

Outbreak Reports

Anaplasma bovis Infection in Fever and Thrombocytopenia Patients — Anhui Province, China, 2021

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Summary

What is already known about this topic?

The genus *Anaplasma* contains seven recognized bacterial species, mainly transmitted by tick bites. The two species, *A. phagocytophilum* and *A. capra*, are known commonly to cause diseases in humans.

What is added by this report?

Anaplasma bovis was initially thought to be only an animal agent until the first patient case was reported in 2019. This study investigated another two patients who became sick within one month in the same township and were infected with *A. bovis* in Anhui Province.

What are the implications for public health practice?

This study suggested that more *A. bovis*-infected patients may exist in this area and that patients with anaplasmosis require an early and specific diagnosis.

Surveillance in Jinzhai County, Lu'an City, Anhui Province, China revealed that between May and June 2021, 2 patients developed fever and thrombocytopenia after being bitten by a tick. The local hospitals and CDC could not determine the pathogen, so blood specimens were sent to the National Institute for Communicable Disease Control and Prevention to investigate this event. Nested polymerase chain reactions (PCR) and indirect immunofluorescence assays (IFAs) identified *Anaplasma bovis* as the pathogen of both cases. Gene sequencing suggested that a tick, *Haemaphysalis longicornis*, was the source of infection. This is the second report of *A. bovis* infections in humans worldwide. Clinicians and public health physicians should be more attentive to these diseases and learn how to diagnose them as early as possible.

INVESTIGATION AND RESULTS

In 2021, 2 patients (Patient A and Patient B)

developed intermittent fever as an initial clinical symptom after a tick bite. Both of them were characterized by fever, thrombocytopenia, rash, asthenia, anorexia, myalgia, chill, diarrhea, and headache, which were not resolved by common antibiotics. In a local hospital, these patients were clinically diagnosed with endemic typhus.

On May 10, 2021, a 67-year-old man (Patient A) developed a fever with chills, asthenia, anorexia, and myalgia after being bitten by a tick in a mountainous area. Even with routine anti-infection treatments, his body was covered with a rash 3 days later. He was brought to the Department of Infectious Disease of Jinzhai County People's Hospital (JCPH; Lu'an City, Anhui Province, China) on May 17, 2021, with persistent intermittent fever and rashes. Based on his medical history and epidemiological and clinical manifestations, he was admitted to JCPH with clinically diagnosed typhus. Blood tests revealed eosinophilic granulocytes of $0.00 \times 10^9/L$, thrombocytes of $87 \times 10^9/L$, alanine aminotransferase of 117.07 U/L, and aspartate transaminase of 104.13 U/L. After treatment with azithromycin for 7 days, his clinical symptoms disappeared.

On June 5, 2021, a 57-year-old man (Patient B) developed a fever reaching 39 °C accompanied by headache, asthenia, anorexia, myalgia, and occasional diarrhea after working in a field. He was brought to the Department of Infectious Disease of JCPH on June 8, 2021, with progressive exacerbation of his symptoms. He was admitted to JCPH with "fever of undetermined origin," and his general status upon hospital admission was poor. Blood tests revealed leukocytes of $3.94 \times 10^9/L$ and thrombocytes of $95 \times 10^9/L$. After treatment with azithromycin for 7 days, his clinical symptoms disappeared, and his general status improved greatly.

The blood specimens collected from these two patients were sent to determine the pathogen through nested PCR and IFA. DNA was extracted with the

QIAamp DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany), and several human pathogens were tested using nested PCR targeting the 16S ribosomal RNA (*rrs*) gene. Specific immunoglobulin IgG and IgM antibodies against spotted fever group and related rickettsia (*Rickettsia typhi*, *Orientia tsutsugamushi*, *A. phagocytophilum*, *Ehrlichia chaffeensis*, and *A. bovis* in sera) were detected by IFA (Fuller Laboratories, Fullerton, CA, USA). The *A. bovis* substrate slides were developed by our department. According to the manufacturer's instructions, an IgG titer of $\geq 1:64$ and IgM titer of $\geq 1:20$ denoted a positive result.

