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Research article

Human pathogens in ticks removed from humans in Hebei, China

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ABSTRACT

Ticks are the hosts or vectors of many human pathogens, including viruses, bacteria and protozoa, and can transmit these causative agents to humans when feeding on human bodies. In this study, 26 ticks removed from humans in Hebei, China were tested for the presence of human-pathogenic microorganisms by Polymerase Chain Reaction (PCR) or Reversed Transcript PCR (RT-PCR). As a result, 11 ticks tested positive for at least one human pathogen. Specifically, four validated human pathogens, including *Rickettsia raoultii*, *Candidatus* Rickettsia tarasevichiae, *Babesia venatorum*, and *Borrelia garinii*, as well as *Anaplasma ovis* with zoonotic potential, were identified in *Ixodes persulcatus*, *Dermacentor silvarum* and *Haemaphysalis concinna*. Importantly, this is the first report of *Anaplasma* and *Babesia* species pathogenic to humans in Hebei province. Moreover, the co-infections, including double infection and quadruple infection were observed. In addition, *Candidatus* R. principis with unknown pathogenicity was identified in one tick, which may be the same species as *Candidatus* R. hongyuanensis based on the nucleotide identity and phylogenetic analysis. Concluding, four validated tick-borne pathogens and one with zoonotic potential were identified in ticks parasitizing humans, suggesting the potential high public health risk in the local human population.

1. Introduction

Ranking second only to mosquitoes as vectors of many human infectious diseases, ticks can harbor and transmit a great variety of viruses, bacteria and protozoa, and many of which are identified in recent two decades [1]. China is seriously affected by tick-borne pathogens and associated diseases. In mainland China, approximately 50 tick-borne human pathogens belonging to the three major groups of microorganisms mentioned above have been identified in the first 20 years of the 21st century [2]. Importantly, human cases, caused by tick-borne agents including at least six species of genus *Rickettsia*, two species of genus *Anaplasma*, one species of genus *Ehrlichia*, one species of genus *Neoehrlichia*, five species of genus *Borrelia* and four viruses, have been reported in China [2,3]. Additionally, many field surveys have been performed and revealed that more human pathogens were circulating in ticks [2,3].

Ticks removed from human bodies and associated causative agents have been reported in several European countries, USA, Brazil, Turkey, Argentina, and South Korea [4,5,6,7,8,9,10,11,12,13,14]. The ticks removed from humans are of great diversity, including *Haemaphysalis*, *Dermacentor*, *Rhipicephalus*, *Ixodes*, *Hyalomma* and *Amblyomma*. More importantly, a variety of microorganisms

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pathogenic to humans, including *Anaplasma*, *Ehrlichia*, *Candidatus* Neoehrlichia, *Rickettsia*, *Borrelia*, *Babesia*, Severe Fever with Thrombocytopenia Syndrome Virus (SFTSV), and tick-borne encephalitis virus (TBEV), have been identified [4,5,6,7,8,9,10,11,12,13,14]. In mainland China, although various pathogens mentioned above were identified in both questing and engorged ticks, fed on animals, no causative agent in human-parasitizing ticks have been investigated until now [2,3]. Ticks can transmit the pathogens to humans when they feed on humans. Therefore, identification of pathogens in ticks parasitizing may be benefit for the diagnosis and prevention of tick-borne disease in local population.

The northern part of Hebei province, located in north China, is a plateau with dense vegetation, conducive to the survival of ticks. Notably, many novel human pathogens have been identified in ticks collected in China, indicating that China is a hotspot of tick-borne pathogens and tick-borne diseases [3]. Therefore, tick samples were collected from human bodies in Hebei Province in this study, aiming to screen the presence of tick-borne bacteria, parasites, and viruses, to assess the transmission risk of these pathogens to humans. In addition, the results of this study are helpful to provide basis for the treatment of diseases if these patients present clinical symptoms later.

Table 1
Primer sequences used in this study.

