 1) and 4.1% to 43.8% in the 28 districts. Within nomadic camps, 39.2% (95% CI: 38.2–41.0) had at least one

Table 2. Number of nomadic camps with members seropositive for *Brucella* spp., Mongolia, 2010 to 2012

Province	Number of nomadic camps surveyed	Positive	% of positivity*	95% confidence interval
Arkhangai	79	7	8.9	1.89–15.9
Govi-Altai	80	28	35.0	32.0–37.9
Khuvsgul	82	35	42.7	40.2–45.1
Umnugovi	80	33	41.3	38.1–44.5
Uvs	58	13	22.4	17.5–27.2
Selenge	78	40	51.3	49.1–53.4
Sukhbaatar	83	56	67.5	65.9–69.0
Zavkhan	69	27	39.1	36.1–42.0
Total	609	239	39.2	38.2–41.0

* Based on parallel interpretation of the RBT and ELISA test

to four seropositive members (Table 2). This equated to an annual incidence of seroconversion of 1145 per 100 000 and an overall annual incidence of 229 clinical cases per 100 000.

Seroprevalence was higher in females than in males (11.2% compared with 10.9%, $P = 0.029$). By age group, the highest seroprevalence was found in those 45 years and above at 15.5% (95% CI: 13.9–17.0), with the lowest in the four to 10 year age group at 2.6% (95% CI: 1.5–20.4). All occupation categories included seropositive cases ranging between 2.8% and 30.0% (Table 3).

Analysis of risk factors for brucellosis

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

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

History of human brucellosis and clinical symptoms

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

Reported clinical symptoms at the time of the study were compared to the sero-status of participants. Overall, 165 of the 316 (52.2%) brucellosis seropositive participants and 1186 of the 2540 (46.7%) seronegative participants reported symptoms. Among all seropositives, 36.7% reported more than three symptoms; among the seronegatives, 23.1% reported more than three symptoms ($P < 0.001$). Headache; joint, back and muscle pain; night sweats and sleeping disturbances were significantly associated with brucellosis seropositivity (Table 5).

DISCUSSION

We report a seroprevalence of *Brucella* spp. among rural people of 11.1% (with a range between provinces from 2.3% to 22.6%) and an annual incidence of 229 per 100 000. The high incidence in the study likely reflects an increase in human brucellosis after

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

Characteristic	Number of participants	Number seropositive (%)	OR (95% CI)	p value
<i>Age group (years)</i>				
4–9	39	1 (2.6)	1.0	–
10–14	69	4 (5.8)	2.3 (1.2–4.1)	0.440
15–19	96	3 (3.1)	1.2 (0.6–2.7)	0.864
20–44	1769	171 (9.7)	3.9 (1.2–7.6)	0.151
45 and above	883	137 (15.5)	6.6 (4.5–10.2)	0.046
<i>Sex</i>				
Males	1181	132 (11.2)	1.0	–
Females	1675	184 (10.9)	1.0 (0.9–1.2)	0.968
<i>Occupation</i>				
Herder	2260	263 (11.6)	1.3 (0.9–2.5)	0.087
Student	142	4 (3.0)	0.9 (0.3–2.5)	0.345
Office worker	96	7 (7.3)	0.7 (0.2–1.6)	0.267
Worker	70	7 (10.0)	0.9 (0.5–2.0)	0.733
Retired	37	7 (18.9)	2.0 (0.8–4.2)	0.112
Veterinarian	20	6 (30.0)	3.5 (1.6–7.9)	0.016
Entrepreneur	18	4 (22.2)	2.3 (1.0–4.6)	0.119
Unemployed	16	1 (6.3)	0.5 (0.3–1.3)	0.521
Children under six	13	1 (7.7)	0.7 (0.3–1.6)	0.708
Other	184	16 (8.7)	0.8 (0.4–1.7)	0.328
<i>Risk factors</i>				
Animal obstetric work	778	93 (11.9)	1.5 (0.9–2.5)	0.121
Contact with aborted animal fetuses and placentas	769	104 (13.5)	1.4 (1.0–2.1)	0.016
Consumption of raw milk	295	32 (10.8)	1.2 (0.7–1.8)	0.546
Consumption of raw liver	38	11 (28.9)	0.8 (0.5–1.2)	0.612
Consumption of undercooked liver	1067	146 (13.7)	1.5 (0.9–4.3)	0.001
Consumption of fresh animal blood	143	12 (8.4)	1.5 (1.0–1.7)	0.332

OR, odds ratio; CI, confidence interval.

* Based on parallel interpretation of RBT and ELISA

the transition in Mongolia from socialism to a market economy leading to livestock privatization and collapse of the veterinary sector.⁴

Although several earlier studies also estimated the seroprevalences of *Brucella* spp. in Mongolia among high-risk people including herders, veterinarians and raw animal processing technicians,^{11–14} these differed from our study in time, study design and methodology and should not be compared. The result from our study was higher than the 0.1% to 10.1% reported among high-risk people in other countries,^{10,15–21} which is not surprising as Mongolia is ranked second in the world for brucellosis incidence.⁵ We also estimated a much higher incidence compared with that reported from notification data,²² despite the fact that we have taken a conservative assumption that 20% of seropositive cases are clinical cases.

According to the multivariate analysis, adults aged 45 years and above and veterinarians had a higher risk for brucellosis. This age group plays an important role in livestock herding and birthing, and veterinarians have direct contact with animals and aborted materials when doing veterinary examinations. We also found seropositives in all age groups, including in young children (four to nine years), which may indicate ongoing exposure and transmission of brucellosis in rural Mongolia. These groups should be targeted with material about protection against brucellosis infection.

This study will serve as a baseline of the seroprevalence of *Brucella* spp. in rural people in Mongolia before the implementation of a nationwide livestock vaccination campaign; it also will be used for ongoing brucellosis surveillance. A decrease of human incidence and repeated sero-surveillance surveys

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

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

* Either derived from the χ^2 test or Fisher's exact test.

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

Clinical symptoms		Number of participants	Number seropositive (%)	p value
Fever	No	2721	301 (11.1)	0.561
	Yes	135	15 (11.1)	
Headache	No	1588	167 (10.5)	< 0.001
	Yes	1268	149 (11.8)	
Joint pain	No	1569	155 (9.9)	0.014
	Yes	1287	161 (12.5)	
Back pain	No	1505	151 (10.0)	0.038
	Yes	1351	165 (12.2)	
Muscle pain	No	2266	234 (10.3)	0.009
	Yes	590	82 (13.9)	
Weight loss	No	2623	287 (10.9)	0.379
	Yes	233	29 (12.4)	
Weakness	No	1892	194 (10.3)	0.058
	Yes	964	122 (12.7)	
Night sweats	No	2520	266 (10.6)	0.013
	Yes	336	50 (14.9)	
Sleeping disturbance	No	2326	242 (10.4)	0.010
	Yes	530	74 (14.0)	
Miscarriage	No	1644	182 (11.1)	0.713
	Yes	31	2 (6.4)	
Testicular pain	No	1171	131 (11.2)	0.620
	Yes	10	1 (10.0)	

in humans will indirectly assess the efficacy of the vaccination campaign in livestock.²³

There were several limitations to the study. First, association between human and livestock seropositivity was not assessed in provinces (with the exception of Zavkhan and Sukhbaatar⁷). There also may have been temporal variations in risk factors for childhood brucellosis, interpretation of reported clinical symptoms for brucellosis based on seropositivity and pathogen exposure that were not captured by the cross-sectional study design.

CONCLUSION

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

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

Conflicts of interests

None declared.

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