



Molecular survey of bovine Babesia species in Bactrian camels (*Camelus bactrianus*) in Mongolia

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journal or publication title	Ticks and Tick-borne Diseases
volume	13
number	1
page range	101871
year	2022-01
URL	http://id.nii.ac.jp/1588/00004760/

doi: info:doi/10.1016/j.ttbdis.2021.101871

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2 **Mongolia**

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26 **ABSTRACT**

27 Bovine babesiosis, which is caused by species of genus *Babesia*, is a leading cause of
28 considerable economic losses to the cattle industry each year. Bovine *Babesia* species have
29 frequently been detected in non-cattle hosts, such as water buffalo (*Bubalus bubalis*), from
30 which the parasites can be transmitted by ticks to cattle. Therefore, *Babesia* infections should
31 be minimized not only in cattle but also in non-cattle carriers. In the present study, we surveyed
32 the Bactrian camels (*Camelus bactrianus*) in Mongolia for three clinically significant bovine
33 *Babesia* species, including *Babesia bovis*, *B. bigemina*, and *Babesia* sp. Mymensingh, which
34 had been detected previously in Mongolian cattle. We screened blood DNA samples from 305
35 Bactrian camels in six Mongolian provinces for these species, using parasite-specific PCR
36 assays. Our findings showed that the Bactrian camels in Mongolia were infected with all three
37 *Babesia* species surveyed. The overall positive rates of *B. bovis*, *B. bigemina*, and *Babesia* sp.
38 Mymensingh were 32.1%, 21.6%, and 24.3%, respectively, whereas 52.5% of the surveyed
39 animals were infected with at least one parasite species. We also found that the female Bactrian
40 camels and the Mongolian native camel breeds had significantly higher *Babesia* positive rates
41 than the male Bactrian camels and the Hos Zogdort breed. In Mongolia, cattle and Bactrian
42 camels usually share common pasture lands for grazing; furthermore, tick species infesting
43 cattle also infest Bactrian camels. Our findings, together with these observations, suggest that
44 the tick transmission of bovine *Babesia* species might be possible between cattle and Bactrian
45 camels. Therefore, strategies for the control of bovine babesiosis in Mongolia should include
46 methods to minimize bovine *Babesia* species infections in Bactrian camels.

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48 **Keywords:** *Babesia bovis*, *Babesia bigemina*, *Babesia* sp. Mymensingh, Bactrian camel,
49 Mongolia, cattle

50

51 **1. Introduction**

52 Bovine babesiosis is a serious disease affecting cattle populations, especially in tropical
53 and subtropical regions of the world (Suarez et al., 2019). The disease is caused by species of
54 intra-erythrocytic protozoan parasites belonging to the genus *Babesia*; these parasites are
55 transmitted by ticks (Bock et al., 2004). The invasion, multiplication, and egress of *Babesia*
56 cause a massive hemolysis, leading to severe anemia in the infected cattle (Hunfeld et al., 2008).
57 Although several *Babesia* species infect cattle, only *Babesia bovis*, *B. bigemina*, *B. divergens*,
58 and *Babesia* sp. Mymensingh are known to cause clinical babesiosis (Bock et al., 2004;
59 Sivakumar et al., 2018; Zintl et al., 2003).

60 Bovine *Babesia* species infect not only cattle but also other animals (Elsify et al., 2015;
61 Jaimes-Dueñez et al., 2018; Sivakumar et al., 2013, 2014, 2020). These non-cattle hosts may
62 act as reservoirs from which the *Babesia* species can be transmitted to cattle via tick vectors
63 (Jaimes-Dueñez et al., 2018). Therefore, to ensure effective control of bovine babesiosis, it is
64 imperative to minimize *Babesia* infections not only in cattle but also in non-cattle hosts
65 (Romero-Salas et al. 2016). However, only a few non-cattle hosts, including water buffalo
66 (*Bubalus bubalis*), goats (*Capra aegagrus hircus*), sheep (*Ovis aries*), dromedary camels
67 (*Camelus dromedaries*), horses (*Equus caballus*), and white-tailed deer (*Odocoileus*
68 *virginianus*), have been studied to date (Cantu-C et al., 2009; Criado-Fornelio et al., 2009;
69 Elsify et al., 2015; Fereig et al., 2017; Jaimes-Dueñez et al., 2018; Sivakumar et al., 2013, 2014,
70 2020). Among them, only water buffalo are considered to play a significant role in the
71 epidemiology of bovine *Babesia* species (Jaimes-Dueñez et al., 2018). Conversely, the role of
72 other host animals remains unclear. For instance, *in vitro* studies have demonstrated that *B.*
73 *bovis* undergoes only limited asexual multiplication in caprine and ovine erythrocytes (Gaffar
74 et al., 2003).

75 Bactrian camels (*Camelus bactrianus*) are found mostly in the desert regions of the
76 countries in Central Asia, such as Mongolia, China, and Kazakhstan (Chuluunbat et al., 2014;
77 Imamura et al., 2017; Ji et al., 2009). Recent studies have reported *Theileria equi* and *T. sinensis*
78 infections in Bactrian camels (Li et al., 2019; Li et al., 2020). However, the infections with
79 bovine *Babesia* species have not been investigated in this animal species.

80 In Mongolia, Bactrian camels are maintained primarily by nomads in the Gobi Desert
81 region under harsh environmental conditions (Chuluunbat et al., 2014). They have long been
82 used for the production of milk, wool, and meat, as well as for the transportation of people and
83 goods. As of 2019, the estimated camel population was approximately 472,000 in Mongolia
84 (National Statistics Office of Mongolia, 2019). The four camel breeds in Mongolia include
85 Mongolian native camel (MNT), Hos Zogdort (HZ), Galbiin Gobiin Ulaan (GGU), and Haniin
86 Hetsiin Huren (HHH) breeds (Chuluunbat et al., 2014). These camels usually co-graze in
87 pasture lands with other livestock, including cattle, as all farm animals are maintained under
88 an extensive management system (Suttie, 2005).

