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First Case of Human Babesiosis in Korea: Detection and Characterization of a Novel Type of *Babesia* sp. (KO1) Similar to Ovine *Babesia*▽

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We report on the first case of human babesiosis in Korea. The intraerythrocytic parasite (KO1) in the patient's blood mainly appeared as paired pyriforms and ring forms; but Maltese cross forms were not seen, and the parasite showed morphological features consistent with those of the genus *Babesia* sensu stricto. The sequence of the 18S rRNA gene of KO1 was closely related to that of *Babesia* spp. isolated from sheep in China (similarity, 98%). The present study provides the first evidence of the presence of a hitherto unidentified, new type of *Babesia* parasite capable of infecting humans.

CASE REPORT

A 75-year-old female residing in Gurae, Jeon-nam Province, Korea, was hospitalized at the Ajou University Medical Center, Suwon, Korea, on 5 June 2005 with symptoms of severe anemia, an irregular high fever, rigor, muscle pain, vomiting, diarrhea, and jaundice. Her medical history included gastric cancer, diagnosed in 1998, for which she had received treatments including gastric resection and splenectomy. Because of those operations, she had received blood transfusions twice in 1999. Initially, her disease was diagnosed as malaria because the clinical symptoms and morphologies of the parasites found in her blood smear were quite similar to those of the malaria parasite and because tertian malaria has often been found often in Korea in recent years. She was treated with quinine, an antimalarial drug, but it was apparently ineffective. Therefore, the patient's blood sample was transferred to the Division of Malaria and Parasitic Diseases, Korean Centers for Disease Control and Prevention, for systematic diagnosis on 20 June 2005.

On 22 June, her disease was diagnosed as babesiosis by microscopic examination and molecular biological methods. The patient was then treated with clindamycin on 23 June, which successfully reduced the parasitemia. The patient was discharged from the hospital on 1 July. When the second examination was done on 6 July, no parasite was detected by microscopic examination or PCR analysis.

Giemsa-stained blood smears from the patient revealed that intraerythrocytic parasites were most frequently observed as paired pyriforms and ring forms, but Maltese cross forms were not seen in most smears (Fig. 1). In general, the genus *Babesia* has been divided into two groups, namely "large" and "small" *Babesia*; the former is larger than 3 μm and frequently appears as unique paired pyriforms, while the latter is smaller than 3 μm and mainly appears as ring forms and, occasionally, Maltese cross forms (10). Hence, the parasite found in this study (KO1) can be classified as large *Babesia* on the basis of its size and shape.

To investigate the parasite's molecular characteristics, we performed nested PCR to detect the 18S rRNA gene of *Babesia microti* (12, 17), because it was regarded as the most probable cause of human babesiosis and it has been observed in wild mice in Korea. The parasite DNAs, prepared from the patient's blood sample by using a Nucleospin blood DNA extraction kit (Macherey-Nagel, Germany), were used for the first round of PCR with the primer set of Bab1A (5′-GTCTT AGTATAAGCTTTTATACAGCG-3′) and Bab4A (5′-GAT AGGTCAGAAACTTGAATGATACATCG-3′), followed by a second round of PCR with 1 μl of the first-round PCR product with the primer set Bab2A (5′-CAGTTATAGTTTA TTTGATGTTCGTTTTAC-3′) and Bab3A (5′-CGGCAAAG CCATGCGATTCGCTAAT-3′) (17). The results were negative, indicating that *B. microti* was not present. At the same time, another nested PCR was conducted independently with primer sets that universally detect the 18S rRNA genes of most *Babesia* spp., namely, primers Bab5 (5′-AATTACCCAATCC TGACACAGG-3′) and Bab8 (5′-TTTGGCAGTAGT TCGT CTTTAACA-3′) for the first round of amplification and primers Bab6 (5′-GACACAGGG AGGTAGTGACAAGA-3′) and Bab7 (5′-CCCAACTGCTCCTATTAACCATTAC-3′) for the second round of PCR (17). As a result, a PCR product of about 400 bp was obtained. The PCR product was purified and cloned into a plasmid vector by using a Zero Blunt TOPO PCR cloning kit (Invitrogen, Carlsbad, CA), according to the manufacturer's instructions. The nucleotide sequences were determined by using a BigDye chain termination kit (Applied Biosystems, Foster City, CA) with an ABI 3100 automated sequencer (Applied Biosystems). The sequence was used to search the database of the National Center for Biotechnology

