■ **BRIEF COMMUNICATION**

Molecular Detection and Seroprevalence of *Babesia microti* among Stock Farmers in Khutul City, Selenge Province, Mongolia

Sung-Hee Hong[1], Davaasuren Anu[2], Young-Il Jeong[1], Davaajav Abmed[2], Shin-Hyeong Cho[1], Won-Ja Lee[1], Sang-Eun Lee[1,*]

[1]*Division of Malaria &Parasitic Diseases, Korea National Institute of Health, Korea Centers for Disease Control and Prevention, Osong 363-951, Korea;* [2]*Laboratory of Parasitology, National Center for Communicable Diseases, Ministry of Health, Ulaanbaator-210648, Mongolia*

Abstract: Babesiosis is an emerging tick-borne disease in humans worldwide; however, little is known about the frequency of infection or prevalence of this disease in other parts of the world, excluding North America. In this study, we aimed to investigate *Babesia microti* infection frequency in a human population in Mongolia. One hundred blood samples were collected from stock farmers living in Khutul city of Selenge province, Mongolia. The sera and DNA from blood samples were evaluated for the presence of *B. microti* infection by using indirect fluorescent antibody (IFA) tests and PCR. The positive detection rates obtained using the IFA tests and PCR assays were 7% and 3%, respectively. This study is the first to detect of *B. microti* infections based on antibody seroprevalence or PCR assays for the presence of *B. microti* DNA in a Mongolian population.

Key words: *Babesia microti*, IFA test, PCR, Mongolia

Babesiosis, caused by an intraerythrocytic protozoan parasite, is a common disease in wildlife worldwide and is gaining attention as an emerging tick-borne zoonosis in humans [1,2]. Although approximately 100 *Babesia* species infect animals, only a few have been reported to infect humans [3,4]. Most cases of human babesiosis have been caused by *Babesia divergens*, *Babesia microti*, or *Babesia duncani*, with the cattle-infecting species *B. divergens* being the most common in Europe, and the rodent-infecting species *B. microti* and *B. duncani* being the most common in North America. Together, these species have caused a significant number of human infections [1,5]. Several cases of *B. duncani* and *B. duncani*-type organisms have been identified in patients on the Pacific coast of the United States from northern California to Washington state [6,7]. *B. microti* is a parasite in wild rodents, and most cases of babesiosis in humans caused by *B. microti* are transmitted from wild rodents through *Ixodes* tick bites or inadvertently during blood transfusions [8,9]. In Asia, *B. microti*-like parasites have caused illnesses in Japan and Taiwan [10,11], and a novel Babesia sp. (KO1) has been identified in South Korea [12].

Mongolia, situated in central Asia between Russia to the north and China to the south, is an agricultural country focused on the livestock industry. Animal husbandry of cattle, sheep, goats, and horses brings many people into contact with ticks while herding the livestock pasturing on steppes. Previous reports have confirmed the presence of *Babesia* gene fragments from parasitic species in field-collected *Dermacentor nuttalli* ticks in Mongolia [13], and babesiosis in Mongolian horses is widespread [14]. Also, *B. microti* DNA was reportedly detected in small mammals in Russia [16] and in ticks of Inner Mongolia [17]. Furthermore, the highest incidence of human Lyme borreliosis transmitted by tick has been reported in Selenge province [15].

Similarly, previous reports of *B. microti* infection in Mongolia are limited to ticks and animals, and minimal information exists regarding *B. microti* infection among the Mongolian population involved in the livestock industry, even though they are highly exposed in the *B. microti* infection. The objective of this study was to serologically and molecularly detect *B. microti* infection in small stock farmers in Khutul city of Se-

lenge province in northern Mongolia, which is adjacent to neighboring regions, Russia.

One hundred blood samples were collected in April 2011 from residential stock farmers living in Khutul, near the Russian border (Fig. 1). Genomic DNA was extracted from the blood samples using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions and was stored in 100 µl of Tris-EDTA at 20˚C. The blood samples were separated by centrifugation at 2,000 g for 10 min, and the collected sera were stored at 20˚C until use.