89 Notably, all three tick species known to infest Bactrian camels, including *Dermacentor*
90 *marginatus*, *D. nuttalli*, and *Hyalomma asiaticum*, also infest cattle in Mongolia (Narankhajid
91 et al., 2018). Therefore, we hypothesized that the *Babesia* species infecting cattle may also
92 infect these camels. To test our hypothesis, we surveyed the Bactrian camels reared in various
93 Mongolian provinces for *B. bovis*, *B. bigemina*, and *Babesia* sp. Mymensingh, which had been
94 recently detected in Mongolian cattle (Otgonsuren et al., 2020).

95 **2. Materials and methods**

96 ***2.1. Blood sampling and DNA extraction***

97 In July 2017 and April 2018, we collected blood samples randomly from 305 Bactrian
98 camels reared in the following six Mongolian provinces: Bayan-Ulgii, Govi-Altai, Khovd, Uvs,
99 and Zavkhan in Western Mongolia and Bayankhongor in Southern Mongolia (Fig. 1, Table 1).

100 The sampled animals included 84 females and 221 males and three breeds (MNT, $n = 120$; HZ,
101 $n = 177$; and GGU, $n = 8$).

102 At the time of sampling, all animals were asymptomatic and apparently healthy.
103 Approximately 5 ml of blood was collected from the jugular vein of each animal and placed in
104 a sterile vacutainer tube containing EDTA. Subsequently, we prepared thin blood smears on
105 glass slides using the samples collected from 33, 24, and 12 animals in Khovd, Uvs, and
106 Zavkhan provinces, respectively. We also prepared genomic DNA from each blood sample
107 using a previously reported phenol:chloroform:isoamyl alcohol method (Sambrook and Russell,
108 2001). We then stored the DNA samples at -30°C . All animal protocols were approved by the
109 Animal Care and Use Committee of Obihiro University of Agriculture and Veterinary
110 Medicine, Japan (Approval no. 28-45).

111 **2.2. Microscopic examination for Babesia parasites**

112 We fixed the thin blood smears with absolute methanol, stained with Giemsa, and then
113 observed under a light microscope for detecting *Babesia* parasites within erythrocytes.

114 **2.2. PCR screening for *B. bovis*, *B. bigemina*, and *Babesia* sp. *Mymensingh***

115 We screened all the DNA samples from the Bactrian camels using previously described
116 PCR assays specific to *B. bovis*, *B. bigemina*, and *Babesia* sp. *Mymensingh*. A single-step PCR
117 assay using the inner forward and reverse primers of a previously described nested PCR assay
118 based on rhoptry-associated protein 1 gene (*rap-1*) was used to detect *B. bovis* (Figuroa et al.,
119 1993), while two single-step PCR assays based on apical membrane antigen 1 genes (*ama-1*)
120 were used to detect *B. bigemina* (Sivakumar et al., 2012) and *Babesia* sp. *Mymensingh*
121 (Sivakumar et al., 2018). We have described the primers, reaction mixtures, and cycling
122 conditions for the PCR assays in our previous report (Otgonsuren et al., 2020). We used DNA
123 samples extracted from *B. bovis* and *B. bigemina* *in vitro* cultures and from blood of a cow
124 naturally infected with *Babesia* sp. *Mymensingh* (Sivakumar et al., 2018) as positive controls

125 in the respective PCR assays. A DNA sample from a non-infected cow was used as a negative
126 control, while a no template control was used to monitor the cross-contamination.

127 We resolved the resultant PCR products by agarose gel electrophoresis, stained with
128 ethidium bromide, and then visualized under UV light. The samples were considered to be
129 positive for *B. bovis*, *B. bigemina*, and *Babesia* sp. Mymensingh if the band sizes were
130 approximately 298, 211, and 371 bp, respectively (Otgonsuren et al., 2020).

131 **2.3. Cloning and sequencing**

132 We cloned and sequenced the selected amplicons obtained from each PCR assay after
133 extracting these amplicons using a commercial kit (QIAquick Gel Extraction Kit, Qiagen,
134 Hilden, Germany) and ligating them to a PCR 2.1 plasmid vector (PCR 2.1-TOPO, Invitrogen,
135 Carlsbad, CA, USA). We then sequenced the inserted gene fragments using an ABI PRISM
136 3100 genetic analyzer (Applied Biosystems, Branchburg, NJ, USA). We analyzed the newly
137 generated sequences using a basic local alignment search tool
138 (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) to confirm the origins of the gene sequences and to
139 determine the identity scores shared with the corresponding sequences from GenBank.

140 **2.4. Statistical analyses**

141 We calculated the 95% confidence intervals (CIs) for the positive rates based on
142 Wilson's score interval (Wilson, 1927) using an OpenEpi online software
143 (<https://www.openepi.com/Proportion/Proportion.htm>). We calculated the *P* values for the
144 differences between the positive rates using an “*N*–1” chi-square test
145 (https://www.medcalc.org/calc/comparison_of_proportions.php) (Campbell, 2007;
146 Richardson, 2011). The differences were considered to be statistically significant if the *P* values
147 were <0.05.

148 **3. Results and discussion**

149 We designed the present study to investigate whether Bactrian camels in Mongolia were
150 infected with *Babesia* species, including *B. bovis*, *B. bigemina*, and *Babesia* sp. Mymensingh,
151 that are capable of causing clinical babesiosis in cattle. Bactrian camels are reared mainly in
152 Western and Southern Mongolia. However, except for Bayankhongor, cattle farming is not
153 very common in Southern Mongolia. Therefore, the present survey was conducted only in
154 Western Mongolia and Bayankhongor province (Fig. 1). The sampling was carried out during
155 the tick season in Mongolia (March to September), and therefore tick infestation was common
156 among the sampled camels (data not shown).

