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First Case of Human Brucellosis Caused by *Brucella melitensis* in Korea

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Dear Editor,

Human brucellosis is the most common zoonosis worldwide and is caused by gram-negative bacteria, *Brucella* spp. [1]. Among the *Brucella* spp., *B. abortus*, *B. canis*, *B. melitensis*, and *B. suis* can cause human brucellosis. Human brucellosis involves various organs including bone marrow (BM), shows non-specific clinical manifestations including fever, fatigue, back pain, and arthralgia, and can progress to septicemia or multi-organ failure [1, 2]. In Korea, since the first case of human brucellosis in 2002 [3], all reported 747 cases have been caused by *B. abortus*, and the majority were related to the outbreak of bovine brucellosis in mid-2000 [4]. We report the first Korean case of human brucellosis caused by *B. melitensis*.

A 34-yr-old man presented with high fever (39.2°C), significant weight loss (10 kg/month), and back pain that persisted for three weeks. He was a Korean resident living in northwestern China (Yanji, Jilin) who had been working on a stock farm in Pyeongchang, Gangwon Province, Korea for two months before visiting our hospital. He had hepatosplenomegaly, elevated aminotransferases, and pancytopenia. His complete blood count showed the following: hemoglobin, 8.8 g/dL; white blood cells, 2.1×10^9/L; and platelets, 43×10^9/L. His BM was normocellular with intact trilineage hematopoiesis, and neither granuloma nor necrosis was present. Plasma cells (CD19+/CD56-) were elevated (11.7%), and polyclonal gammopathy was detected via serum protein electrophoresis. A magnetic resonance imaging to evaluate back pain revealed pyogenic spondylitis at L3-4.

The first two blood cultures yielded gram-negative bacilli, which were identified as *B. melitensis* by using Vitek 2 (bioMérieux, Marcy-l'Etoile, France). In a microagglutination test (MAT), the antibody titer was 1:80. The patient received doxycycline and rifampicin for three weeks and was discharged with no symptoms except mild leukopenia. The MAT titer was not rechecked during the recovery period, because he declined further treatment.

The Vitek 2 system database has a limitation in identifying *Brucella* spp.: it misidentifies *B. abortus* as *B. melitensis*. Therefore, the identification result of *B. melitensis* was further con-

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firmed at the Division of Zoonoses at the Korea Centers for Disease Control and Prevention after the approval of the Institutional Review Board of Konkuk University Medical Center, Seoul, Korea. After a blood culture, the cultivated product was streaked on both a blood agar plate and a *Brucella* agar plate and incubated at 37°C and 5% CO_2 atmosphere. After several passages through the *Brucella* agar plate, the pure culture product (the isolate) tested negative for H_2S production and positive for oxidase and urease. DNA was extracted from the isolate to amplify the *Brucella*-specific genes (*16S rRNA*, *BCSP31*, and *Omp2*) [5]. PCR targeting *Omp31*, *IS711*, and *BCSS* confirmed this isolate as *B. melitensis* [6-8] (Table 1). Draft whole genome sequence analysis assembled by CLC bio CLC Genomics Workbench 7.5.1 (Waltham, MA, USA) revealed that the isolate was *B. melitensis* (Fig. 1).

To identify *Brucella* spp. when brucellosis is suspected, a careful and complete history should be taken of the patient's occupation, travel, and unpasteurized diet history [9]. The present patient had worked on a sheep farm for two months before admission and experienced nonspecific symptoms (fever, weight loss, and back pain) for three weeks, indicating *B. melitensis* infection. Before he was in Korea, he had lived in northwestern China, where *B. melitensis* has been dominant [10]. It is not clear whether he had raw food from infected animals in China or in Korea, or whether he acquired brucellosis occupationally by direct skin contact or inhalation of aerosolized infected particles [9]. Although the clinical history appears insufficient, considering that he had been healthy before developing these clinical symptoms, it is unlikely that he already had brucellosis in China.

In the present case, H_2S was negative, and oxidase and urease were positive in the biochemical analysis, suggesting the possibility of *B. melitensis* infection together with the patient's working history on the sheep farm. However, H_2S production can be also negative in the other *Brucella* spp., and molecular identification is necessary to confirm the diagnosis. We used multiple gene targets to detect the *Brucella* genus using a common PCR assay [5] and also used *Omp31*, *IS711*, and *BCSS* gene targets to distinguish *B. melitensis* from the other *Brucella* spp. [6-8] (Table 1).

Conclusively, this is the first official case of human brucellosis caused by *B. melitensis* in Korea that was confirmed by using molecular methods. Careful recording of patient history and molecular identification are necessary in patients with suspected brucellosis to confirm the causative *Brucella* spp.

