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Epidemiological study of equine piroplasmosis in Mongolia

Damdinsuren Boldbaatar[a], Xuenan Xuan[a], Badgar Battsetseg[a], Ikuo Igarashi[a], Banzragch Battur[b], Zayat Batsukh[b], Badarch Bayambaa[b], Kozo Fujisaki[a,*]

[a]*National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Inado-cho, Obihiro, Hokkaido 080-8555, Japan*
[b]*Department of Veterinary Ectoparasitology and Protozoology, Institute of Veterinary Medicine, Zaisan 210153, Ulaanbaatar, Mongolia*

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Abstract

The purpose of this study was to demonstrate the occurrence of equine piroplasmosis in Mongolia, a country in which the disease occurs epidemically in different climatic conditions. Antibodies to *Babesia equi* and *B. caballi* were determined in serum samples of 254 pastured horses in different locations of Mongolia using an enzyme-linked immunosorbent assay with recombinant antigens. One hundred and eighty-five (72.8%) and 102 (40.1%) of all serum samples were positive for *B. equi* and *B. caballi* infections, respectively. In addition, 78 (30.7%) samples were positive for both *B. equi* and *B. caballi* infections. These results indicate that equine piroplasmosis is widespread in Mongolia. To our knowledge, this is the first report describing an epidemiological study on equine piroplasmosis in different geographic regions in Mongolia.

Keywords: *Babesia equi*; *Babesia caballi*; ELISA; EMA-1; P48

Equine piroplasmosis, caused by *Babesia equi* and *B. caballi*, is considered to be the most important tick-borne disease of horses in tropical and subtropical areas (Schein, 1988). Babesiosis is generally characterized by fever, anemia, jaundice, and edema. In some cases, it causes the death (Freidhoff, 1982; Bruning et al., 1997; Schein, 1988; De Waal, 1992). Mongolia, a country located in a landlocked plateau of Central Asia, covering an area of 1,566,500 km^2, has a human population of 2.5 million and a horse population of 2.2 million and is known for its pasture animal husbandry, which raises thousands of livestock species, including horses. The country is mountainous, with an average altitude of 1580 m a.s.l. The geography of the country is characterized by great diversity, such as a mountain-forest steppe, a mountain steppe, a semi-desert, and a desert. The climate is continental, with long cold, dry winters and short warm summers. The average temperature ranges from 20 to 25 °C in summer (July) and −20 to −32 °C in winter (January). In Mongolia, the provinces of Tuv, Sukhbaatar, Selenge, Khovd, Uvs, and Umnugobi are

* Corresponding author. Tel.: +81 155 49 5646; fax: +81 155 49 5643.
E-mail address: fujisaki@obihiro.ac.jp (K. Fujisaki).

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situated in different climatic zones. Using the conventional thin blood smear examination, indirect fluorescence antibody test (IFAT), enzyme-linked immunosorbent assay (ELISA), and polymerase chain reaction (PCR) babesiosis has been documented as widespread in horses of Central Mongolia (Dash, 1966; Avarzed et al., 1997; Xuan et al., 1998; Ikadai et al., 2000). Tick infestation with *Dermacentor nuttalli*, *Dermacentor silvarum*, and *Hyalomma dromedari* is common in Mongolian horses (Dash, 1966; Byambaa et al., 1994). Amplification of specific equine *Babesia* gene fragments in field-collected blood samples, *D. nuttalli* adult ticks (i.e., unfed), and *D. nuttalli* ticks (i.e., partially engorged) on horses in Mongolia has been reported. These reports suggest the role of tick vector in *Babesia* transmission. Furthermore, the detection of parasite DNA in eggs and larvae is suggestive of transovarial parasite transmission in this species (Battsetseg et al., 2001, 2002). In endemic countries, the control of equine piroplasmosis is important to keep international markets open to the horse industry (Freidhoff, 1988). *Babesia* seroprevalence in horses is a good indicator of tick distribution (Tenter and Friedhoff, 1986). In the present study, we performed a preliminary epidemiological study on equine piroplasmosis in different environmental areas of Mongolia using recombinant ELISA antigens.

ELISA with recombinant *B. equi* merozoite antigen-1 (EMA-1) expressed by baculovirus in insect cells for the diagnosis of *B. equi* infection in horses was performed as described elsewhere (Xuan et al., 2001a, 2001b). EMA-1 was purified from a recombinant baculovirus AcEMA-1-infected Sf9 cell culture and used as an ELISA antigen for detecting antibodies to *B. equi* in horses. The ELISA using recombinant P48 protein expressed in *Escherichia coli* by the pGEX vector for the diagnosis of *B. caballi* infection in horses was carried out as described elsewhere (Ikadai et al., 1999). To evaluate whether the recombinant EMA-1 expressed by baculovirus and recombinant P48 expressed by *E. coli* can be suitable antigens for use in the diagnosis of *B. equi* and *B. caballi* infections in horses, the related antibodies were tested in an ELISA. Serum samples from horses, experimentally infected with *B. equi* and *B. caballi,* and healthy horses in Japan were used as positive and negative controls, respectively. The serum samples from horses experimentally infected with *B. equi* reacted positively to recombinant EMA-1 antigen (optical densities >0.1), while serum samples from 10 normal horses and 10 horses experimentally infected with *B. caballi* were negative (optical densities >0.1). The serum samples from horses experimentally infected with *B. caballi* reacted positive to recombinant P48 antigen (optical densities >0.1), while serum samples from 10 normal horses and 10 horses experimentally infected with *B. equi* were negative (optical densities >0.1). The ELISA titer was expressed as the reciprocal of the maximum dilution that showed an ELISA value equal to or greater than 0.1, which is the difference in absorbance between that for the recombinant EMA-1 antigen or for the control lacZ antigen well and recombinant P48 antigen or for the control GST antigen well, respectively.

A total of 254 serum samples were collected from pastured horses in the Tuv, Sukhbaatar, Selenge, Khovd, Uvs, and Umnugobi provinces in Mongolia (Table 1). Although no parasites were detected in the Giemsa-stained blood smears from any of 254 horses, 185 (72.8%) and 102 (40.1%) were ELISA positive for *B. equi* and *B. caballi* infections, respectively (Table 1). Seventy-eight (30.7%) samples were positive and 45 (17.7) samples were negative for both *B. equi* and *B. caballi* infections (Table 2). The ages of the positive horses varied from months to 20 years. *B. equi* and

Table 1
Prevalence of equine piroplasmosis in Mongolia[a]

Province	No. of examined	No. of positive (%)	
		B. equi[b]	*B. caballi*[c]
Tuv	46	36 (78.2)	29 (63.0)
Selenge	39	22 (56.4)	7 (17.9)
Sukhbaatar	47	39 (82.9)	19 (40.4)
Khovd	48	19 (39.5)	10 (20.8)
Uvs	23	20 (86.9)	6 (26.1)
Umnugobi	51	49 (96.0)	31 (60.7)
Total	254	185 (72.8)	102 (40.1)

[a] Values in parentheses are in percentage.

[b] Antibodies to *B. equi* were detected by ELISA using the recombinant EMA-1 expressed in insect cells. The ELISA was considered positive when an optical density at 415 nm equal to or greater than 0.1 was observed at dilutions of 1:100 and above.

[c] Antibodies to *B. caballi* were detected by ELISA using the recombinant P48 expressed in *E. coli*. The ELISA was considered positive when an optical density at 415 nm equal to or greater than 0.1 was observed at dilutions of 1:100 and above.