Serosurvey of Babesia bovis and Babesia bigemina in cattle in Mongolia

Serosurvey of *Babesia bovis* and *Babesia bigemina* in cattle in Mongolia

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ABSTRACT

Mongolia is an agriculturally rich country with large livestock populations that contribute significantly to its national economy. However, the export market for live animals and livestock products is often constrained for various reasons including infectious diseases. *Babesia bovis* and *B. bigemina*, which are bovine hemoprotozoan parasites, cause severe forms of clinical babesiosis, in cattle. However, a country-wide survey to determine the exposure rates in various provinces in Mongolia was not conducted to determine the risk for infections with these parasite species. Therefore, we investigated the frequency of antibodies to *B. bovis* and *B. bigemina* in cattle reared throughout Mongolia. *B. bovis*-and *B. bigemina*-specific enzyme-linked immunosorbent assays (ELISAs) were used to screen the serum samples sourced from 1946 cattle in 19 of 21 provinces and a provincial municipality (Ulaanbaatar) in Mongolia. We found 351 (18.0%) samples positive for *B. bovis* and 435 (22.4%) samples positive for *B. bigemina* infections. The *B. bovis*- and *B. bigemina*-positive rates ranged from 0.8 to 61.5% and 4.0 to 50.6%, respectively, among the surveyed provinces. The positive rates of *B. bovis* and *B. bigemina* infections were relatively higher in the provinces located in northernmost, northern, eastern, southeastern, and southern Mongolia. Additionally, the *B. bovis*- and *B. bigemina*-positive rates were not significantly different between females (18.2 and 22.2%, respectively) and males (17.2 and 18.8%, respectively) or

between the 1–3-year-old (16.2 and 19.4%, respectively) and >3-year-old (17.1 and 20.9%, respectively) age groups. The differential seropositivity for *B*. *bovis* and *B*. *bigemina* infections among the provinces may reflect the variations in the risk of cattle being infected with these parasite species. The findings of the present study highlight the need for country-wide control measures, including tick control programs, to minimize the rates of *B*. *bovis* and *B*. *bigemina* infections in Mongolian cattle.

Keywords: Babesia bovis, *Babesia bigemina*, Cattle, ELISA, Mongolia

48 **1. Introduction**

49

50 Mongolia is an agricultural country in which the agriculture industry contributes
51 over 30% to the national gross domestic product. The industry is dominated by the
52 livestock sector, which generates approximately 80% of the gross agriculture product
53 (Shagdar, 2002). The cattle population of Mongolia is estimated to be about 4 million
54 head, which is higher than the human population of the country (National Statistics
55 Office of Mongolia, 2017). With such a large cattle population, Mongolia should be
56 able to hold a significant share of the international market. However, the export of live
57 animals, meat, and dairy products has been severely constrained by international trade
58 regulations for various reasons, including the prevalence of infectious diseases among
59 Mongolian cattle (World Bank, 2009). Thus, the control of such infectious diseases is of
60 paramount importance to upgrade the Mongolian cattle industry and thereby increase
61 the export of live animals and livestock products.

62 Bovine babesiosis caused by *Babesia bovis* and *B*. *bigemina* is widespread in
63 tropical and sub-tropical regions of world (Bock et al., 2004). The infections with these
64 parasite species in susceptible cattle often result in severe clinical disease characterized
65 by fever, hemoglobinuria, anemia, jaundice, and, in the case of *B*. *bovis*, neurological
66 and respiratory syndromes (Everitt et al., 1986; Mosqueda et al., 2012). Early diagnosis
67 and treatment with babesiacidal drugs are essential for a better prognosis, and deaths are

not uncommon among the untreated clinical cases of bovine babesiosis (Bock et al., 2004; Mosqueda et al., 2012). Live-attenuated vaccines are used to immunize the susceptible cattle populations against *B*. *bovis* and *B*. *bigemina* infections in some of the endemic countries (Callow et al., 1979; Bock and de Vos, 2001; Shkap et al., 2007; Sivakumar et al., 2013). However, the wide use of live vaccines is limited for several reasons, including transmission of other blood-borne pathogens, development of clinical disease in older and pregnant animals, and vaccine breakthrough due to strain variations (Bock et al., 1992; Berens et al., 2005; Leroith et al., 2005). Similarly, tick control measures are not always successful because of the rapid development of acaricide resistance (George et al., 2004). Recent PCR-based studies have detected *B*. *bovis* and *B*. *bigemina* in Mongolian cattle in three different provinces (Altangerel et al., 2012; Sivakumar et al., 2012). However, as the PCR assays detect only active infections, the rates of animals exposed to *B*. *bovis* and *B*. *bigemina* have not yet been analyzed in Mongolia. Such an investigation is of high importance to estimate the risk for infections with *B*. *bovis* and *B*. *bigemina* in various provinces in this country. With such data, policy makers and veterinary authorities could set up a proper strategy to minimize the population of infected cattle based on the relative risk in each province. Therefore, we conducted a serosurvey of *B*. *bovis* and *B*. *bigemina* infections in cattle reared in 19 of 21 provinces and a provincial municipality (Ulaanbaatar) in Mongolia to map the epidemiology of these harmful parasitic species.