To investigate the presence of infections in relevant ticks, we collected parasitic and free-living ticks from the regions where the two patients lived and worked. All ticks were identified morphologically by an entomologist based on differences in their bodies and basis capituli. All tick DNA was extracted individually. We tested for the presence of Rickettsiales bacteria using a nested or semi-nested PCR targeting the *rrs*, citrate synthase (*gltA*), and 60-kDa heat shock protein (*groEL*) genes as described previously (Supplementary Table S1, available in <http://weekly.chinacdc.cn/>)(1). Phylogenetic trees of the data were created using the Maximum Likelihood (ML) method by employing the GTR+ Γ +I model of substitution, as implemented in PhyML (version 3.0) <http://www.atgc-montpellier.fr/phyml/> (2).

The *rrs* gene was amplified from Patient A using a PCR assay, while it was not detected from Patient B. Using BLASTN with a nucleotide collection, genetic analysis of the recovered *rrs* sequence revealed that it was most closely related to that of the *A. bovis* isolate

Zhouzhi-cattle-10 (GenBank: MH255937.1, 99.75%), and we named it "*A. bovis* strain JZPA." The IgM titer against *A. bovis* of these 2 patients was 1:80, whereas the IgG titer of Patient A was 1:256, and that of Patient B was 1:1,024 (Figure 1). Therefore, PCR and IFA indicated that both patients had been infected with *A. bovis*.

To investigate the source of this pathogen, we carried out an investigation into the vectors present in the areas where the two patients lived and worked. We collected 270 tick samples, which were tested for the *A. bovis* strain JZPA. The *rrs*, *gltA*, and *groEL* genes were detected in 63 samples, and the positive rate in ticks was 23.3%. Each of the 63 samples contained all the three genes. In the *rrs* tree (Figure 2), sequences from Patient A's sample (*A. bovis* strain JZPA) and sequences of the tick (*A. bovis* strain JZT018) clustered together and formed a distinct lineage. There was 100% similarity of the *rrs* gene sequences for the patient and the tick (*H. longicornis*). In the *gltA* and *groEL* trees (Supplementary Figure S1, available in <http://weekly.chinacdc.cn/>), the amplified tick sequences also clustered with *A. bovis*, and they were closely related to sequences from other *A. bovis* strains. These results suggested that *A. bovis* was prevalent in ticks within certain areas of Lu'an City and that these pathogens may infect humans to cause fevers and other symptoms (Table 1).

DISCUSSION

Rickettsiales are obligate intracellular microbes responsible for a wide range of important human

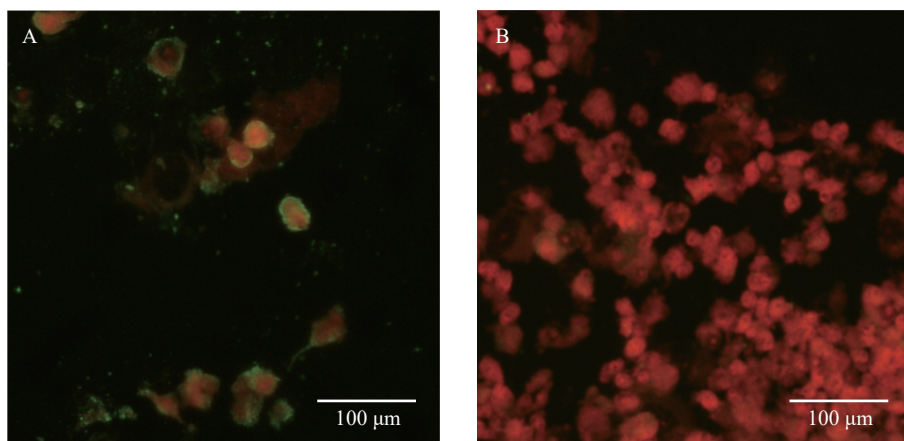


FIGURE 1. Photomicrographs of an IFA test with *Anaplasma bovis* infected patients' sera. (A) IFA of patient sera; (B) The IFA assays tested with negative control.

Note: Green fluorescence shows the positive cells for *A. bovis* bacteria, and red fluorescence shows the host cells. Abbreviation: IFA=immunofluorescence assay.