157 Our microscopic examination revealed that 22 (31.9%) of 69 examined camels,
158 including 10 of 33, 8 of 24, and 4 of 12 animals in Khovd, Uvs, and Zavkhan provinces,
159 respectively, were positive for *Babesia* parasites (Fig. 2). Although species differentiation was
160 not possible with microscopy, our PCR results demonstrated that the camels were infected with
161 all three *Babesia* species surveyed (Fig. 1, Table 1). All microscopy-positive animals were also
162 positive by PCR assays. Furthermore, the infections were particularly common in camels
163 considering the fact that 160 (52.5%) of the 305 Bactrian camels surveyed were infected with
164 at least one *Babesia* species. The overall positive rates were 32.1%, 21.6%, and 24.3% for *B.*
165 *bovis*, *B. bigemina*, and *Babesia* sp. Mymensingh, respectively (Table 1).

166 We cloned and sequenced nine, eight, and nine randomly selected amplicons from *B.*
167 *bovis*, *B. bigemina*, and *Babesia* sp. Mymensingh PCR assays, respectively. The newly
168 determined *B. bovis rap-1* sequences (LC598509–LC598517) shared 99.7–100% identities
169 with a sequence reported from Egypt (AB917246), while the *B. bigemina ama-1* sequences
170 (LC598518–LC598525) shared 99.5–100% identity scores with the sequences from Sri Lanka
171 (LC438499), Israel (KU557538), Turkey (KP000033), South Africa (KF626599), and Italy
172 (GQ257740). Similarly, the *ama-1* sequences generated from the amplicons of *Babesia* sp.
173 Mymensingh PCR assays (LC598526–LC598534) shared 99.2–100% identities with the

174 sequences from several countries, including Mongolia (LC506534), Vietnam (LC486036),
175 Argentina (LC486028), and Uganda (LC486021). These findings confirmed that the respective
176 PCR assays specifically detected the targeted *Babesia* species.

177 A previous study found that cattle in all provinces surveyed in our present investigation
178 were infected with bovine *Babesia* species (Otgonsuren et al., 2020). Similarly, Bactrian
179 camels from all surveyed provinces, except for the Bayan-Ulgii, were positive for at least one
180 *Babesia* species (Fig. 1, Table 1). The negative results obtained in the PCR assays do not
181 necessarily mean that camel population in Bayan-Ulgii is free from *Babesia* infections, as only
182 10 animals were surveyed. *Babesia bovis*, *B. bigemina*, and *Babesia* sp. Mymensingh were
183 identified in three (Uvs, Khovd, and Zavkhan), five (Uvs, Zavkhan, Khovd, Govi-Altai, and
184 Bayankhongor), and four provinces (Uvs, Khovd, Zavkhan, and Govi-Altai), respectively. The
185 positive rates of *B. bovis*, *B. bigemina*, and *Babesia* sp. Mymensingh in the provinces where
186 they were detected ranged 16.8–67.5%, 14.8–37.5%, and 9.1–53.3%, respectively (Table 1).
187 However, a fair comparison among the positive rates in different provinces was not possible
188 because of the low sample numbers in each area surveyed. Therefore, large-scale
189 epidemiological surveys of bovine *Babesia* species in Bactrian camels are required.

190 The *B. bovis* and *B. bigemina* positive rates in Bactrian camels (32.4% and 21.6%,
191 respectively) were comparable to those in cattle (27.9% and 23.6%, respectively) in Mongolia
192 (Otgonsuren et al., 2020). In addition, the positive rate of *Babesia* sp. Mymensingh (24.3%)
193 was higher than that in cattle (5.4%) (Otgonsuren et al., 2020). These observations indicate that
194 the Bactrian camels are an important host for the bovine *Babesia* species we surveyed. As
195 reported in previous study, the tick species infesting camels (*D. marginatus*, *D. nuttalli*, and *H.*
196 *asiaticum*) also infest cattle in Mongolia, and therefore, tick transmission of bovine *Babesia*
197 species might be possible between these two host animals. However, our assumption can be
198 confirmed only if additional experiments demonstrate that the tick species infesting camels are

199 capable of transmitting bovine *Babesia* species, as their vector competence is currently
200 unknown.

201 Co-infections with two or three *Babesia* species were common among the surveyed
202 camels. Of 160 animals infected with at least one *Babesia* species, 66 (41.3%) had co-infections
203 (Table 2). Further studies are now essential to investigate whether the co-infections were due
204 to shared tick vectors (Cutler et al., 2021).

205 As shown in a previous study, positive rates of hemoprotozoan parasites may increase
206 with rising age if the infections persist (Rüegg et al., 2007). In a recent PCR-based longitudinal
207 study conducted in Sri Lanka, most of the *B. bovis*- and *B. bigemina*-positive cattle turned
208 negative within 3 months, which suggests that the infection persistence of these parasite species
209 is less pronounced (Sivakumar et al., 2016). In agreement with this observation, our previous
210 study found that the positive rates of *Babesia* species were comparable between the different
211 age groups of cattle in Mongolia (Otgonsuren et al., 2020). Similarly, in the present study, the
212 positive rates of *B. bovis* and *B. bigemina* in Bactrian camels were comparable between the
213 1–4-year and >4-year age groups, but the *Babesia* sp. Mymensingh-positive rate was higher (P
214 = 0.0027) in the >4-year age group (28.0%) than that in the 1–4-year age group (9.7%) (Table
215 3). These findings suggest that in Bactrian camels, the persistence of *Babesia* sp. Mymensingh
216 infection might be pronounced as compared to that of *B. bovis* and *B. bigemina*. However, this
217 argument is inconclusive, as our findings might have been confounded by several factors, such
218 as parasitemia levels and period between the time of infection and sampling.