2. Materials and methods

2.1. Serum samples

In total, 1946 blood samples were collected from cattle that had been reared in 19 of 21 provinces and a provincial municipality (Ulaanbaatar) in Mongolia (animals in Orhon and Darkhan-Uul provinces were not sampled) during June–October in 2014, April–June in 2015, and March–September in 2016 (Table 1). The sampling was conducted for three consecutive years, because of long distance between provinces and limited number of staff members. Each year, the animals were sampled in different locations. The sampling was carried out in spring (March – May), summer (June – August) or autumn (September and October), while no animals were sampled in winter (November – February). In Mongolia, temperatures drop far below 0ºC during winter. The temperature gradually increases from March, reaches as high as 40ºC in summer, and then starts to decrease from September. Ticks are known to be active in spring, early and late summer, and autumn but not in winter. Cattle breed identification for most of the surveyed animals was almost impossible due to extensive crossbreeding between Ala-Tau, Holstein, and local Mongolian breeds. Serum samples were prepared from the blood samples and then stored at –30°C until use. All animals were apparently healthy at the time of sampling.

108

109 *2.2. Enzyme-linked immunosorbent assays (ELISAs)*

110 *Babesia bovis*- and *B. bigemina*-specific recombinant rhoptry-associated protein 1
111 (RAP-1)-based ELISAs were used to determine the seropositivity for these parasite
112 species in Mongolian cattle, as described previously with some modifications
113 (Sivakumar et al., 2016). Briefly, the N-terminal regions of *B. bovis* (741 bp) and *B.*
114 *bigemina* (513 bp) *rap-1* gene fragments amplified from four parasite-positive archived
115 blood DNA samples sourced from Mongolian cattle (Altangerel et al., 2012) were
116 cloned and sequenced, as previously described (Sivakumar et al., 2018). Sequencing
117 analyses showed that the *rap-1* gene sequences of *B. bovis* (LC323189-LC323192) and
118 *B. bigemina* (LC323193-LC323196) were conserved in Mongolia as they shared high
119 identity (99.6–100% and 99.2–100%, respectively) and similarity scores (99.6–100%
120 and 99.4–100%, respectively).

121 Subsequently, *B. bovis* and *B. bigemina* RAP-1 antigens (rRAP-1) were produced
122 as previously described (Sivakumar et al., 2016, 2018), based on *B. bovis*- and *B.*
123 *bigemina rap-1* gene sequences (LC323189 and LC323193, respectively) determined in
124 the present study. The ELISAs for detecting *B. bovis*- and *B. bigemina*-specific
125 antibodies among the Mongolian cattle serum samples were conducted using ELISA
126 plates coated with 100 μL of 1 μg/mL *B. bovis* and *B. bigemina* rRAP-1 antigens,
127 respectively, essentially as described previously (Sivakumar et al., 2018). A serum

sample is considered positive if the OD value is higher than the cut-off, which was the sum of the mean OD values of eight negative controls and 5 × their standard deviation (Sivakumar et al., 2016).

2.3. Statistical analysis

An OpenEpi software program (http://www.openepi.com/Proportion/Proportion.htm) was used to analyze the positive rates and determine the 95% confidence intervals (CI) using a Wilson score interval (Wilson, 1927). An "N-1" chi-squared test (https://www.medcalc.org/calc/comparison_of_proportions.php) was used to calculate *P* values when comparing the positive rates (Campbell, 2007; Richardson, 2011). A *P* value < 0.05 was considered to indicate a significant difference between the positive rates.