Pathogens	Target gene	Primer	Oligonucleotide sequences (5′- 3′)	References
Anaplasmataceae	*rrs*	Ehr1	AACGAACGCTGGCGGCAAGC (+)	[19]
		Ehr2	AGTAYCGRACCAGATAGCCGC (−)	
		Ehr3	TGCATAGGAATCTACCTAGTAG (+)	
		Ehr4	CTAGGAATTCCGCTATCCTCT (−)	
Rickettsia	*ompA*	Rr190k.70p	TGGCGAATATTTCTCCAAAA(+)	[21]
		Rr190k.720n	TGCATTTGTATTACCTATTGT (−)	
		Rr190k.602n	AGTGCAGCATTCGCTCCCCCT (−)	
		Rr190k.70p	TGGCGAATATTTCTCCAAAA(+)	[20,21]
		Ric-R1	ACCTACATTATCAAHGCCTGT	
		Ric-R2	ACCTSTTAATACTGCATTTRCAT	
	gltA	CS1d	ATGACTAATGGCAATAATAA	[27]
		CSEndr	CTTATACTCTCTATGTACA	
		RpCS1258n	ATTGCAAAAAGTACAGTGAACA	
Anaplasma ovis	*gltA*	AP1SPglF1	ATGBTAGAAAARGCTGTTTTRGMGTGT (+)	[28]
		AP1168SPglR1	TCATACCATTGMGATRCCCATCC (−)	
		APJ10F2	AAKGCTGTTTTAGCGTGTGGTGATCTT (+)	
		APJ932R2	ATTTTCGCCCTCGGGTCGTGA (−)	
Babesia	*18S*	BS1	GACGGTAGGGTATTGGCCT (+)	[22]
		PiroC	CCAACAAAATAGAACCAAAGTCCTAC (−)	
		PiroA	ATTACCCAATCCTGACACAGGG (−)	
	ITS	ITS F	GAGAAGTCGTAACAAGGTTTCCG (+)	[30]
		ITS 2	ACAATTTGCGTTCAATCCCA (−)	
	cytb	Bgcytb-F	AGTGAAGGAAYTTGACAGGT (+)	[29]
		Bgcytb-R	CTTTCCTATTCCTTACGTAC (−)	
Borrelia	*flagellin*	Bor-flaF	TCACAAGCTTCTAGAAAYAC(+)	This study
		Bor-flaR1	GCYACAACCTCATCTGTCAT(−)	
		Bor-flaR2	ATYCTTTATRGAYTCAAGTCTAT(−)	
SFTSV	S gene	F1S	CAGCCACTTTACCCGAACAT(+)	[26]
		R1S	GGAAAGACGCAAAGGAGTGA(−)	
		F2S	CTGGTCTCTGCCCTCTCAAC(+)	
		R2S	GGATTGCAGTGGAGTTTGGTG(−)	
	L gene	F1L	GGCAGCAAACCAGAAGAAAG(+)	
		R1L	CATTTCTCCGAGGGCATTTA(−)	
		F2L	GGGTCTCCTGCTTAGCACAGG(+)	
		R2L	TCAGAFAAFACCCTGCCAGT(−)	
JMTV	segment 2	seg2-1279F	TCACGGGAAGGAGAGGGC(+)	[23]
		seg2-1630R	ACGGCCACAAGCACTGTTGG(−)	
	segment 4	seg4-1783R	ACTCCCATATCGCCTCTTCTGTC(+)	
		seg4-1243F	CTGGCCGTTGGGGTGTACCT(−)	
ALSV	segment 1	MiassF	GGTACACGGACCTGGGATCCTATTG(+)	[24]
		MiassR	TCTCTGACTCCTGTTCTAATC(−)	
	segment 2	Miass_gly_3F	TGGATCAGCTCACACCACAC(+)	
		Miass_gly_3R	TCACCGTCACAGTGGAATGG(−)	
TBEV	RNA polymerase	FSM-1	GAGGCTGAACAACTGCACGA(+)	[25]
		FSM-2i	GAACACGTCCATTCCTGATCT(−)	
		FSM-1	ACGGAACGTGACAAGGCTAG(+)	
		FSM-2i	GCTTGTTACCATCTTTGGAG(−)	

2. Materials and methods

2.1. Ethics statement

This study was approved by the ethics committee of the Chengde Medical University (No. 202004). Oral informed consent was obtained from all the humans who were bitten by ticks.