The DNA samples were used as templates for a nested-PCR to amplify the *B. microti* 18S ribosomal ribonucleic acid (18S rRNA) gene [18]. The PCR primers included the 1st forward (5′-GCCAGTAGTCATATGCTTGTGTTA-3′) and reverse (5′-CTCCTTCCTY TAAGTGATAAGGTTCAC-3′) for the initial reactions and 2nd forward (5′-CCATGCAT GTCTWAGTAY-AARCTTTTA-3′) and reverse (5′-CCTYTAAGTGATAAGGTTCA-CAA AACTT-3′) for the subsequent reactions. Amplifications were performed in 20-µl reactions by using AccuPower PCR master mix (Bioneer, Daejeon, Korea) containing 1 µM each of the 1st forward and reverse primers, sterile water, and 1 µg of DNA sample. The second PCR was then performed using 2 µl of the 1st PCR product and the 2nd PCR primers. The thermal cycler conditions were 94˚C for 5 min; 35 cycles of 94˚C for 60 sec, 59˚C for 45 sec, and 72˚C for 45 sec; and a final extension at 72˚C for 10 min. Amplification products were resolved by
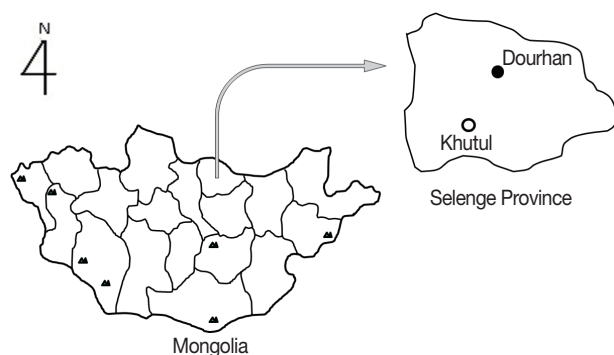

Fig. 1. The survey region for *Babesia microti* infections among stock farmers living in Khutul City in Selenge Province, northern Mongolia.

electrophoresis on 1.5% agarose gels in Tris-acetate-EDTA buffer and were visualized using Safe-Pinky DNA gel staining solution (GenDepot, Houston, Texas, USA). The positive PCR products were then purified using an agarose gel DNA purification kit (Qiagen) and sequenced using an ABI PRISM 3730xl Analyzer (Applied Biosystems, Foster City, California, USA).

IgG antibodies to *B. microti* were detected in the serum samples by using an indirect fluorescent antibody (IFA) test kit (Focus Technologies, Inc., Cypress, California, USA). A cut-off value of 1:64 was used in accordance with the manufacturer's recommendations. The serum samples were diluted 64-fold in PBS, and 25 µl of each was placed on slides coated with fixed *B. microti* antigen derived from RBCs from *B. microti*-infected hamsters. Proper positive and negative controls in IFA test kit were performed in all testing. The slides were incubated in a humid environment at 37˚C for 30 min and washed 5 times with PBS. Diluted fluorescent-labeled anti-human IgG conjugate was placed on the slides before they were incubated in a humid environment at 37˚C for 30 min. The slides were washed 5 times with PBS and air-dried prior to examination by fluorescence microscopy at × 400 magnification. Positive samples were consecutively diluted 2-fold to establish the endpoint titer. The IFA test detected antibodies to *B. microti* in 7% of the samples, with reported titers ranging between 64 and 1,024. The PCR assay detected *B. microti* 18S rRNA in 3% of the samples. Three of the samples tested positive in both assays, while 4 were seropositive in the IFA test but negative in the PCR assay (Table 1). The PCR-positive samples had IFA titers of both 512 and 1,024. Sequencing results showed that all PCR-positive samples were similar to *B. microti*. One of the 2 sequences obtained from positive samples were similar to a *Clethrionomys* sp. isolated from Russia (AY144693) and the other was similar to a human isolate from China (KF4110827). Five of the 7 *B. microti*-positive samples were from females, and 2 additional positive results were obtained for subjects of unknown sex (Table 2). No statistical differences were observed between the positive and negative results with regard to age and history of tick bites.