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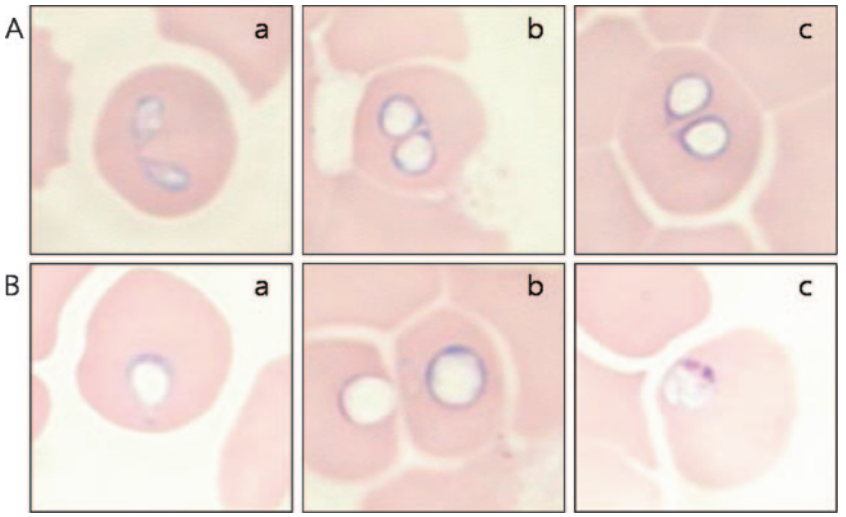

FIG. 1. Giemsa-stained smears of blood obtained on 2 July 2005 from a patient in Gurae, Korea, who had acquired babesiosis. (A) Paired pyriform parasites; (B) ring-form parasites. Bars, 5 μm.

Information (NCBI) with the Basic Local Alignment Search Tool (http://www.ncbi.nlm.nih.gov/BLAST/) for similarity analysis. Interestingly, the result revealed that the sequence from the patient was virtually identical to that of a *Babesia* sp. isolated from a sheep in Hebei, China (GenBank accession number DQ159074). Thus, for a more accurate analysis we again amplified the full-sized sequence (~1.7 kb) encoding the 18S rRNA gene of the *Babesia* parasite from the patient with the primer set A (5′-ACCTGGTTGATCCTGCCAGT-3′) and B (5′-TGATCCTTCTGCAGGTTCACCTAC-3′) described by Medlin et al. (9), cloned the PCR product, and sequenced it as described above. The sequence obtained was highly similar (98%) to that registered in GenBank under accession number DQ159074. Phylogenetic analysis was carried out with the MacVector software package (version 8.1.1; Genetics Computer Group, Inc., Madison, WI). A total of 1,684 bp of the 18S rRNA sequence obtained in this study and the 18S rRNA gene sequences available from NCBI's database were aligned with the program ClustalW, followed by alignment with the phylogenetic analysis program available in the same package. The phylogenetic tree constructed by the neighbor-joining method is depicted in Fig. 2, which clearly demonstrates their close relationship. The *Babesia* sp. from the Korean patient formed a clade that included several *Babesia* spp. previously reported from sheep in China (18). Meanwhile, the *Babesia* spp. previously known to be the agents of human babesiosis, such as *B. divergens*, *B. microti*, and *B. duncani*, were placed into the phylogenetic tree very distant from the *Babesia* sp. found in this study. It was also shown that the new *Babesia* sp. was apparently included in the genus *Babesia* sensu stricto (i.e., large *Babesia*), but morphologically it was clearly distinguishable from *B. divergens* parasites and some other *B. divergens*-like parasites, such as MO-1 (5) and EU-1 (6), which are the sole species in the genus *Babesia* sensu stricto that have been confirmed to infect humans in Europe (4, 6) and, very recently, in the United States (7). The great resemblance of the morphologies of the intraerythrocytic parasites found in the Korean patient and those reported for the *Babesia* sp. from sheep in Gansu, China (1), is also noteworthy. Whether or not the patient was infected with parasites naturally maintained in sheep in Korea, however, must await further epizootiologic investigations, because our preliminary surveys have yet to provide any evidence of the natural vertebrate hosts or the ticks involved in transmission. Until enough knowledge is accumulated to give certain specific nomenclature, we temporarily designate this parasite strain KO1.