2.2. Collection of ticks and DNA/RNA extraction

Between 2019 and 2021, ticks were collected from humans with tick bites and without any symptoms who presented to the hospital of Weichang Manchu and Mongolian Autonomous County, Hebei Province, China. All the recovered ticks were stored at −80 °C. All ticks were adults, already engorged; they were firstly morphologically identified based on the taxonomical keys used for tick identification [15,16,17]. Furthermore, the tick species was confirmed by analyzing the partial cytochrome *c* oxidase I (*COI*) gene obtained by PCR with primers LCO1490 and HCO2198 [18].

All the tick specimens were washed three times with 75% alcohol and then two times with phosphate buffered saline (PBS). Both total DNA and RNA were extracted from the whole body of each tick using the Total DNA/RNA Isolation Kit (Omega, Norcross, GA, USA) according to the instructions. The DNA samples were eluted with 50 μL elution buffer and RNA samples with 25 μL RNase-free (DEPC-treated) water. All the DNA and RNA samples were stored in −80 °C before pathogens detection.

2.3. Screening and molecular characterization of pathogens

All the DNA samples were detected for the presence of tick-borne pathogens by Polymerase Chain Reaction (PCR). All the primers used in this study are shown in Table 1. Specifically, the Anaplasmataceae bacteria were detected by amplifying the partial 16S ribosomal RNA (rRNA) gene with primer pairs described by Rar et al. [19]. Semi-nested PCR was used to detect genus *Rickettsia* with the primer pairs targeting the *ompA* gene described previously [20,21]. Genus *Babesia* was detected with the primer pairs targeting the 18S rRNA gene described by Armstrong et al. [22]. Genus *Borrelia* was detected by amplifying the *flagellin* gene with the primer pairs Bor-flaF/Bor-flaR1 for the first round and Bor-flaF/Bor-flaR2 for the second, which were designed by ourselves. Viruses, including Severe fever with thrombocytopenia syndrome virus (SFTSV), Jinmen tick virus (JMTV), Alongshan virus (ALSV), and Tick-borne encephalitis virus (TBEV) were detected according to the methods described previously [23,24,25,26].

To better characterize the pathogens, the *citrate synthase* (*gltA*) gene of *Anaplasma* and *Rickettsia* was amplified by (semi-)nested PCR with the primers described previously, respectively [27,28]. The ITS region and *cytb* of genus *Babesia* were amplified using previously-mentioned primers [29,30].

For detection of genus *Borrelia* by semi-nested PCR, a 20 μL reaction mixture was used for the first round, including 10 μL *Premix Taq* (Takara, Dalian, China), 1.0 μL total DNA sample as template, 1.0 μL of each primer (10 pmol) and 7.0 μL ddH_2O. For the second round, the PCR reaction was performed in a 40 μL reaction mixture, containing 20 μL *Premix Taq* (Takara, Dalian, China), 2.0 μL of the first-round PCR product as template, 2.0 μL of each primer (10 pmol) and 14.0 μL ddH_2O. The same PCR amplifications were used for both rounds of the semi-nested PCR, including an initial denaturation performed at 94 °C for 5 min, followed by 30 cycles of 94 °C for 40 s, 52 °C for 40 s, and 72 °C for 50 s, and a final extension at 72 °C for 8 min.

In order to avoid contamination, the operations of DNA extraction, PCR mixture preparation, template addition, PCR amplification and electrophoresis were performed in a fume hood in five separated rooms and dedicated pipets and tips with filter elements inside were used in all operations. In addition, ddH_2O was used as a negative control.

