Short Communication

Case of Human Infection with *Anaplasma phagocytophilum* in Inner Mongolia, China

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SUMMARY: *Anaplasma phagocytophilum* is an obligate intracellular bacterium that causes febrile illness in humans and livestock. A 49-year-old woman was suffering from feverish symptoms, fatigue, arthralgia, general body pain, and anorexia for 2 weeks. Later, she visited the Bayannur Centers for Disease Control and Prevention Hospital in Inner Mongolia, China. Molecular-based diagnostic analysis of the patient's blood revealed that *A. phagocytophilum* *p44* DNA was positive, but *Brucella omp31*, spotted fever group *Rickettsia gltA*, *Orientia tsutsugamushi* 16S rDNA, and *Ehrlichia p28* were negative. The amino acid sequences of 9 *A. phagocytophilum* *p44* clones obtained from the patient shared 44–100% similarity among them and were closely related to those of previously identified *p44* clones from *Canis familiaris* (accession no. KJV64194) and from *Ixodes persulcatus* tick (no. BAN28309). Serological tests using the patient's serum showed that immunoglobulin M (IgM) and IgG titers to *A. phagocytophilum* antigens were 160 and 20, respectively, determined using indirect immunofluorescence assay, and the reaction to recombinant P44 proteins (rP44-1, rP44-18ES, and/or rP44-47) was confirmed using Western blot analysis. Thus, the results obtained in this study strongly suggest that the patient was infected with *A. phagocytophilum*. To our knowledge, this is the first case of human anaplasmosis infection in the Inner Mongolia Autonomous Region.

Human granulocytic anaplasmosis (HGA) is a febrile and emerging tick-borne infectious disease. It is caused by *Anaplasma phagocytophilum*, an obligate intracellular gram-negative bacterium. In East Asia, the first case reports of HGA from China, Japan, and South Korea were published in 2008, 2013, and 2014, respectively (1–3). The publication from China in 2008 was also the only report claiming nosocomial (human-to-human) infection of *A. phagocytophilum* (1). However, Wormser (4) strongly suggests that 9 HGA cases in the first report from China (1) seem to be infection by severe fever with thrombocytopenia syndrome virus (SFTSV) (5) rather than *A. phagocytophilum* based on clinical and laboratory data comparison. Additional cases of HGA were reported from Hebei Province, Beijing city, Tianjin city, Shandong Province, Henan Province, and Hubei Province of China (6,7). In Inner Mongolia, the incidence of

brucellosis is high (8), but non-brucellosis cases with fever of unknown origin were often recognized. In this study, we found a case of *A. phagocytophilum* infection from non-brucellosis patients in Inner Mongolia by molecular-based diagnostic and serological tests.

We conducted nested PCR with primers based on the highly conserved regions of *A. phagocytophilum* *p44* multigenes as described previously (2) in 261 blood samples from non-brucellosis patients with fever of unknown origin in the Bayannur Centers for Disease Control and Prevention Hospital, Bayannur city, Inner Mongolia, China. A 400-bp amplicon was detected from one patient and was TA-cloned. The phylogenetic tree was constructed based on amino acid sequence alignment of the cloned *p44*s from the patient and the closely-related *p44* clone relatives using MEGA 7 (Fig. 1). The sequences of 9 *p44* clones obtained from the patient shared 44–100% similarity among them and fell into 2 major clades in the tree. BLAST search showed that the closest relatives of 6 identical clones (H8-2 as a representative in Fig. 1) and 2 other clones (H8-3 and H8-4) were P44-31 (KJV64194) (92%) from *Canis familiaris* in the Netherlands, and another clone (H8-1) was closely related to P44 (BAN28309) (99%) from *Ixodes persulcatus* in Russia. Nested PCRs for *Brucella omp31*, spotted fever group *Rickettsia gltA*, *Orientia tsutsugamushi* 16S rDNA, and *Ehrlichia p28* in the patient sample were also conducted according to the pro-

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Fig. 1. Phylogenetic classification of *A. phagocytophilum* *p44* multigene clones detected in blood from a patient in Inner Mongolia, China. The tree was constructed based on *p44*s (123–127 amino acids) and their close relatives using the neighbor-joining method. Boldfaced characters show 4 representative *p44* clones from the patient in China. Numbers on the tree indicate bootstrap values for branch point. Data in parentheses indicate the number of *p44* clones with identical sequences and the accession numbers. Scale bar shows sequence divergence.