We conducted further epidemiological surveys with blood samples from the residents in the patient's village, Gurae, in southwestern South Korea. For this survey we were able to collect blood samples from 68 residents (30 males and 38 females; age range, 37 to 100 years), and we extracted DNA for parasite detection by PCR. PCR amplification was done with primers Bab5 and Bab8 for the first round of PCR and primers Bab6 and Bab7 for the second round of PCR. Three cases were confirmed to be positive (Fig. 3). Sequencing analysis of the three PCR products showed sequences identical to those detected in the patient (KO1) (data not shown). These results indicate that there may be some asymptomatic carriers in this village. We have also attempted to detect the *Babesia* parasite in 17 goats raised in the village, but unfortunately, no positive signal was detected.

Babesiosis, caused by infection with parasites of the genus *Babesia*, is one of the most ubiquitous infections in wild and domestic animals worldwide. The disease is transmitted by ixodid ticks to vertebrate hosts. Recently, it has increasingly gained attention as an emerging zoonosis in humans (4, 8). Two main species of *Babesia* parasites, namely, *B. microti* (so-called small *Babesia*) and *B. divergens* (large *Babesia*, or the genus *Babesia* sensu stricto) have been known to be involved in human infections in the United States and Europe, respectively (4, 6, 7). In addition, a newly emerging *Babesia* species, referred to as WA1 (13, 16) and CA1 (11), that causes human babesiosis has been reported and was recently identified as *B.*