3. Results and discussion

Of the 1946 bovine serum samples collected in Mongolia, 351 (18.0%) were positive for *B. bovis*, while 435 (22.4%) samples were positive for and *B. bigemina* infections (Table 1). The highest positive rates for *B. bovis* (61.5%) and *B. bigemina* (50.6%) were determined in Bulgan and Khentii provinces, respectively. In contrast, the

148 lowest positive rates for *B*. *bovis* and *B*. *bigemina* were determined in Zavkhan (0.8%)
149 and Govi-Altai (4.0%) provinces, respectively. In addition, Khovsgol, Selenge, Khentii,
150 Dornogovi, and Omnogovi provinces had a higher frequency of *B*. *bovis* (>25%) than
151 the rest of the provinces surveyed. Similarly, *B*. *bigemina*-positive rates were higher
152 (>25%) in Bulgan, Khovsgol, Selenge, Dornogovi, Ovorkhangai, and Khovd provinces
153 than those in other provinces (Table 1, Fig. 1). Geographically, high rates of *B*. *bovis*
154 and *B*. *bigemina* positivity were observed in the northernmost, northern, eastern,
155 southeastern, and southern provinces (Fig. 1). In contrast, the frequency was relatively
156 low in the central, easternmost, and western provinces, except for Khovd, where 28.5%
157 of the surveyed animals were positive for *B*. *bigemina*.

158 Co-infection with *B*. *bovis* and *B*. *bigemina* was very common among the surveyed
159 cattle (Table 1). Among 618 (31.8%) animals that were infected with *B*. *bovis* and/or *B*.
160 *bigemina*, 168 (27.2%) were co-infected with both parasite species. On a per province
161 basis, the rates of co-infected animals ranged from 0 to 51.9%. Relatively high rates of
162 co-infections were observed in Bayan-Ulgii (26.3%), Bulgan (44.2%), Dornogovi
163 (50.0%), Govi-Altai (28.6%), Govisumber (37.5%), Khentii (33.7%), Khovsgol (51.9%),
164 and Selenge (47.5%) (Table 1).

165 Among the 1946 animals surveyed in the present study, 1562 were females and 384
166 were males. The overall positive rates of *B*. *bovis* and *B*. *bigemina* infections were not
167 significantly different between the females (18.2 and 17.2%, respectively) and males

168 (23.2 and 18.8%, respectively) (Table 2). In contrast, the *B*. *bovis*-and *B*.
169 *bigemina*-positive rates between females and males were significantly different in
170 Bayan-Ulgii, Khentii, Selenge, Tov, and in Ulaanbaatar and Arkhangai, Bulgan, Khentii,
171 Khovsgol, Selenge, and Tov, respectively. However, the numbers of female samples
172 were several times greater than those of males in these locations, making a fair
173 comparison difficult.

174 We also analyzed the positive rates of *B*. *bovis* and *B*. *bigemina* infections based on
175 the age of the animals. However, records on the age of 312 animals were unavailable.
176 Therefore, only 1634 samples were analyzed for parasite positivity based on the age
177 groups (Table 3). The present findings showed that between the 1–3-year and >3-year
178 age groups, the overall positive rates of *B*. *bovis* (16.2 and 17.1%, respectively) and *B*.
179 *bigemina* (19.4 and 20.9%, respectively) were not significantly different (Table 3). In
180 contrast, the positive rates between these age groups were significantly different for *B*.
181 *bovis* in Bayan-Ulgii, Khentii, Khovd, and Ovorkhangai, and for *B*. *bigemina* in Bulgan
182 and Dornogovi. However, the small sample size and the difference between the numbers
183 of samples in each age category may not allow us to make a fair comparison on a per
184 province basis. Age of animal is considered to be risk factor for *B*. *bovis* and *B*.
185 *bigemina* infections (Awad et al., 2011; Terkawi et al., 2011). However, in some of the
186 epidemiological surveys, statistically significant differences were not observed between
187 males and females or between age groups (Iseki et al., 2010; Tembue et al., 2011). Most

188 cattle in Mongolia are managed extensively throughout their life irrespective of the
189 gender. Therefore, the age and gender may not be associated with the risk of getting
190 exposed to infected ticks in Mongolia. This could explain why the overall positive rates
191 were not different between age groups or males and females in this country. However,
192 these findings may not be conclusive, as the sample sizes were not statistically
193 determined in the present study. Therefore, future investigations in Mongolia should be
194 based on statistically calculated sample sizes in order to confirm our findings.