Some parasitic *Babesia* isolates that are extensively distribut-

Table 1. Indirect fluorescent antibody (IFA) and PCR assay results for *Babesia microti* infections among residents of Khutul city in Selenge province, Mongolia

Number tested	Total no. positive in IFA		64	128	Titer 256	512	>1,024	Total no. positive in PCR	No. positive in both IFA and PCR
100	≥ 64		64	128	256	512	>1,024	3 (3%)	3 (3%)
	7 (7%)		3	1	0	2	1		

Table 2. Positive rate of *B. microti* in IFA and PCR according to age, gender, and tick bite exposure

Variables	No.	Positive (%) for *B. microti* in IFA	Positive (%) for *B. microti* in PCR
Age (years)			
<10	2	-	-
11–20	16	2 (12.5)	1 (6.3)
21–30	16	-	-
31–40	15	-	-
41–50	17	1 (5.9)	1 (5.9)
60>	16	1 (6.3)	-
Unknown	18	3 (16.7)	1 (5.6)
Sex			
Male	42	-	-
Female	47	5 (10.6)	2 (4.3)
Unknown	11	2 (18.2)	1 (9.1)
Tick bite exposure			
Yes	59	3 (5.1)	2 (3.4)
No	41	4 (9.8)	1 (2.4)
Total	100	7 (7)	3 (3)

ed in Mongolia are considered pathogenic only in animals, such as *B. equi* (EMA-1) from pasture ticks and *B. caballi* (BC48) [14,19] and *B. bigemina* from Mongolian cattle [20]. Different parasitic *Babesia* spp. can cause babesiosis in humans, resulting in distinct geographical distributions determined by the presence of the intermediate hosts. In Eurasia, babesiosis caused by *B. divergens* is rare, but it is more lethal to human and bovine hosts [1,21] than North American hosts, infected predominantly by *B. microti* [22].

Our study is the first to detect *B. microti* in human subjects in Mongolia by utilizing seroprevalence and molecular assays, and our results may provide insights into the diagnosis and control of this disease in Mongolia. A broader and more detailed survey for other human infections of zoonotic *Babesia* spp., such as *B. divergens* and *B. duncani* is warranted in other regions of Mongolia. Traditionally, the diagnosis of babesiosis in humans has been based on detection of parasites in blood smears from patients with clinical symptoms of the disease. Because the clinical symptoms of babesiosis is usually mild in humans and the infection rate of RBCs is less than 1% [23,24], it is difficult to diagnose babesiosis by microscopic examination. The absence or presence of a laboratory manifestation depends on the level of parasitemia in the host patient.

Although *B. microti* is widely distributed throughout the world, symptomatic infections in humans are seldom reported [25]. In the present study, none of the individuals who tested positive exhibited the associated clinical symptoms of fever or anemia. Another difficulty with diagnosing *Babesia*-specific antibodies in human sera has been the lack of commercially available standardized tests for use in diagnostic laboratories [26]. However, the IFA tests now available have been shown to be sensitive, specific, and reproducible methods for detecting *B. microti*-specific antibodies in human sera [27]. In this study, we evaluated a commercially available IFA test to detect antibodies to *B. microti* in the sera of individuals. In addition, we screened specifically for the *B. microti* 18S rRNA gene by analyzing blood samples by using a PCR assay. The results of the IFA tests and PCR assays were similar, suggesting that both methods could be utilized for diagnosing *B. microti* infections in humans. Poyraz et al. [28] previously reported that no statistical differences exist in the seropositivity between sexes or age groups in Turkey, the same results found in our study. Another study found that the prevalence of antibodies to *B. microti* or *B. divergens* was considerably higher in tick-exposed individuals than that found in a control group of healthy blood donors [27].

Interestingly, our study found no significant differences in the prevalence of *B. microti* infections between individuals who had been bitten by ticks and those who had not. This suggests the need for an investigation into what vector species carrying *B. microti*, including ticks, are present in Khutul, Mongolia. This study is the first to report *B. microti* infections among stock farmers in Khutul city of Selenge Province, Mongolia, based on antibody seroprevalence and a PCR assay for the presence of *B. microti* 18S rRNA in the blood of subjects. Although no correlation was found between positive tests for infection and tick bite exposure, the risk of infection from tick-borne diseases may still exist in this region. The IFA test and PCR assay proved to be useful tools for screening of *B. microti* infections, and further serological and molecular epidemiological surveys should be performed with larger sample sizes throughout Mongolia. These studies should be conducted in conjunction with an investigation of the prevalence of ticks and rodents in order to determine which species is the primary *B. microti* infection vector in Mongolia.