219 Comparison of the positive rates between the male and female animals revealed that
220 the rates for all three tested *Babesia* species were significantly higher ($P < 0.0001$) in females
221 than in males (Table 4). Furthermore, we also analyzed the differential positive rates among
222 the camel breeds (MNT, HZ, and GGU) (Table 5). A comparative analysis indicated that the
223 positive rates of *B. bovis*, *B. bigemina*, and *Babesia* sp. Mymensingh were significantly higher

224 in MNT than in HS ($P < 0.0001$, 0.0285 , < 0.0001 , respectively). We sampled only eight GGU
225 Bactrian camels and therefore did not consider this breed in our analysis. The observed
226 discrepancies could be associated with the differences in the tick burden. Therefore, further
227 studies investigating the tick burden and the predisposing factors of tick infestation, such as
228 coat and skin characteristics (Ibelli et al., 2012; Shyma et al., 2015) in males and females as
229 well as in MNT and HZ breeds, may elucidate the differences in the positive rates of the tested
230 *Babesia* species. *Babesia* infections in cattle are known to be influenced by grazing
231 management practices (Rubaire-Akiiki et al., 2004). In the present study, the comparison of
232 the positive rates based on management practices was not possible because all Bactrian camels
233 in Mongolia are managed extensively from birth.

234 The bovine *Babesia* species tend to have a minimum clinical significance in water
235 buffalos (Benitez et al. 2018). However, the clinical significance of these species is not clear
236 in Bactrian camels. Therefore, experimental infections are essential to determine the clinical
237 relevance of bovine *Babesia* species in Bactrian camel. Even if the detected parasites are of
238 clinical significance in Bactrian camels, clinical babesiosis may be a relatively uncommon
239 condition in Mongolia, considering that the high positive rates may indicate an endemically
240 stable situation (Bock et al., 2004; Mahoney and Ross, 1972). It is imperative to conduct a
241 serological survey of the bovine *Babesia* species in Mongolian Bactrian camels to confirm our
242 assumption related to the endemic stability.

243 **4. Conclusion**

244 In conclusion, Bactrian camels in Mongolia are commonly infected with at least three
245 *Babesia* species that are capable of causing clinical babesiosis in cattle. Our study is possibly
246 the first to report the existence of bovine *Babesia* species in Bactrian camels.

247 **Declarations of interest:** none

248

249 **Acknowledgments**

250 We thank the veterinarians and farm owners for their cooperation and assistance with
251 sampling. We also thank Ms. Hiroko Yamamoto (National Research Center for Protozoan
252 Diseases, Obihiro University of Agriculture and Veterinary Medicine, Japan) for her excellent
253 technical support. This study was supported by grants from the Japan Society for the Promotion
254 of Science (JSPS KAKENHI nos. 19KK0174 and 19K23704); by Grants-in-Aid for Graduate
255 Students from the World-leading Innovative and Smart Education (WISE) Program (1801)
256 from the Ministry of Education, Culture, Sports, Science, and Technology, Japan; and from the
257 Science and Technology Research Partnership for Sustainable Development (SATREPS)
258 project sponsored by the Japan Agency for Medical Research and Development, Japan
259 International Cooperation Agency (Grant no. 17jm0110006h0005).

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261 **References**

262

263 Benitez, D., Mesplet, M., Echaide, I., Torioni de Echaide, S., Schnittger, L., Florin-Christensen,
264 M., 2018. Mitigated clinical disease in water buffaloes experimentally infected with
265 *Babesia bovis*. Ticks Tick Borne Dis. 9, 1358–1363.
266 <https://doi.org/10.1016/j.ttbdis.2018.04.012>.

267 Bock, R., Jackson, L., de Vos, A., Jorgensen, W., 2004. Babesiosis of cattle. Parasitology 129
268 (Suppl), S247–S269. <https://doi.org/10.1017/s0031182004005190>.

269 Campbell, I., 2007. Chi-squared and Fisher-Irwin tests of two-by-two tables with small sample
270 recommendations. Stat. Med. 26, 3661–3675. <https://doi.org/10.1002/sim.2832>.

271 Cantu-C, A., Ortega-S, J.A., García-Vázquez, Z., Mosqueda, J., Henke, S.E., George, J.E.,
272 2009. Epizootiology of *Babesia bovis* and *Babesia bigemina* in free-ranging white-tailed
273 deer in northeastern Mexico. J. Parasitol. 95, 536–542. <https://doi.org/10.1645/GE-1648.1>.

274 Chuluunbat, B., Charruau, P., Silbermayr, K., Khorloojav, T., Burger, P.A., 2014. Genetic
275 diversity and population structure of Mongolian domestic Bactrian camels (*Camelus*
276 *bactrianus*). Anim. Genet. 45, 550–558. <https://doi.org/10.1111/age.12158>.

277 Criado-Fornelio, A., Buling, A., Asenzo, G., Benitez, D., Florin-Christensen, M., Gonzalez-
278 Oliva, A., Henriques, G., Silva, M., Alongi, A., Agnone, A., Torina, A., Madruga, C.R.,
279 2009. Development of fluorogenic probe-based PCR assays for the detection and
280 quantification of bovine piroplasmids. Vet. Parasitol. 162, 200–206.
281 <https://doi.org/10.1016/j.vetpar.2009.03.040>.

282 Cutler, S.J., Vayssier-Taussat, M., Estrada-Peña, A., Potkonjak, A., Mihalca, A.D., Zeller, H.,
283 2021. Tick-borne diseases and co-infection: Current considerations. Ticks Tick Borne Dis.
284 12, 101607. <https://doi.org/10.1016/j.ttbdis.2020.101607>.

285 Elsify, A., Sivakumar, T., Nayel, M., Salama, A., Elkhtam, A., Rizk, M., Mosaab, O., Sultan,
286 K., Elsayed, S., Igarashi, I., Yokoyama, N., 2015. An epidemiological survey of bovine
287 *Babesia* and *Theileria* parasites in cattle, buffaloes, and sheep in Egypt. *Parasitol. Int.* 64,
288 79–85. <https://doi.org/10.1016/j.parint.2014.10.002>.