195 The positive rates of *B. bovis* and *B. bigemina* could also be influenced by other
196 factors, such as cattle movement, tick active season, irregular use of acaricides, and
197 distribution and density of tick vectors. Although cattle movement is very common in
198 Mongolia, most of the animals are moved within the province, while only a small
199 proportion of animals are moved to the neighboring provinces. In the present study,
200 samples were collected in spring, summer or autumn, when the ticks are known to be
201 active Mongolia. Tick control measures, including application of acaricides, are not
202 widely practiced among cattle herds in Mongolia. Therefore, the distribution and
203 density of specific tick vectors might explain the different rates of positivity among the
204 provinces. The factors such as environmental humidity and temperature might influence
205 the density and activity of the tick vectors and prevalence of tick-borne diseases (Süss et
206 al., 2008). For example, 4 (Khovsgol, Bulgan, Selenge, and Khentii) of 6 and 7
207 provinces with seropositive rates more than 25% for *B. bovis* and *B. bigemina*,

208 respectively, are located in areas receiving high precipitation (Rao et al., 2015). The
209 increased relative humidity due to high precipitation might favor the tick population,
210 leading to high rate of *B. bovis*- and *B. bigemina*-seropositivity in these provinces.
211 *Rhipicephalus* (*Boophilus*) tick species that are known as the common vector of *B. bovis*
212 and *B. bigemina* were not detected in Mongolia in previous investigations (Bock et al.,
213 2004; Hunfeld et al., 2008). However, *Dermacentor nuttalli*, *Ixodes persulcatus*, and
214 *Hyalomma asiaticum* were reported in Mongolia (Altangerel et al., 2011; Boldbaatar et
215 al., 2017; Tuvshintulga et al., 2015), but the capacity of these tick species as
216 transmission vectors of *B. bovis* and *B. bigemina* has not been investigated yet. High
217 rates of both *B. bovis*- and *B. bigemina*-positivity in some of the provinces suggested
218 that both of the parasite species could have been transmitted by the same vector ticks.
219 The high rates of co-infection observed in such provinces further support our
220 assumption. Thus, future studies in Mongolia should attempt to show *B. bovis* and *B.*
221 *bigemina* infections in tick species collected in various provinces. Importantly, the
222 transmission experiments in cattle are of paramount importance using the tick species
223 positive for these parasites in Mongolia. Such investigations should confirm that the
224 parasites acquired by the vector ticks from experimentally infected cattle can be
225 transmitted to uninfected cattle during blood feeding.

226 If the seroprevalence of *B. bovis* and *B. bigemina* infections is more than 75%, an
227 endemic stability can be achieved in the cattle populations (L'Hostis and Seegers, 2002).

There will be no or few clinical cases among the animals under the endemically stable situation (Bock at al., 2004). The rates of seropositive cattle in Mongolia are far less than those required for endemic stability, suggesting that the clinical babesiosis among Mongolian cattle may not be uncommon. However, such clinical cases could have been unnoticed due to various reasons, such as lack awareness among farmers, not reporting to veterinary authorities, lack of veterinary investigation, and potential resistance to clinical babesiosis in Mongolian cattle. In addition, immunity acquired during early life as the herds are managed by an extensive system might account for the lower frequency of clinical babesiosis (Regassa et al., 2003). Nevertheless, the veterinarians in Mongolia should look for the clinical cases of babesiosis among cattle populations, especially when the animals are anemic. Although the very low seropositive rates in some of the Mongolian provinces indicate a low risk for *Babesia* infection, the mortality rate might increase if the animals develop clinical babesiosis (L'Hostis and Seegers, 2002).

In summary, the country-wide survey conducted in the present study found that the cattle populations bred throughout Mongolia have been exposed to *B*. *bovis* and *B*. *bigemina* and that the frequency of these parasite species varies among the provinces. Therefore, the government of Mongolia and the related authorities should formulate and implement control measures, including tick control programs, to minimize infections by *B*. *bovis* and *B*. *bigemina* in cattle throughout the country.

Conflict of interest statement

The authors declare no conflicts of interest in association with the present study.

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361

362 **Figure legend**

363

364 **Fig. 1.** Epidemiological mapping of *B*. *bovis* and *B*. *bigemina* infections.
365 Epidemiological maps were prepared based on the seropositive rates of *B*. *bovis* (panel
366 A) and *B*. *bigemina* (panel B) infections in cattle in various provinces of Mongolia.
367 Different colors are used to indicate the levels of positive rates. Note that the *B*. *bovis*-
368 and *B*. *bigemina*-positive rates were relatively higher in Bulgan, Khovsgol, Selenge,
369 Khentii, Dornogovi, and Omnogovi, and in Khentii, Bulgan, Khovsgol, Selenge,
370 Dornogovi, Ovorkhangai, and Khovd, respectively, than in the rest of the surveyed
371 provinces.