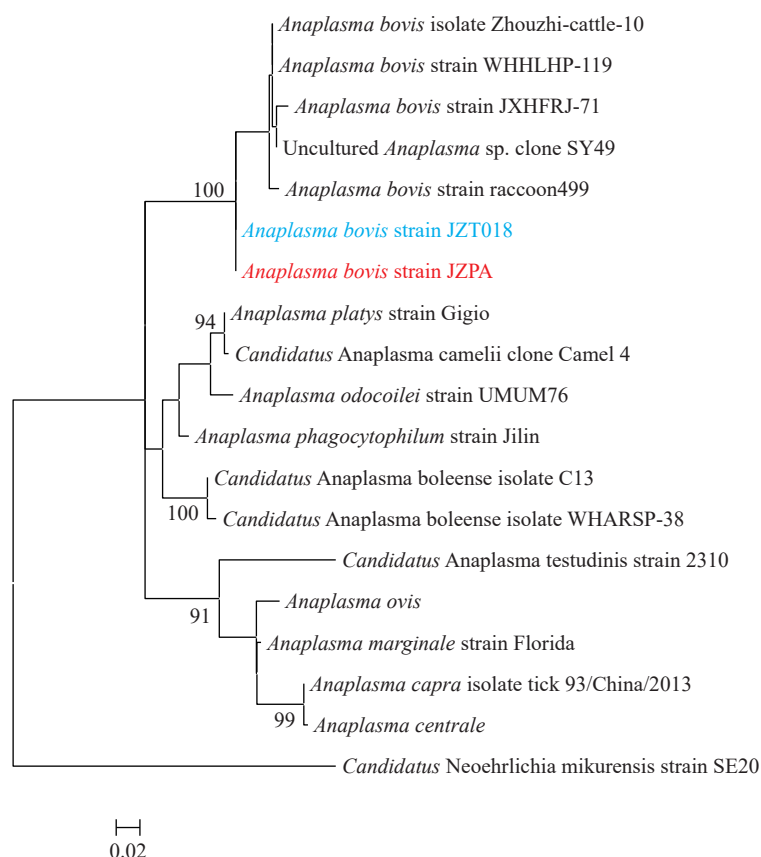


FIGURE 2. Phylogenetic relationship of *Anaplasma* species based on *rrs* sequences.

Note: All trees were mid-point rooted for clarity only. Bootstrap values (>70%) were shown for appropriate nodes. Scale bar represents number of nucleotide substitutions per site. The sequence obtained from Patient A is marked in red, and the sequence obtained from ticks is marked in blue.

diseases, including anaplasmosis, ehrlichiosis, rickettsioses, and scrub typhus. Rickettsial diseases are not being effectively controlled worldwide (3). Several *Anaplasma* species, such as *A. phagocytophilum*, *A. capra*, and *A. bovis*, are considered human pathogens (1,4–5). Incidences of human granulocytic anaplasmosis have increased steadily since its discovery in the 1990s (4). A second *Anaplasma* spp., *A. capra*, was determined to be a human pathogen in 2015 (5). In Jiangxi Province in 2017, a patient reportedly became infected with *A. bovis*, which was previously considered to be a bovine-specific pathogen (1). In recent decades, novel pathogens have been increasingly identified due to the application of molecular diagnostic methods (6). Furthermore, numerous bacteria previously considered non-pathogenic are now commonly associated with human diseases (7). Because commonly administered antibiotics are ineffective for anaplasmosis, early diagnosis is important. If the identity of the pathogens is not promptly and accurately determined, these diseases may become

severe and lead to death.

Both patients lived in the same township close to Jinzhai County, and neither had a history of traveling. We defined *A. bovis* as the cause of the illness based on PCR, IFA, and tick investigations. Unfortunately, the pathogens of *A. bovis* were isolated from ticks, but not from patients. This is the second report of *A. bovis*-infected humans worldwide, with the first being reported by Lu et al. in Jiangxi Province, China (1). Although the clinical symptoms were atypical, clinicians should pay attention to patients experiencing intermittent fever as an initial clinical symptom and thrombocytopenia after a tick bite or exposure to wildlife. Patients exhibiting these features should be considered to potentially have *A. bovis* infection.

Currently, the genus *Anaplasma* contains seven recognized bacterial species (6), most of which are known to be animal-specific pathogens; for example, *A. marginale*, *A. centrale*, and *A. ovis* are specific to ruminants; *A. platys* is a causative agent of infectious cyclic thrombocytopenia in dogs and cats (6); and *A.*

TABLE 1. Clinical manifestations and laboratory findings of two patients infected with *Anaplasma bovis* in Jinzhai County, Anhui Province, China, 2021.