289 Fereig, R.M., Mohamed, S.G.A., Mahmoud, H.Y.A.H., AbouLaila, M.R., Guswanto, A.,
290 Nguyen, T.T., Ahmed Mohamed, A.E., Inoue, N., Igarashi, I., Nishikawa, Y., 2017.
291 Seroprevalence of *Babesia bovis*, *B. bigemina*, *Trypanosoma evansi*, and *Anaplasma*
292 *marginale* antibodies in cattle in southern Egypt. *Ticks Tick Borne Dis.* 8, 125–131.
293 <https://doi.org/10.1016/j.ttbdis.2016.10.008>.

294 Figueroa, J.V., Chieves, L.P., Johnson, G.S., Buening, G.M., 1993. Multiplex polymerase
295 chain reaction based assay for the detection of *Babesia bigemina*, *Babesia bovis* and
296 *Anaplasma marginale* DNA in bovine blood. *Vet. Parasitol.* 50, 69–81.
297 [https://doi.org/10.1016/0304-4017\(93\)90008-b](https://doi.org/10.1016/0304-4017(93)90008-b).

298 Gaffar, F.R., Franssen, F.F., de Vries, E., 2003. *Babesia bovis* merozoites invade human, ovine,
299 equine, porcine and caprine erythrocytes by a sialic acid-dependent mechanism followed
300 by developmental arrest after a single round of cell fission. *Int. J. Parasitol.* 33, 1595–1603.
301 [https://doi.org/10.1016/s0020-7519\(03\)00254-6](https://doi.org/10.1016/s0020-7519(03)00254-6).

302 Hunfeld, K.P., Hildebrandt, A., Gray, J.S., 2008. Babesiosis: recent insights into an ancient
303 disease. *Int. J. Parasitol.* 38, 1219–1237. <https://doi.org/10.1016/j.ijpara.2008.03.001>.

304 Ibelli, A.M., Ribeiro, A.R., Giglioti, R., Regitano, L.C., Alencar, M.M., Chagas, A.C., Paço,
305 A.L., Oliveira, H.N., Duarte, J.M., Oliveira, M.C., 2012. Resistance of cattle of various
306 genetic groups to the tick *Rhipicephalus microplus* and the relationship with coat traits.
307 *Vet. Parasitol.* 186, 425–430. <https://doi.org/10.1016/j.vetpar.2011.11.019>.

308 Imamura, K., Salmurzauli, R., Iklasov, M.K., Baibayssov, A., Matsui, K., Nurtazin, S.T., 2017.
309 The distribution of the two domestic camel species in Kazakhstan caused by the demand

310 of industrial stockbreeding. *J. Arid. Land. Stud.* 26, 233–236.
311 https://doi.org/10.14976/jals.26.4_233

312 Jaimes-Dueñez, J., Triana-Chávez, O., Holguín-Rocha, A., Tobon-Castaño, A., Mejía-
313 Jaramillo, A.M., 2018. Molecular surveillance and phylogenetic traits of *Babesia*
314 *bigemina* and *Babesia bovis* in cattle (*Bos taurus*) and water buffaloes (*Bubalus bubalis*)
315 from Colombia. *Parasit Vectors* 11, 510. <https://doi.org/10.1186/s13071-018-3091-2>.

316 Ji, R., Cui, P., Ding, F., Geng, J., Gao, H., Zhang, H., Yu, J., Hu, S., Meng, H., 2009.
317 Monophyletic origin of domestic Bactrian camel (*Camelus bactrianus*) and its
318 evolutionary relationship with the extant wild camel (*Camelus bactrianus ferus*). *Anim.*
319 *Genet.* 40, 377–382. <https://doi.org/10.1111/j.1365-2052.2008.01848.x>.

320 Li, J., Jian, Y., Jia, L., Galon, E.M., Benedicto, B., Wang, G., Cai, Q., Liu, M., Li, Y., Ji, S.,
321 Tumwebaze, M.A., Ma, L., Xuan, X., 2020. Molecular characterization of tick-borne
322 bacteria and protozoans in yaks (*Bos grunniens*), Tibetan sheep (*Ovis aries*) and Bactrian
323 camels (*Camelus bactrianus*) in the Qinghai-Tibetan Plateau Area, China. *Ticks Tick*
324 *Borne Dis.* 11, 101466. <https://doi.org/10.1016/j.ttbdis.2020.101466>.

325 Li, Y., Li, X., Liu, J., Wang, J., Jia, D., Liu, A., He, Z., Guan, G., Liu, Z., Liu, G., Luo, J., Yin,
326 H., 2019. First report of *Theileria* Infection of Bactrian camels (*Camelus bactrianus*) in
327 Xinjiang, China. *Acta Parasitol.* 64, 923–926. [https://doi.org/10.2478/s11686-019-00086-](https://doi.org/10.2478/s11686-019-00086-0)
328 0.

329 Mahoney, D.F., Ross, D. R., 1972. Epizootiological factors in the control of bovine babesiosis.
330 *Aust. Vet. J.* 48, 292–298. <https://doi.org/10.1111/j.1751-0813.1972.tb05160.x>.

331 Narankhajid, M., Yeruult, C., Gurbadam, A., Battsetseg, J., Aberle, S.W., Bayartogtokh, B.,
332 Joachim, A., Duscher, G.G., 2018. Some aspects on tick species in Mongolia and their
333 potential role in the transmission of equine piroplasms, *Anaplasma phagocytophilum* and

334 *Borrelia burgdorferi* L. Parasitol. Res. 117, 3557–3566. <https://doi.org/10.1007/s00436->
335 018-6053-x.

336 National statistics office of Mongolia, Livestock, 2019. Available at:
337 http://www.1212.mn/stat.aspx?LIST_ID=976_L10_1, Accessed 28 December 2020.