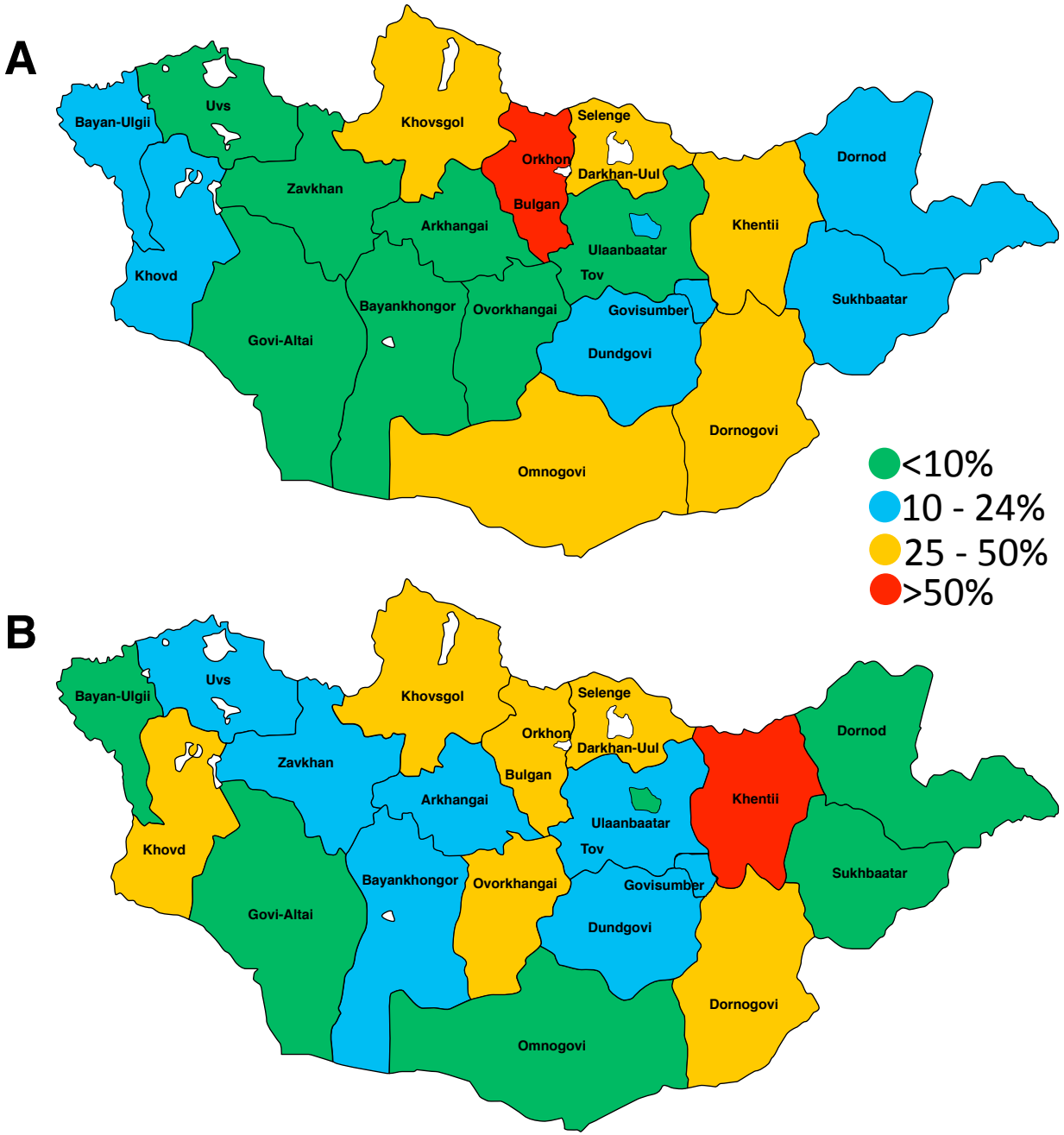
A
Bayan-Ulgii
Uvs
Khovsgol
Selenge
Orkhon
Darkhan-Uul
Dornod
Zavkhan
Bulgan
Arkhangai
Khentii
Ulaanbaatar
Khovd
Tov
Sukhbaatar
Bayankhongor
Ovorkhangai
Govisumber
Govi-Altai
Dundgovi
Dornogovi
Omnogovi
<10%
10 - 24%
25 - 50%
>50%
B
Bayan-Ulgii
Uvs
Khovsgol
Selenge
Orkhon
Darkhan-Uul
Dornod
Zavkhan
Bulgan
Arkhangai
Khentii
Ulaanbaatar
Khovd
Tov
Sukhbaatar
Bayankhongor
Ovorkhangai
Govisumber
Govi-Altai
Dundgovi
Dornogovi
Omnogovi

Fig. 1

Table 1
Summary of ELISA results.