ACKNOWLEDGMENTS

CONFLICT OF INTEREST

We have no conflict of interest related to this study.

REFERENCES

1. Homer MJ, Aguilar-Delfin I, Telford SR 3rd, Krause PJ, Persing DH. Babesiosis. Clin Microbiol Rev 2000; 13: 451-469.

2. Herwaldt BL, Caccio S, Gherlinzoni F, Aspock H, Slemenda SB, Piccaluga P, Martinelli G, Edelhofer R, Hollenstein U, Poletti G, Pampiglione S, Loschenberger K, Tura S, Pieniazek NJ. Molecular characterization of a non-*Babesia divergens* organism causing zoonotic babesiosis in Europe. Emerg Infect Dis 2003; 9: 942-948.

3. Spielman A. Human babesiosis on Nantucket Island: transmission by nymphal *Ixodes* ticks. Am J Trop Med Hyg 1976; 25: 784-787.

4. Vannier E, Gewurz BE, Krause PJ. Human babesiosis. Infect Dis Clin North Am 2008; 22: 469-488.

5. Hunfeld KP, Brade V. Zoonotic *Babesia*: possibly emerging pathogens to be considered for tick-infested humans in Central Europe. Int J Med Microbiol 2004; 293 (supp. 37): 93-103.

6. Persing DH, Herwaldt BL, Glaser C, Lane RS, Thomford JW, Mathiesen D, Krause PJ, Phillip DF, Conrad PA. Infection with a *Babesia*-like organism in northern California. N Engl J Med 1995; 332: 298-303.

7. Conrad PA, Jemtrup AM, Carreno RA, Thomford J, Wainwright K, Eberhard M, Quick R, Telford SR3rd, Herwaldt BL. Description of *Babesia duncani* n.sp. (Apicomplexa: Babesiidae) from humans and its differentiation from other piroplasms. Int J Parasitol 2006; 36: 779-789.

8. Leiby DA. Babesiosis and blood transfusion: flying under the radar. Vox Sang 2006; 90: 157-165.

9. Popovsky MA. Transfusion-transmitted babesiosis. Transfusion 1991; 31: 296-298.

10. Shih CM, Liu LP, Chung WC, Ong SJ, Wang CC. Human babesiosis in Taiwan: asymptomatic infection with a *Babesia microti*-like organism in a Taiwanese woman. J Clin Microbiol 1997; 35: 450-454.

11. Saito-Ito A, Tsuji M, Wei Q, He S, Matsui T, Kohsaki M, Arai S, Kamiyama T, Hioki K, Ishihara C. Transfusion-acquired, autochthonous human babesiosis in Japan: isolation of *Babesia microti*-like parasites with hu-RBC-SCID mice. J Clin Microbiol 2000; 38: 4511-4516.

12. Kim JY, Cho SH, Joo HN, Tsuji M, Cho SR, Park IJ, Chung GT, Ju JW, Cheun HI, Lee HW, Lee YH, Kim TS. First case of human babesiosis in Korea: detection and characterization of a novel type of *Babesia* sp. (KO1) similar to ovine *Babesia*. J Clin Microbiol 2007; 45: 2084-2087.

13. Battsetseg B, Xuan X, Ikadai H, Bautista JL, Byambaa B, Boldbaatar D, Battur B, Battsetseg G, Batsukh Z, Igarashi I, Nagasawa H, Mikami T, Fujisaki K. Detection of *Babesia caballi* and *Babesia equi* in *Dermacentor nuttalli* adult ticks. Int J Parasitol 2001; 31:

384-386.

14. Battsetseg B, Lucero S, Xuan X, Claveria F, Byambaa B, Battur B, Boldbaatar D, Batsukh Z, Khaliunaa T, Battsetseg G, Igarashi I, Nagasawa H, Fujisaki K. Detection of equine *Babesia* spp. gene fragments in *Dermacentor nuttalli* Olenev 1929 infesting mongolian horses, and their amplification in egg and larval progenies. J Vet Med Sci 2002; 64: 727-730.