338 Otgonsuren, D., Sivakumar, T., Amgalanbaatar, T., Enkhtaivan, B., Narantsatsral, S.,
339 Tuvshintulga, B., Zoljargal, M., Munkhgerel, D., Davkharbayar, B., Baatarjargal, P.,
340 Davaasuren, B., Myagmarsuren, P., Battsetseg, B., Battur, B., Yokoyama, N., 2020.
341 Molecular epidemiological survey of *Babesia bovis*, *Babesia bigemina*, and *Babesia* sp.
342 Mymensingh infections in Mongolian cattle. Parasitol. Int. 77, 102107.
343 <https://doi.org/10.1016/j.parint.2020.102107>.

344 Richardson, J.T., 2011. The analysis of 2×2 contingency tables—yet again. Stat. Med. 30,
345 890. <https://doi.org/10.1002/sim.4116>.

346 Romero-Salas, D., Mira, A., Mosqueda, J., García-Vázquez, Z., Hidalgo-Ruiz, M., Vela, N.A.,
347 de León, A.A., Florin-Christensen, M., Schnittger, L., 2016. Molecular and serological
348 detection of *Babesia bovis*- and *Babesia bigemina*-infection in bovines and water
349 buffaloes raised jointly in an endemic field. Vet. Parasitol. 217, 101–107.
350 <https://doi.org/10.1016/j.vetpar.2015.12.030>.

351 Rubaire-Akiiki, C., Okello-Onen, J., Nasinyama, G.W., Vaarst, M., Kabagambe, E.K., Mwayi,
352 W., Musunga, D., Wandukwa, W., 2004. The prevalence of serum antibodies to tick-borne
353 infections in Mbale District, Uganda: the effect of agro-ecological zone, grazing
354 management and age of cattle. J. Insect. Sci. 4, 8. <https://doi.org/10.1093/jis/4.1.8>.

355 Rüegg, S.R., Torgerson, P., Deplazes, P., Mathis, A., 2007. Age-dependent dynamics of
356 *Theileria equi* and *Babesia caballi* infections in southwest Mongolia based on IFAT
357 and/or PCR prevalence data from domestic horses and ticks. Parasitology 134, 939–947.
358 <https://doi.org/10.1645/18-189>.

359 Sambrook, J., Russell. D.W., 2001. Molecular Cloning: A Laboratory Manual Cold Spring
360 Harbor Laboratory Press, NY, USA.

361 Shyma, K.P., Gupta, J.P., Singh, V., 2015. Breeding strategies for tick resistance in tropical
362 cattle: a sustainable approach for tick control. J. Parasit. Dis. 39, 1–6.
363 <https://doi.org/10.1007/s12639-013-0294-5>.

364 Sivakumar, T., Altangerel, K., Battsetseg, B., Battur, B., Aboulaila, M., Munkhjargal, T.,
365 Yoshinari, T., Yokoyama, N., Igarashi, I., 2012. Genetic detection of *Babesia bigemina*
366 from Mongolian cattle using apical membrane antigen-1 gene-based PCR assay. Vet.
367 Parasitol. 187, 17–22. <https://doi.org/10.1016/j.vetpar.2012.01.008>.

368 Sivakumar, T., Lan, D.T., Long, P.T., Yoshinari, T., Tattiyapong, M., Guswanto, A., Okubo,
369 K., Igarashi, I., Inoue, N., Xuan, X., Yokoyama, N., 2013. PCR detection and genetic
370 diversity of bovine hemoprotozoan parasites in Vietnam. J. Vet. Med. Sci. 75, 1455–1462.
371 <https://doi.org/10.1292/jvms.13-0221>.

372 Sivakumar, T., Kothalawala, H., Weerasooriya, G., Silva, S.S.P., Puvanendiran, S.,
373 Munkhjargal, T., Igarashi, I., Yokoyama, N., 2016. A longitudinal study of *Babesia* and
374 *Theileria* infections in cattle in Sri Lanka. Vet. Parasitol. Reg. Stud. Reports 6, 20–27.
375 <https://doi.org/10.1016/j.vprsr.2016.11.009>.

376 Sivakumar, T., Tattiyapong, M., Fukushi, S., Hayashida, K., Kothalawala, H., Silva, S.S.,
377 Vimalakumar, S.C., Kanagaratnam, R., Meewewa, A.S., Suthaharan, K., Puvirajan, T., de
378 Silva, W.K., Igarashi, I., Yokoyama, N., 2014. Genetic characterization of *Babesia* and
379 *Theileria* parasites in water buffaloes in Sri Lanka. Vet. Parasitol. 200, 24–30.
380 <https://doi.org/10.1016/j.vetpar.2013.11.029>.

381 Sivakumar, T., Tuvshintulga, B., Kothalawala, H., Silva, S.S.P., Lan, D.T.B., Long, P.T.,
382 Ybañez, A.P., Ybañez, R.H.D., Benitez, D.F., Tayebwa, D.S., De Macedo, A.C.C.,
383 Schnittger, L., Yokoyama, N., 2020. Host range and geographical distribution of *Babesia*

384 sp. Mymensingh. *Transbound. Emerg. Dis.* 67, 2233–2239.
385 <https://doi.org/10.1111/tbed.13546>.

386 Sivakumar, T., Tuvshintulga, B., Zhyldyz, A., Kothalawala, H., Yapa, P.R., Kanagaratnam, R.,
387 Vimalakumar, S.C., Abeysekera, T.S., Weerasingha, A.S., Yamagishi, J., Igarashi, I.,
388 Silva, S.S.P., Yokoyama, N., 2018. Genetic analysis of *Babesia* isolates from cattle with
389 clinical babesiosis in Sri Lanka. *J. Clin. Microbiol.* 56, e00895–18.
390 <https://doi.org/10.1128/JCM.00895-18>.