Item	Patient A	Patient B
Clinical characterization		
Fever	+	+
Rash	+	-
Asthenia	+	+
Anorexia	+	+
Myalgia	+	+
Chill	+	-
Headache	-	+
Dizziness	-	-
Nausea	-	-
Lymphadenopathy	-	-
Vomiting	-	-
Diarrhea	-	+
Eschar	-	-
Cough	-	-
Arthralgia	-	-
Laboratory findings*		
White blood cell count	6.41×10 ⁹ /L	3.94×10 ⁹ /L
Platelet count	87×10 ⁹ /L	95×10 ⁹ /L
CRP	62.75 mg/L	4.93 mg/L
ALT	117.07 U/L	14 U/L
AST	104.13 U/L	24 U/L

*Normal ranges: white-cell count: 4.0–10.0 × 10⁹ /L, platelet count: 100–300 × 10⁹ /L, CRP 0–8 mg/L, ALT: 0–40 U/L, AST: 5–40 U/L.

Abbreviations: CRP=C-reaction protein; ALT=alanine aminotransferase; AST=glutamic oxaloacetic acid transferase.

bovis was thought to only infect bovine to cause bovine ehrlichiosis, which frequently occurs in Africa and Asia, until the first case was reported in humans. Animals infected with *A. bovis* are characterized by fluctuating fever, lymphadenopathy, depression, and occasionally death. When humans are infected with these pathogens, they display fever, rash, asthenia, anorexia, rigor, headache, myalgia, eschar, and lymphadenopathy.

A novel tick-borne bunyavirus was first discovered in Hubei and Henan provinces in 2009 (8), and human granulocytic anaplasmosis was reported in these regions (9). Bunyavirus and human granulocytic anaplasmosis infections can elicit similar symptoms, so distinguishing between them by using clinical manifestations can be difficult. For these patients, we used PCR and IFA to exclude the diagnoses of severe

fever with thrombocytopenia syndrome and infection with other types of bacteria of the order Rickettsiales, and the results showed that both patients were infected with *A. bovis*.

We screened 13 patients with Rickettsiae and *Anaplasma* infection symptoms, and only 2 patients were determined with *A. bovis* infection. Although surveillance found only two patients in this study, these patients became sick within one month of each other and lived in the same township. The close timing of their illness, their adjacent place of residence, and the high prevalence rate of *A. bovis* in ticks near their work and living places suggest that more *A. bovis*-infected patients may exist in this area.

Human infections with several types of tick-borne pathogens in mainland China have been documented in recent years, and they are an increasing threat to public health (10). Missed diagnoses and misdiagnoses can lead to poor outcomes or even death, so an early and specific diagnosis is important. The recommendations from this case study are the following: 1) a rapid detection method for diagnosis (e.g., nested PCR or real-time PCR) should be established; 2) the high prevalence rate of *A. bovis* in ticks suggests that potential outbreaks may occur in this region, and more investigations should be conducted in arthropods and wild animals in these areas; and 3) people should be educated on how to protect themselves against tick bites, especially those in susceptible populations (e.g., farm workers).