cedure described previously (9,10), and the results were all negative. For serological evidence, indirect immunofluorescence assay (IFA) using *A. phagocytophilum* HZ strain (US-human isolate) cultured with THP-1, HL60, and NB4 cells as antigens was performed using the procedure as described previously (2). Fluorescein isothiocyanate-labeled rabbit anti-human immunoglobulin M (IgM) and IgG antibodies (Kirkegaard & Perry Laboratories, Gaithersburg, MD, USA) were used as secondary antibodies. The IFA results showed that the patient's serum had IgM and IgG titers of 160 and 20, respectively,

against the antigens of the 3 infected cell lines (Table 1). The IgG reaction in IFA was weak. This weakness might be generated by the antigenic differences between human infectious *A. phagocytophilum* in the USA and Asia. Western blot analysis was further conducted using 4 different recombinant P44 antigenic proteins (rP44-1, rP44-18ES, rP44-47E, and rP44-60) as described previously (2,11). The results showed that IgM bound to 3 rP44s (rP44-1, weak rP44-18ES, and rP44-47E) and IgG reacted weakly with rP44-1 alone (Fig. 2).

The case record of the patient is that a 49-year-old woman who is a shepherd was suffering from sporadic feverish symptoms (body temperature was not measured), fatigue, arthralgia, general body pain, and anorexia for 2 weeks, and these symptoms did not improve. Then, she visited the Bayannur Centers for Disease Control and Prevention Hospital, Inner Mongolia, in July 26, 2016. The physician suspected brucellosis, but results of the serological test commonly used for brucellosis were negative. The laboratory tests (reference values) showed 3.96×10^9 leukocytes/L ($3.5\text{--}9.2 \times 10^9$), 145×10^9 thrombocytes/L ($155\text{--}365 \times 10^9$), 66 IU/L of aspartate aminotransferase (< 38), 46 IU/L of alanine aminotransferase (< 36), and 6.0 mg/dL of C-reactive protein (CRP; < 0.3).

In China, cases of human anaplasmosis have increasingly been reported since 2008 (1). However, it seems that those cases include SFTSV infection (4). Hence, we usually consider at least 2 clinical and laboratory parameters (diarrhea and CRP) to distinguish between SFTS and HGA. In Japan (2,11,12), SFTSV and *A. phagocytophilum* caused diarrhea in 37/48 (77%) and 0/6 patients (0%), respectively ($p = 0.000479$, Fisher's exact test), and high CRP levels (> 0.8 mg/dL) were found in 0/47 SFTS patients (0%; range from 0.05 to 0.78 mg/dL) and in 5/6 HGA patients (83%; range from

Table 1. IFA antibody titer in serum from the patient against *Anaplasma phagocytophilum* cultured in 3 different infected cell lines

Patient	Location	Sex	Age	Antigen titer					
				THP-1 cell		HL60 cell		NB4 cell	
				IgM	IgG	IgM	IgG	IgM	IgG
Case 1 ¹⁾	Bayannur, Inner Mongolia	woman	49	160	20	160	20	160	20

¹⁾ Case 1 patient was *A. phagocytophilum*-*p44*-PCR positive shown in Fig. 1. Blood was corrected from the patient before antibiotics treatment on July 26, 2016, and the serum was prepared from a part of the whole blood sample. Both whole blood and serum samples were stored at -80°C until use.

Fig. 2. Western blot analyses of a serum from a patient who has infected with *A. phagocytophilum* in Inner Mongolia, China, using 4 different P44 recombinant antigens (rP44-1, rP44-18ES, rP44-47E, and rP44-r60). Arrows indicate the rP44 proteins that reacted with the serum sample from the patient as well as positive rabbit serum in this study.

0.7 to 17.2 mg/dL) ($p = 0.000002$, Fisher's exact test). The patient in this study had no diarrhea and 6.0 mg/dL of CRP. Furthermore, the inhabitation of a main transmission tick vector, *Haemaphysalis longicornis*, for SFTSV has not been confirmed in Inner Mongolia so far. Additional consideration is infection with *Anaplasma capra*, a novel human pathogen, in China that was reported in 2015 (13). Genetically, *A. capra* seems to be similar with *Anaplasma centrale* Aomori that was originally identified from cattle in Japan (14) by Inokuma et al. Our previous study showed that *p44* primers used in this study did not amplify any DNA fragment from deer infected with *A. centrale* Aomori (15), suggesting the *A. phagocytophilum p44*-specific primers. Serologically, it was reported that *A. capra* and *A. phagocytophilum* antigens in IFA appear to be weakly cross-reactive (13), but the recombinant P44 proteins specific for *A. phagocytophilum* reacted with serum from the patient in this study. Taken together, our results strongly suggest human *A. phagocytophilum* infection in the patient. As far as we know, this is the first case of HGA caused by *A. phagocytophilum* in the Inner Mongolia Autonomous Region in China.

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Conflict of interest None to declare.

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