Province	No. samples	*B. bovis*		*B. bigemina*		No. *Babesia* positive[b]	No. co-infected samples (%[c])
		No. positive	% (CI[a])	No. positive	% (CI)		
Arkhangai	68	3	4.4 (1.5-12.2)	12	17.6 (10.4-28.4)	14	1 (7.1)
Bayankhongor	77	3	3.9 (1.3-10.8)	14	18.2 (11.2-28.2)	16	1 (6.3)
Bayan-ulgii	150	17	11.3 (7.2-17.4)	7	4.7 (2.3-9.3)	19	5 (26.3)
Bulgan	78	48	61.5 (50.4-71.6)	27	34.6 (25.0-45.7)	52	23 (44.2)
Dornod	142	30	21.1 (15.2-28.6)	12	8.5 (4.9-14.2)	38	4 (10.5)
Dornogovi	42	15	35.7 (23.0-50.8)	15	35.7 (23.0-50.8)	20	10 (50.0)
Dundgovi	38	5	13.2 (5.8-27.3)	5	13.2 (5.8-27.3)	8	2 (25.0)
Govi-Altai	124	4	3.2 (1.3-8.0)	5	4.0 (1.7-9.1)	7	2 (28.6)
Govisumber	124	16	12.9 (8.1-20.0)	28	22.6 (16.1-30.7)	32	12 (37.5)
Khentii	178	45	25.3 (19.5-32.1)	90	50.6 (43.3-57.8)	101	34 (33.7)
Khovd	151	33	21.9 (16.0-29.1)	43	28.5 (21.9-36.1)	69	7 (10.1)
Khovsgol	90	36	40 (30.5-50.3)	43	47.8 (37.8-58.0)	52	27 (51.9)
Omnogovi	101	29	28.7 (20.8-38.2)	8	7.9 (4.1-14.9)	32	5 (15.6)
Ovorkhangai	98	6	6.1 (2.8-12.7)	37	37.8 (28.8-47.7)	41	2 (4.9)
Selenge	113	42	37.2 (28.8-46.4)	45	39.8 (31.3-49.0)	59	28 (47.5)
Sukhbaatar	102	11	10.8 (6.1-18.3)	7	6.9 (3.4-13.5)	16	2 (12.5)
Tov	69	3	4.3 (1.5-12.0)	13	18.8 (11.4-29.6)	15	1 (6.7)
Ulaanbaatar	13	2	15.4 (4.3-42.2)	1	7.7 (1.4-33.3)	3	0 (0)
Uvs	65	2	3.1 (0.8-10.5)	7	10.8 (5.3-206)	8	1 (12.5)
Zavkhan	123	1	0.8 (0.1-4.5)	16	13 (8.2-20.1)	16	1 (6.3)
Total	**1946**	**351**	**18 (16.4-19.8)**	**435**	**22.4 (20.6-24.3)**	**618**	**168 (27.2)**

ELISA = enzyme-linked immunosorbent assay.
[a] 95% confidence interval.
[b] Numbers of samples positive for *B. bovis* and/or *B. bigemina.*
[c] Expressed as a percentage of the numbers of animals that were positive for *B. bovis* and/or *B. bigemina.*

Table 2
Seroprevalence of *B. bovis* and *B. bigemina* in female and male cattle in Mongolia.