391 Suarez, C.E., Alzan, H.F., Silva, M.G., Rathinasamy, V., Poole, W.A., Cooke, B.M., 2019.
392 Unravelling the cellular and molecular pathogenesis of bovine babesiosis: is the sky the
393 limit? *Int. J. Parasitol.* 49, 183–197. <https://doi.org/10.1016/j.ijpara.2018.11.002>.

394 Suttie, J.M., 2005. Grazing management in Mongolia, in: Suttie, J.M., Reynolds, S.G., Batello,
395 C. (Eds.), *Grasslands of the world*. Food and Agriculture Organization of the United
396 Nations, Rome, pp. 265–304.

397 Wilson, E.B., 1927. Probable inference, the law of succession, and statistical inference. *J. Am.*
398 *Stat. Assoc.* 22, 209–212. <https://doi.org/10.2307/2276774>.

399 Zintl, A., Mulcahy, G., Skerrett, H.E., Taylor, S.M., Gray, J.S., 2003. *Babesia divergens*, a
400 bovine blood parasite of veterinary and zoonotic importance. *Clin. Microbiol. Rev.* 16,
401 622–636. <https://doi.org/10.1128/cmr.16.4.622-636.2003>.

402

403 **Figure legends**

404 **Fig. 1.** Map showing the Mongolian provinces where the Bactrian camels were surveyed for
405 *Babesia bovis*, *B. bigemina*, and *Babesia* sp. Mymensingh. The map was created with ArcGIS
406 v10.1 software program (Environmental Systems Research Institute, Redlands, CA, USA).
407 Among the six provinces surveyed (marked with lines), five had animals infected with the
408 tested *Babesia* species.

409

410 **Fig. 2.** Micrographs of *Babesia* parasites detected in Bactrian camels. Giemsa-stained thin
411 blood smears prepared from Bactrian camels were observed under a light microscope for
412 detecting *Babesia* parasites (indicated by arrows) within erythrocytes.

Table 1. PCR detection of *B. bovis*, *B. bigemina*, and *Babesia* sp. Mymensingh in 305 Bactrian camels from Mongolia

Province	No.	<i>B. bovis</i>		<i>B. bigemina</i>		<i>Babesia</i> sp. Mymensingh	
		No. positive	% (CI ^a)	No. positive	% (CI)	No. positive	% (CI)
Bayankhongor	8	0	0.0 (0.0–32.4)	3	37.5 (13.7–69.4)	0	0.0 (0.0–32.4)
Bayan-Ulgii	10	0	0.0 (0.0–34.5)	0	0.0 (0.0–34.5)	0	0.0 (0.0–34.5)
Govi-Altai	22	0	0.0 (0.0–14.9)	7	31.8 (16.4–52.7)	2	9.1 (2.5–27.8)
Khovd	155	26	16.8 (11.7–23.4)	23	14.8 (10.1–21.3)	15	9.7 (6.0–15.4)
Uvs	30	18	60.0 (42.3–75.4)	5	16.7 (7.3–33.6)	16	53.3 (36.1–69.8)
Zavkhan	80	54	67.5 (56.6–76.8)	28	35.0 (25.5–45.9)	41	51.3 (40.5–61.9)
Total	305	98	32.1 (27.1–37.6)	66	21.6 (17.4–26.6)	74	24.3 (19.8–29.4)

^a95% confidence interval.

Table 2. Co-infection of *Babesia* species in Bactrian camels surveyed in Mongolia

Parasite species	No. positive (% ^a)
<i>B. bovis</i> + <i>B. bigemina</i> + <i>Babesia sp.</i> Mymensingh	12 (7.5)
<i>B. bovis</i> + <i>B. bigemina</i>	17 (10.6)
<i>B. bovis</i> + <i>Babesia sp.</i> Mymensingh	32 (20.0)
<i>B. bigemina</i> + <i>Babesia sp.</i> Mymensingh	5 (3.1)
Total	66 (41.3)

^aCo-infection rates are expressed as the percentage of the number of animals infected ($n = 160$) with at least one *Babesia* species.

Table 3. Positive rate of *Babesia* species in 1–4-year and > 4-year age groups

Parasites	1–4-year (<i>n</i> = 62)		>4-year (<i>n</i> = 243)		<i>P</i> value
	No. positive	% (CI ^a)	No. positive	% (CI)	
<i>B. bovis</i>	17	27.4 (17.9–39.6)	81	33.3 (27.7–39.5)	0.3752
<i>B. bigemina</i>	15	24.2 (15.3–36.2)	51	21 (16.3–26.5)	0.5856
<i>Babesia</i> sp. Mymensingh	6	9.7 (4.5–19.6)	68	28 (22.7–33.9)	0.0027

^a95% confidence interval.

Table 4. Positive rates of *Babesia* species in female and male Bactrian camels

Parasites	Female (<i>n</i> = 84)		Male (<i>n</i> = 221)		<i>P</i> value
	No. positive	% (CI ^a)	No. positive	% (CI)	
<i>B. bovis</i>	58	69.1 (58.5–77.9)	40	18.1 (13.6–23.7)	< 0.0001
<i>B. bigemina</i>	45	53.6 (43.0–63.9)	21	9.5 (6.3–14.1)	< 0.0001
<i>Babesia</i> sp. Mymensingh	48	57.1 (46.5–67.2)	26	11.8 (8.2–16.7)	< 0.0001

^a95% confidence interval.