After electrophoresis analysis, each PCR product of the expected size was purified using a MiniBEST Agarose Gel DNA Extraction Kit Ver.4.0 (TaKaRa, Dalian, China). After purification, the amplicons less than 700 bp were bidirectionally sequenced using the primers as for the PCR. Alternatively, the amplicons were also cloned into pMD19-T vector and positive clones were identified for further sequencing with universal M13-47 forward and RV-M reverse primers when clear double peaks were visible on the sequencing chromatograms. In addition, the amplicons greater than 700 bp were sequenced using the same strategy as those amplicons less than 700 bp with ambiguous sequencing results.

2.4. Nucleotide sequence identity and phylogenetic analyses

Bioedit v.7.1.11 was used to assemble and edit all sequences obtained in this study [31]. All the sequences were subjected to a BLASTN search against the GenBank database. The MegAlign program in Lasergene was used to calculate the nucleotide sequence identity [32]. The Maximum Likelihood tree was reconstructed using PhyML 3.0 [33] based on the best-fit evolutionary model (GTR+Γ+I) evaluated using MEGA 6.0.6 [34]. The bootstrap analysis was performed with 1000 replicates. The nucleotide sequences obtained in this study were deposited in GenBank under the accession numbers OP363198-OP363200, OP363203-OP363204, OP382364-OP382390.

3. Results

3.1. Identification of tick-borne pathogens

A total of 26 ticks removed from human bodies were collected and identified as *Ixodes persulcatus* (N = 13), *Dermacentor silvarum* (N = 9), and *Haemaphysalis concinna* (N = 4) by both morphology and molecular identification by analyzing the partial *COI* gene.

After amplification, sequencing of the PCR products and further blast showed that eleven ticks (42.3%) tested positive for at least one causative agent in the three above-mentioned ticks (Table 2). Totally, four pathogens were identified, namely *R. raoultii*, *Candidatus* R. tarasevichiae, *Bo. garinii* and *Ba. venatorum*. In addition, *A. ovis*, a potential zoonotic pathogen, was identified. Specifically, *A. ovis* was identified in one *I. persulcatus*. *Rickettsia raoultii* was detected in one *I. persulcatus*, three *D. silvarum* and one *H. concinna*, and *Candidatus* R. tarasevichiae in five *I. persulcatus*. *Borrelia garinii* was identified in three *I. persulcatus*, and *Ba. venatorum* in one *D. silvarum* and one *H. concinna*. In addition, one *H. concinna* tested positive for *Candidatus* R. principis, and one *I. persulcatus* positive for *Ba. caballi*. Because *Candidatus* R. principis belongs to the spotted fever group rickettsiae (SFGR), it has the potential to be pathogenic to humans and the positive sample was analyzed furtherly. In contrast, *Ba. caballi* has been confirmed to be non-pathogenic to humans and the positive sample was excluded for further analysis. Furthermore, co-infection with different pathogens in one tick was expected, being identified in three ticks (11.5%) (Table 2). Specifically, double infection with *Bo. garinii* and *Candidatus* R. tarasevichiae, was observed in two ticks, as well as *Ba. caballi* and *Candidatus* R. tarasevichiae in one tick. Moreover, quadruple infection with *Bo. garinii*, *Candidatus* R. tarasevichiae, *A. ovis*, and *Ba. venatorum* was also identified in one tick. However, no other pathogens were detected, even if *A. phagocytophilum*, *A. capra*, and *E. chaffeensis*, as well as SFTSV, are widely distributed in mainland China.

3.2. Molecular characterization of tick-borne pathogens

For *A. ovis*, one partial 16S rRNA gene sequence shared more than 99.0% nucleotide identities with those of *A. ovis*. Furthermore, the partial *gltA* gene sequence was also obtained, and presented the highest nucleotide identity of 99.6% with the strain Dongwangmang-goat-15 (MG869297) from Xi'an of China. Consistently, this sequence was most closely related to this strain in the phylogenetic tree (Fig. 1).