15. Holger C, Scholz. Margos G, Derschum H, Speck S, Tserennorov D, Erdenebat N, Undraa M, Enkhtuja B, Battsetseg J, Otgonchimeg C, Otgonsuren G , Nymadulam B, Romer A, Thomas A, Essbauer S, Wolfel R, Kiefer D, Zoller L, Otgonbaatar D, Fingerle V. High prevalence of genetically diverse *Borrelia bavariensis*-like strains in *Ixodes persulcatus* from Selenge Aimag, Mongolia. Ticks Tick Borne Dis 2013; 4: 89-92.

16. Rar VA, Epikhina TI, Livanova NN, Pukhovskaia NM, Vysochina NP, Ivanov LI. Detection of *Babesia* spp. DNA in small mammals and ixodic ticks on the territory of north Ural, west Siberia and far east of Russia. Mol Gen Microbiol Virusol 2010; 3:26-30.

17. Hao GF, Li H, Sun Y, Ge RP, Qia GQ, Li B, Tian WZ, Shi NX, Yang XY. Detection of tick and tick-borne pathogen in some ports of Inner Mongolia. Zhonghua Liu Xing Bing Xue Za Zhi 2009; 30: 365-367.

18. Zamoto A, Tsuji M, Kawabuchi T, Wei Q, Asakawa M, Ishihara C. U.S.-type *Babesia microti* isolated from small wild mammals in Eastern Hokkaido, Japan. J Vet Med Sci 2004; 66: 919-926.

19. Avarzed A, De Waal DT, Igarashi I, Saito A, Oyamada T, Toyoda Y, Suzuki N. Prevalence of equine piroplasmosis in Central Mongolia. Onderstepoort J Vet Res 1997; 64: 141-145.

20. Sivakumar T, Altangerel K, Battsetseg B, Battur B, Aboulaila M, Munkhjargal T, Yoshinari T, Yokoyama N, Igarashi I. Genetic detection of *Babesia bigemina* from Mongolian cattle using apical membrane antigen-1 gene-based PCR assay. Vet Parasitol 2012; 187: 17-22.

21. Malandrin L, L Hostis M, Chauvin A. Isolation of *Babesia divergens* from carrier cattle blood using in vitro culture. Vet Res 2004; 35: 131-139.

22. Krause PJ, Telford SR 3rd, Spielman A, Sikand V, Ryan R, Christianson D, Burke G, Brassard P, Pollack R, Peck J, Persing DH. Concurrent Lyme disease and babesiosis. Evidence for increased severity and duration of illness. JAMA 1996; 275: 1657-1660.

23. Vannier E, Krause PJ. Human babesiosis. N Engl J Med 2012; 366: 2397-2407.

24. Welc-Faleciak R, Bajer A, Bednarska M, Paziewska A, Sinski E. Long term monitoring of *Babesia microti* infection in BALB/c mice using nested PCR. Ann Agric Environ Med 2007; 14: 287-290.

25. Sun Y, Liu G, Yang L, Xu R, Cao W. *Babesia microti*-like rodent parasites isolated from *Ixodes persulcatus* (Acari: Ixodidae) in Heilongjiang Province, China. Vet Parasitol 2008; 156: 333-339.

26. Hunfeld KP, Lambert A, Kampen H, Albert S, Epe C, Brade V, Tenter AM. Seroprevalence of *Babesia* infections in humans exposed to ticks in midwestern Germany. J Clin Microbiol 2002; 40: 2431-2436.

27. Krause PJ, Telford SR 3rd, Ryan R, Conrad PA, Wilson M, Thomford JW, Spielman A. Diagnosis of babesiosis: evaluation of a serologic test for the detection of *Babesia microti* antibody. J Infect Dis 1994; 169: 923-926.

28. Poyraz O, Gunes T. Seroprevalance of *Babesia microti* in humans living in rural areas of the Sinop region. Turkiye Parazitol Derg 2010; 34: 81-85.