Table 5. Positive rates of *Babesia* species in three Bactrian camel breeds in Mongolia

Parasites	MNT ^a (<i>n</i> = 120)		HZ (<i>n</i> = 177)		GGU (<i>n</i> = 8)	
	No. positive	% (CI ^b)	No. positive	% (CI)	No. positive	% (CI)
<i>B. bovis</i>	72	60.0 (51.1–68.3)	26	14.7 (10.2–20.7)	0	0.0 (0.0–32.4)
<i>B. bigemina</i>	33	27.5 (20.3–36.1)	30	16.9 (12.1–23.2)	3	37.5 (13.7–69.4)
<i>Babesia</i> sp. Mymensingh	57	47.5 (38.8–56.4)	17	9.6 (6.1–14.8)	0	0.0 (0.0–32.4)

^aThree Bactrian camel breeds, including Mongolian native camel (MNT), Hos Zogdort (HZ), and Galbiin Gobiin Ulaan (GGU), were sampled.

^b95% confidence interval.

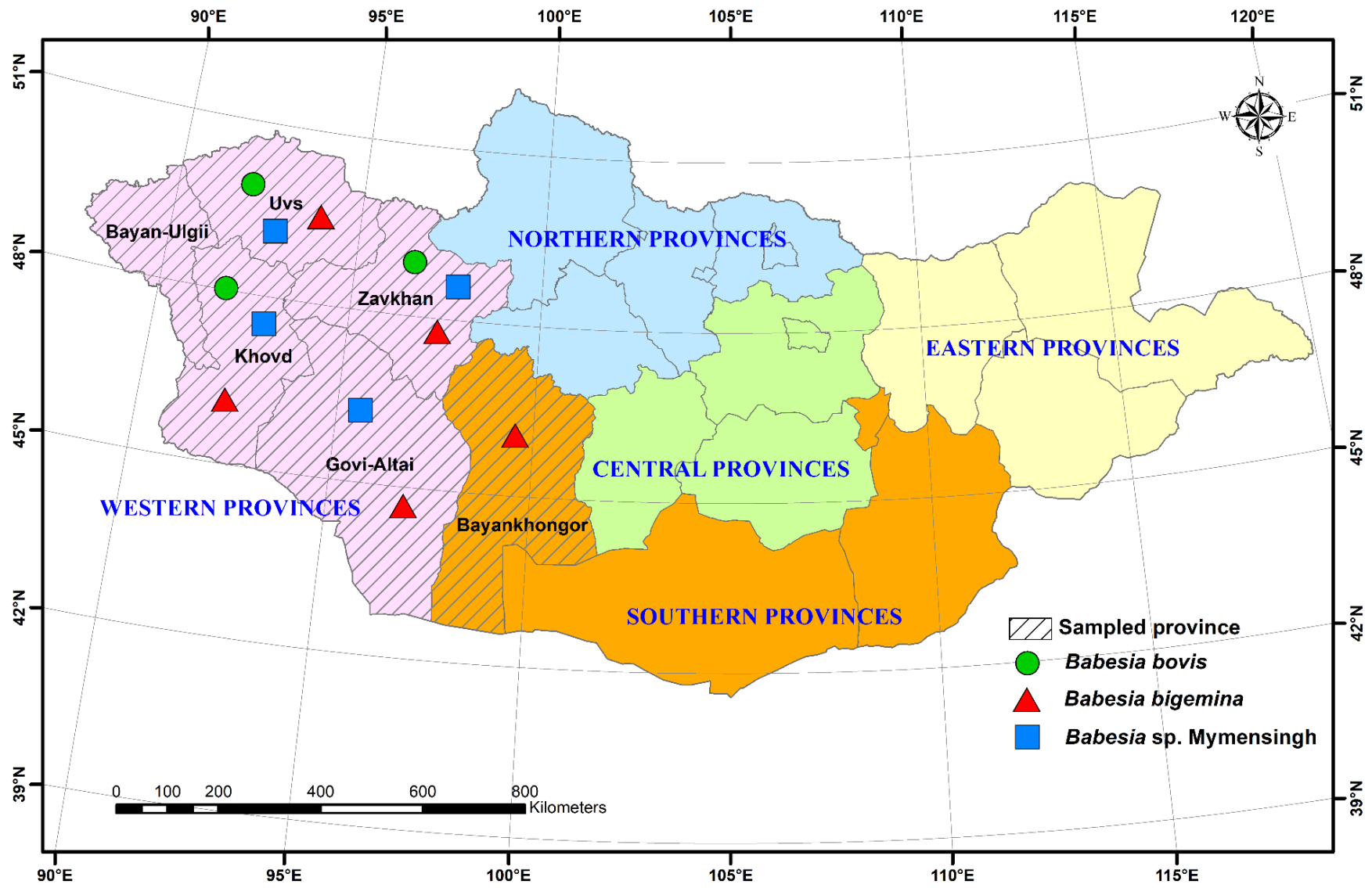


Fig. 1

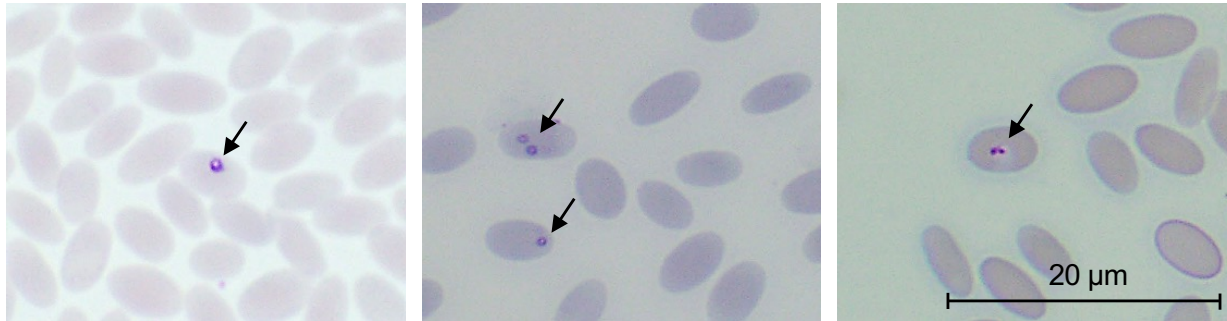


Fig. 2