For genus *Rickettsia*, all eleven *ompA* gene sequences shared 74.6–100% nucleotide identities with each other. Specifically, five sequences shared 98.5–100% nucleotide identities with the known ones of *R. raoultii*, another five shared 99.0–100% nucleotide identities with the known ones of *Candidatus* R. tarasevichiae, and the last one shared 97.5–99.1% nucleotide identities with the known ones of *Candidatus* R. principis, respectively. Furthermore, the *gltA* gene sequences were also recovered from all the positive samples, and presented 90.8–100% nucleotide identities with each other. They shared 99.9% nucleotide identities with the three above-mentioned one validated and two candidate *Rickettsia* species, respectively. Interestingly, all the *ompA* gene sequences of *Candidatus* R. principis, including those herein, shared 97.6–98.3% nucleotide identities with that of *Candidatus* R. hongyuanensis clone tick61 from Sichuan Province of China [35]. Moreover, all the *gltA* gene sequences of *Candidatus* R. principis shared 100% nucleotide identities with that of *Candidatus* R. hongyuanensis clone tick61. In the phylogenetic tree of the polymorphisms from the *ompA* and *gltA* genes, eleven sequences were classified into three groups, corresponding to *R. raoultii*, *Candidatus* R. tarasevichiae, and *Candidatus* R. principis, respectively (Fig. 2). Especially, Weichang-HcRp18 clustered with both the strains Huaian-HF of *Candidatus* R. principis (from Jiangsu province of China) and *Candidatus* R. hongyuanensis clone tick61.

All three newly generated *flagellin* gene sequences from genus *Borrelia* had the highest nucleotide identities with the known sequences of *Bo. garinii* (98.0–100%). The remarkable thing is that the *flagellin* gene sequences of Weichang-IpBg5 and Weichang-IpBg11 shared the highest nucleotide identity with that of the strain JEM5 (D63370) from Japan, and Weichang-IpBg11 with the isolate BgVir from Russian (CP003151). In the phylogenetic tree, these three sequences also were clustered with the known *flagellin* gene sequences of *Bo. garinii* (Fig. 3). Specifically, Weichang-IpBg5 and Weichang-IpBg11 exhibited the closest relationship with the strain JEM5, and Weichang-IpBg11 with the isolate BgVir.

Two 18S rRNA gene sequences of *Ba. venatorum* shared 99.7% nucleotide identity with each other, and had the highest nucleotide identities with the known sequences of *Ba. venatorum* (99.7–100%). Furthermore, the partial ITS sequences recovered from these two positive samples shared 100% nucleotide identities with that of the *Ba. venatorum* strain HLJ602 (KU377434). Moreover, the partial *cytb* gene sequence was obtained from one positive sample and shared the highest nucleotide identities of approximately 81% with that

Table 2
Pathogens detected in different tick species.

Pathogens	Tick species		
	I. persulcatus	*D. silvarum*	*H. concinna*
A. ovis	number 5	–	–
R. raoultii	number 6	numbers 10, 13, 22	number 12
Candidatus R. tarasevichiae	numbers 5, 9, 11, 15 and 16	–	–
Candidatus R. principis	–	–	number 18
Bo. garinii	numbers 5, 11 and 16	–	–
Ba. venatorum	number 5	–	number 3

"-" indicates that ticks tested negative for the pathogen.