Province	No. samples		*B. bovis*					*B. bigemina*				
	Female	Male	Female		Male		*P* value	Female		Male		*P* value
			No. positive	% (CI[a])	No. positive	% (CI)		No. positive	% (CI)	No. positive	% (CI)	
Arkhangai	92	12	2	2.2 (0.6-7.6)	1	8.3 (1.5-35.4)	0.2388	8	8.7 (4.5-16.2)	4	33.3 (13.8-61.0)	0.0125
Bayankhongor	69	8	2	2.9 (0.8-10.0)	1	12.5 (2.2-47.0)	0.1870	13	18.8 (11.4-29.6)	1	12.5 (2.2-47.0)	0.6637
Bayan-Ulgii	56	36	15	26.8 (17-39.6)	2	5.6 (1.5-18.1)	0.011	6	10.7 (5.0-21.4)	1	2.8 (0.5-14.1)	0.1654
Bulgan	62	16	41	66.1 (53.7-76.7)	7	43.8 (23.1-66.8)	0.0259	27	43.5 (31.9-55.9)	0	0.0 (0.0-43.4)	0.0012
Dornod	127	33	23	18.1 (12.4-25.7)	7	21.2(10.7-37.8)	0.6853	10	7.9 (4.3-13.9)	2	6.1 (1.7-19.6)	0.7278
Dornogovi	114	7	13	11.4 (6.8-18.5)	2	28.6 (8.2-64.1)	0.1819	14	12.3 (7.5-19.6)	1	14.3 (2.6-51.3)	0.8767
Dundgovi	33	5	4	12.1 (4.8-27.3)	1	20 (3.6-62.4)	0.6306	5	15.2 (6.6-30.9)	0	0.0 (0.0-43.4)	0.3558
Govi-Altai	35	14	3	8.6 (3.0-22.40	1	7.1 (1.3-31.4)	0.8639	4	11.4 (4.5-26.0)	1	7.1 (1.3-31.5)	0.6561
Govisumber	82	33	11	13.4 (7.7-22.4)	5	15.2 (6.6-30.9)	0.8017	16	19.5 (12.4-29.4)	12	36.4 (22.2-53.4)	0.0572
Khentii	109	25	43	39.4 (30.8-48.8)	2	8.0 (2.2-25.0)	0.0028	80	73.4 (64.4-80.8)	10	40.0 (23.4-59.3)	0.0014
Khovd	151	24	25	16.6 (11.5-23.30	8	33.3 (18.0-53.2)	0.0529	39	25.8 (19.5-33.3)	4	16.7 (6.7-35.9)	0.3374
Khovsgol	61	21	29	47.5 (35.5-59.8)	7	33.3 (17.2-54.6)	0.261	36	59.0 (46.5-70.5)	7	33.3 (17.2-54.6)	0.0432
Omnogovi	76	19	21	27.6 (18.8-38.6)	8	42.1 (23.1-63.7)	0.2219	8	10.5 (5.4-19.4)	0	0.0 (0.0-16.8)	0.1421
Ovorkhangai	92	22	5	5.4 (2.3-12.1)	1	4.5 (0.8-21.8)	0.8653	29	31.5 (22.9-41.6)	8	36.4 (19.7-57.0)	0.6606
Selenge	42	21	35	83.3 (69.4-91.4)	7	33.3 (17.2-54.6)	0.0001	39	92.9 (90-97.5)	6	28.6 (13.8-50)	<0.0001
Sukhbaatar	153	18	9	5.9 (2.3-12.1)	2	11.1 (3.1-32.8)	0.3969	7	4.6 (2.2-9.1)	0	0.0 (0.0-17.6)	0.3542
Tov	91	8	1	1.1 (0.2-6.0)	2	25.0 (7.1-59.0)	0.0002	8	8.8 (4.5-16.4)	5	62.5 (30.6-86.3)	< 0.0001
Ulaanbaatar	110	8	0	0.0(0.0-3.4)	2	25.0 (7.1-59.0)	0.0001	1	0.9 (0.2-5.0)	0	0.0 (0.0-32.4)	0.7885
Uvs	84	23	2	2.4 (0.6-8.3)	0	0.0 (0.0-14.3)	0.4553	4	4.8 (1.8-11.6)	3	13.0 (4.5-32.1)	0.1614
Zavkhan	69	31	1	1.4 (0.3-7.8)	0	0.0 (0.0-11.0)	0.5101	9	13.0 (7.0-23.0)	7	22.6 (11.4-40.0)	0.2279
Total	1562	384	285	18.2 (16.4-20.3)	66	17.2 (13.7-21.3)	0.6478	363	23.2 (21.2-25.4)	72	18.8 (15.2-23.0)	0.0637

[a] 95% confidence interval.

Table 3
Seroprevalence of *B. bovis* and *B. bigemina* in different age groups of cattle in Mongolia.