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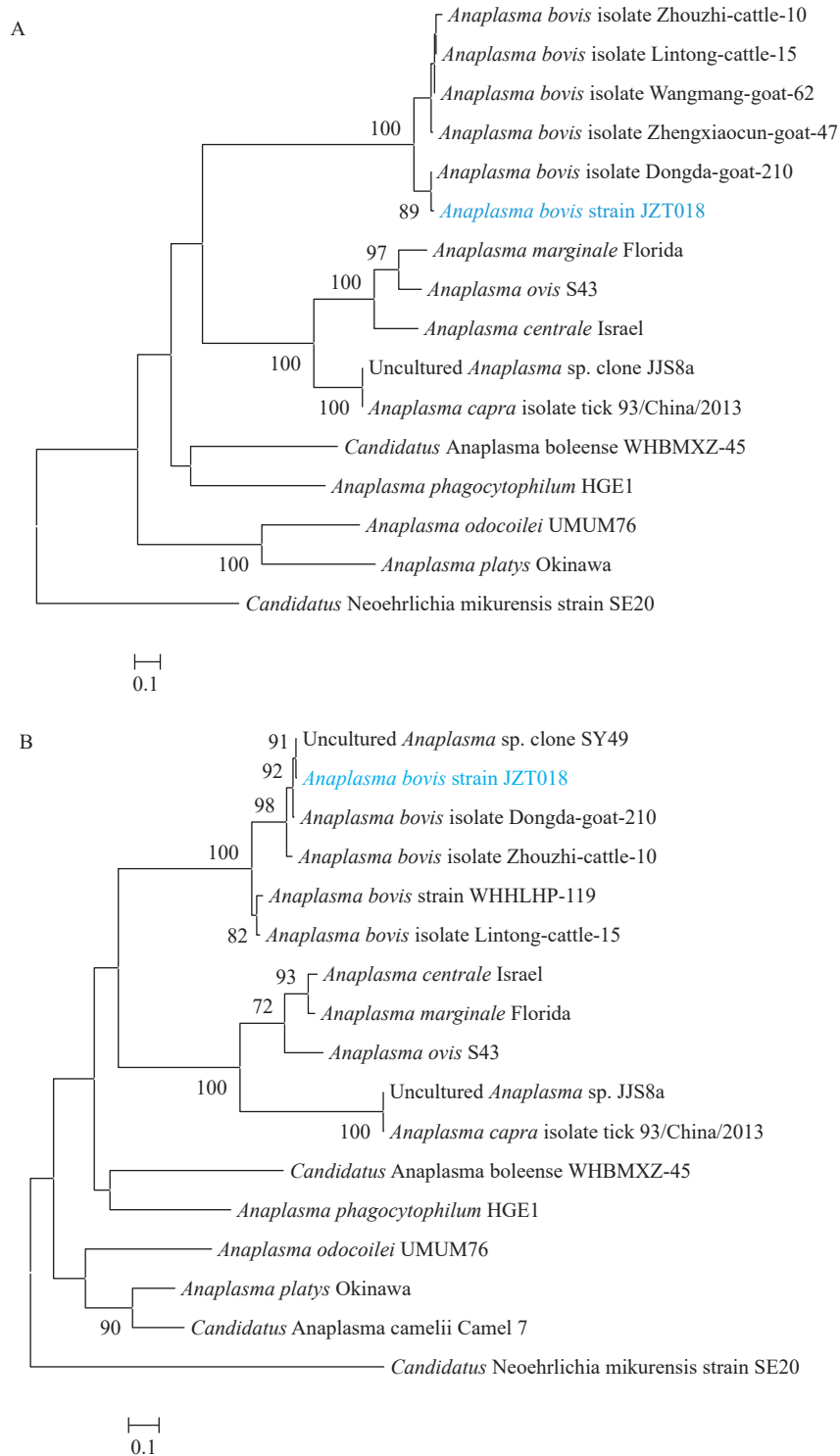
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SUPPLEMENTARY FIGURE S1. Phylogenetic relationship of *Anaplasma* species based on gene sequences of *gItA* (A) and *groEL* (B).

Note: All trees were mid-point rooted for clarity only. Bootstrap values (>70%) were shown for appropriate nodes. The scale bar represented number of nucleotide substitutions per site. The sequence obtained from ticks was marked in blue. Abbreviation: PCR=polymerase chain reaction.

SUPPLEMENTARY TABLE S1. The PCR primers used in this study.

Gene	Name	Primers (5'-3')	Fragment size
<i>rrs</i>	fd1	AGAGTTTGATCCTGGCTCAG	1,500 bp
	rp2	ACGGCTACCTTGTRACGACTT	
<i>groEL</i>	bovis-groF1	GTATGCARTTTGATCGYGGAT	1,330 bp
	bovis-groF2	GAAGTTGGAAGRGAYGGDGT	
	bovis-groR	GCCTWACAGCDGCAACTTG	
<i>gltA</i>	bovis-gltA-F1	TACATCWACWGTAAGAATGG	1,100 bp
	bovis-gltA-F2	ACWGTAAAGATGGTKGGCTC	
	bovis-gltA-R	CCRGCAGTDCGTCCCAGTGC	
<i>COI</i>	Ron	GGAGCYCCWGATATAGCTTTCCC	488 bp
	Nancy	CCTGGTAAAATTTAAAATATAAACTTC	

Abbreviations: PCR=polymerase chain reaction; *rrs*=16S ribosomal RNA; *groEL*=60-kDa heat shock protein; *gltA*=citrate synthase; *COI*=cytochrome-cytochrome oxidase.