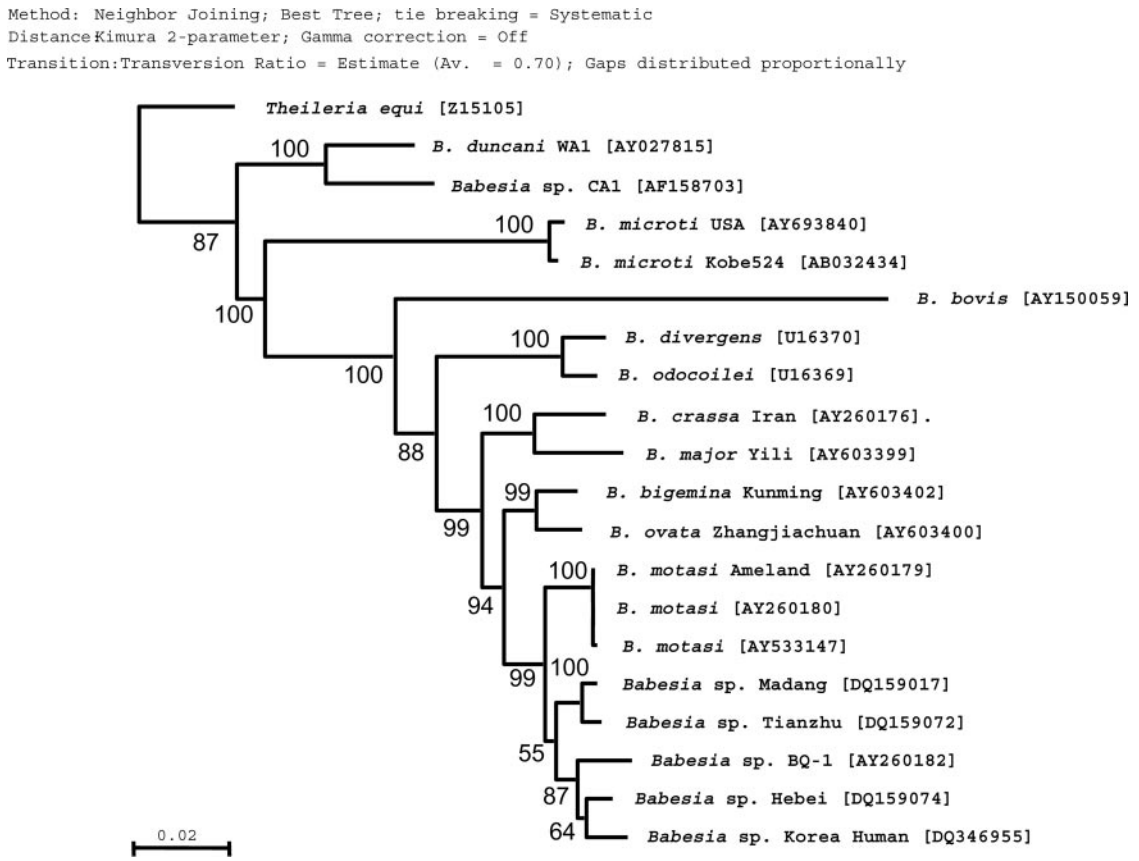

FIG. 2. Phylogenetic analysis of the 18S rRNA gene sequences of *Babesia* sp. strain KO1 (newly found in the Korean index case patient) and closely related *Babesia* spp. The phylogenetic tree was built by using the neighbor-joining method. Multiple-sequence alignment was carried out by use of the ClustalW alignment program in the MacVector software package, and the aligned sequence was used to construct phylogenetic trees by using the Phylogenetic Analysis program in the same software package. The numbers on the nodes indicate the percentage of replicates of 1,000 samplings in which the given branching pattern was obtained. The scale bar indicates 0.02 nucleotide substitutions per site. The designations in brackets are GenBank accession numbers.

duncani sp. nov. in the United States (3). In East Asia, cases of human babesiosis have been reported in Taiwan (15) and Japan (14). Both of these were caused by *B. microti*-like parasites, which often appear asymptomatically. Although field surveys of wild rodents and cattle have suggested the presence of *Babesia* parasites in Korea (2, 19), human babesiosis has not yet been reported. In this study we report the first case of human babesiosis in Korea, which was found to be a novel large *Babesia* parasite infecting a human and which was nearly fatal.

In conclusion, this is the first report of human babesiosis in Korea which was apparently caused by a hitherto unidentified, new type of *Babesia* sp. capable of infecting humans. The sequence of this new parasite is similar (98%) to that of *Babesia* spp. detected from sheep in China. Research collaboration will apparently be needed to further investigate the interrelationship between the parasites found in the Korean index case patient and those reported from sheep in China.

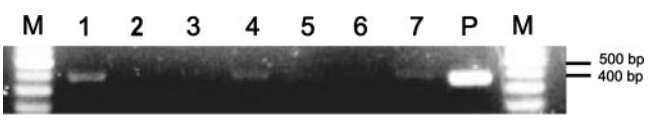

FIG. 3. PCR amplification for detection of *Babesia* spp. from the inhabitants in the village where the patient lives (lanes 1 to 7). DNA from the patient (lane P) was used as a positive control. The products of the second PCR with primers Bab6 and Bab7 are shown. Lanes M, molecular size marker.

Nucleotide sequence accession number. The 18S rRNA gene sequence of strain KO1 can be found in the GenBank database under accession number DQ346955.

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