Table 1. Primers used in this study and the results of PCR and sequencing

Gene	Primer	Sequence (5′-3′)	Size (specific species)	Reference	Result (PCR and sequencing)
16s rRNA	F	TCG AGC GCC CGC AAG GGG	905 bp (*Brucella* spp.)	5	+
	R	AAC CAT AGT GTC TCC ACT AA			
BCSP 31	F	TGG CTC GGT TGC CAA TAT CAA	223 bp (*B.* spp.)	5	+
	R	CGC GCT TGC CTT TCA GGT CTG			
Omp2	F	GCG CTA AGG CTG CCG ACG CAA	193 bp (*B.* spp.)	5	+
	R	ACC AGC CAT TGC GGT CGG TA			
Omp31	F	TGA CAG ACT TTT TCG CCG AA	720 bp (*B. melitensis*)	6	+
	R	TAT GGA TTG CAG CAC CGC			
IS711	Ba F	GAC GAA CGG AAT TTT TCC AAT CCC	498 bp (*B. abortus*)	7	-
	IS711	TGC CGA TCA CTT AAG GGC CTT CAT			
	Bm F	AAA TCG CGT CCT TGC TGG TCT GA	731 bp (*B. melitensis*)	7	+
	IS711	TGC CGA TCA CTT AAG GGC CTT CAT			
	Bo F	CGG GTT CTG GCA CCA TCG TCG	976 bp (*B. ovis*)	7	-
	IS711	TGC CGA TCA CTT AAG GGC CTT CAT			
	Bs F	GCG CGG TTT TCT GAA GGT TCA GG	285 bp (*B. suis*)	7	-
	IS711	TGC CGA TCA CTT AAG GGC CTT CAT			
BCSS	F	CCA GAT AGA CCT CTC TGG A	300 bp (*B. canis*)	8	-
	R	TGG CCT TTT CTG ATC TGT TCT T			

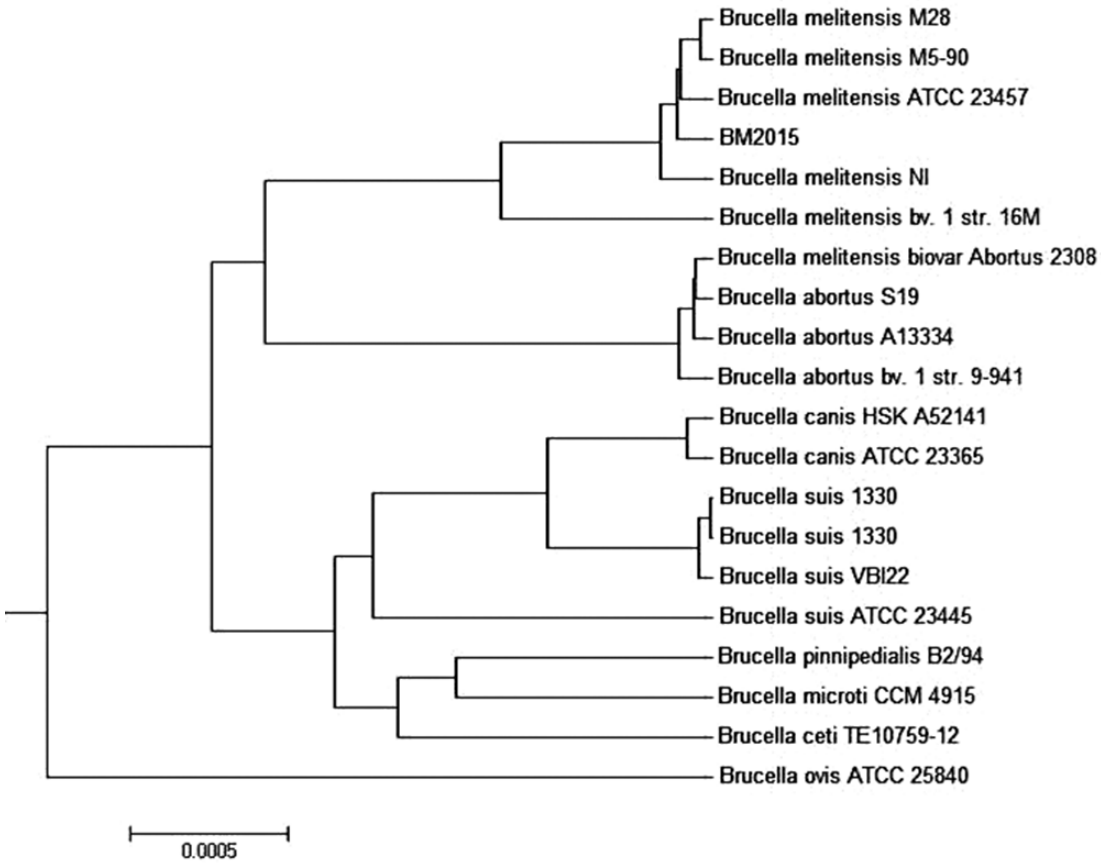

Fig. 1. Average nucleotide identity tree of *Brucella melitensis*. The genome tree was constructed by using BM2015 (the isolate) and related *Brucella* spp.

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Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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