Province	No. samples		*B. bovis*					*B. bigemina*				
	1-3 years	> 3 years	1-3 years		> 3 years		*P* value	1-3 years		> 3 years		*P* value
			No. positive	% (CI[a])	No. positive	% (CI)		No. positive	% (CI)	No. positive	% (CI)	
Arkhangai	1	1	0	0.0	0	0.0		1	100 (20.7-100)	0	0.0	0.3173
Bayankhongor	24	52	1	4.1 (0.7-20.2)	2	3.8 (1.1-13.0)	0.9502	6	25.0 (12.0-44.9)	8	15.4 (8.0-27.5)	0.3189
Bayan-Ulgii	80	69	13	16.3 (9.6-26.0)	3	4.3 (1.5-12.0)	0.0187	3	3.8 (1.3-10.5)	3	4.3 (1.5-12.0)	0.8775
Bulgan	17	17	14	82.4 (59.0-93.9)	14	82.4 (59.0-93.9)	1.0000	3	17.6 (6.2-41.0)	9	52.9 (31.0-73.8)	0.0338
Dornod	67	75	14	20.9 (12.9-32.1)	16	21.3 (13.6-31.9)	0.9537	6	9.0 (7.2-23.6)	6	8.0 (3.7-16.4)	0.8314
Dornogovi	11	22	2	18.2 (5.1-47.7)	11	50 (30.7-69.3)	0.0827	0	0.0	9	40.9 (23.3-61.3)	0.0143
Dundgovi	8	30	1	12.5 (2.2-47.1)	4	13.3 (5.3-29.7)	0.9532	2	25.0 (7.1-59.1)	3	10.0 (3.5-25.6)	0.2712
Govi-Altai	44	78	2	4.5 (1.3-15.1)	2	2.6 (0.7-8.9)	0.5734	2	4.5 (1.3-15.1)	3	3.8 (1.3-10.7)	0.8513
Govisumber	66	58	8	12.1 (6.3-22.1)	8	13.8 (7.2-24.9)	0.7789	17	25.8 (16.8-37.4)	11	19.0 (10.9-30.9)	0.3684
Khentii	73	104	12	16.4 (9.7-26.6)	33	31.7 (23.6-41.2)	0.0217	32	43.8 (33.1-55.2)	57	54.8 (45.2-64.0)	0.1508
Khovd	41	108	15	36.6 (23.6-51.9)	17	15.7 (10.1-23.8)	0.0057	12	29.3 (17.6-44.5)	31	28.7 (21.0-37.9)	0.9426
Khovsgol	19	3	13	68.4 (46.0-84.6)	2	66.7 (20.8-93.9)	0.6561	10	52.6 (31.7-72.7)	1	33.3 (6.2-79.2)	0.5438
Omnogovi	54	47	15	27.8 (17.6-40.9)	14	29.8 (18.7-44.0)	0.8255	3	5.6 (1.9-15.1)	5	10.6 (4.6-22.6)	0.3559
Ovorkhangai	68	30	2	2.9 (0.8-10.1)	4	13.3 (5.3-29.7)	0.0483	26	38.2 (27.6-50.1)	11	36.7 (21.9-54.5)	0.8883
Selenge	5	10	3	60.0 (23.1-88.2)	9	90.0 (59.6-98.2)	0.1859	3	60.0 (23.1-88.2)	6	60.0 (31.3-83.2)	1.0000
Sukhbaatar	37	65	2	5.4 (1.5-17.7)	9	13.8 (7.5-24.3)	0.1901	1	2.7 (0.5-13.8)	6	9.2 (4.3-18.7)	0.2135
Tov	24	45	1	4.2 (0.7-20.2)	2	4.4 (1.2-14.8)	0.9692	4	16.7 (6.7-35.9)	9	20.0 (10.9-33.8)	0.7404
Ulaanbaatar	7	6	2	28.6 (8.2-64.1)	0	0.0	0.1712	0	0.0	1	16.7 (3.0-56.4)	0.2796
Uvs	35	23	0	0.0	2	8.7 (2.4-26.8)	0.0783	4	11.4 (4.5-26.0)	3	13.0 (4.5-32.1)	0.8559
Zavkhan	60	50	0	0.0	1	2.0 (0.4-10.5)	0.2733	9	15.0 (8.1-26.1)	5	10.0 (4.3-21.4)	0.4354
Total	741	893	120	16.2 (13.7-19.0)	153	17.1 (14.8-19.7)	0.6273	144	19.4 (16.8-22.4)	187	20.9 (18.4-23.7)	0.4525

[a] 95% confidence interval