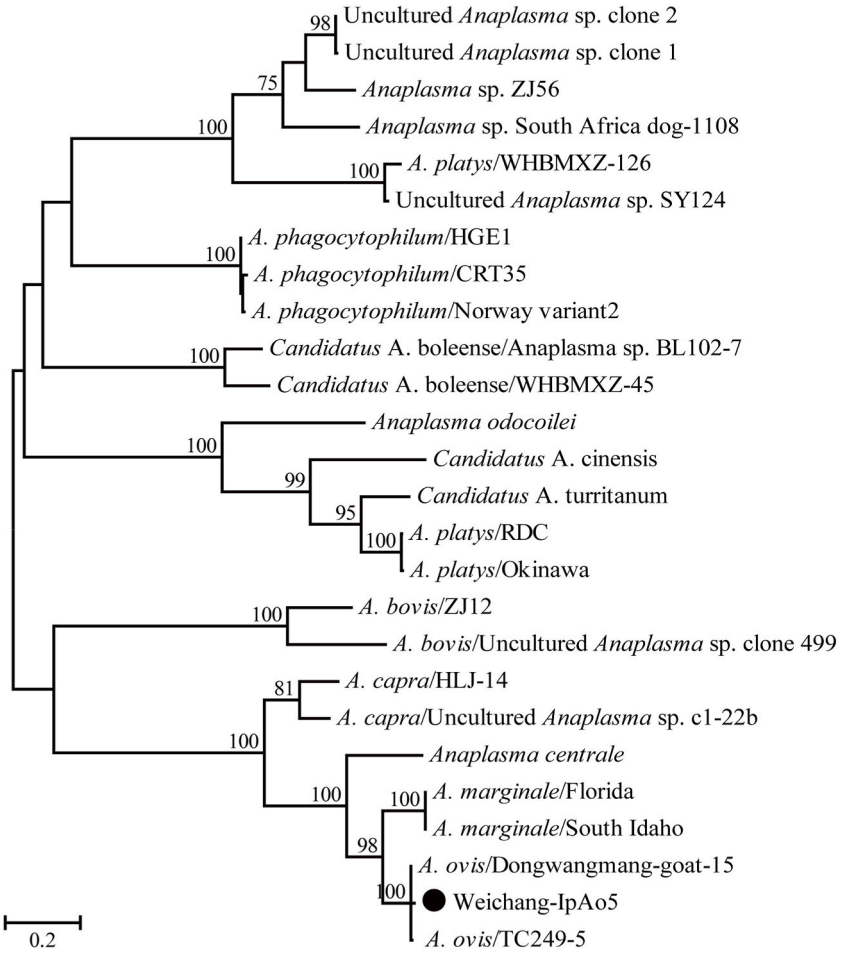

Fig. 1. Phylogenetic tree of the *gltA* gene sequences of the genus *Anaplasma*. Numbers at each node indicate bootstrap values. The tree was mid-point rooted for clarity and the scale bar represents the number of nucleotide substitutions per site. The taxa marked by circles depict taxa that provided sequences obtained in this study.

of *Ba. gibsoni* due to the absence of *cytb* gene sequence of *Ba. venatorum* in GenBank database. Consistent with the similarity analysis, the 18S gene sequences recovered in this study were clustered with *Ba. venatorum* in the phylogenetic tree (Fig. 4).

4. Discussion

This is the first study performed to investigate the presence of tick-borne human pathogens in human-parasitizing ticks in China. In previous study, except for *R. raoultii*, *Candidatus* R. tarasevichiae, *Candidatus* R. hebeiii, and *Bo. burgdorferi*, no other tick-borne human pathogens have been reported in Hebei Province, China, although at least 14 tick species exist [2,3]. Hence, molecular identification of tick-borne human pathogens in tick are needed. Our results provide the first evidence of the presence of *Anaplasma* and *Babesia* species pathogenic to humans in Hebei province, China, and also suggest *Rickettsia* spp. were the most common human causative agents. Our findings provide insights into the distribution and endemicity of these tick-borne agents. In addition, co-infection with different pathogens in one tick was observed. Over the past decade, a great number of metagenomic studies in ticks were performed and diverse viruses, bacterial and protozoa were identified, and these studies have mainly focused on the virus identification [36,37,38,39,40,41]. However, the majority of these tick-borne microorganisms are not pathogenic to humans. Therefore, in this study, the pathogens were identified by PCR targeting the specific causative agent from ticks parasitizing humans.

In this study, *R. raoultii* was mainly identified in *D. silvarum*, consistent with the close association between *R. raoultii* and *Dermacentor* spp [42]. In addition, *R. raoultii* identified *I. persulcatus* and *H. concinna* herein, in line with previous studies that *R. raoultii* has been identified in Heilongjiang Province of China, and Russian Far East [43,44]. Consistent with the close association between *Candidatus* R. tarasevichiae and *I. persulcatus* [2,3], all *Candidatus* R. tarasevichiae were identified in *I. persulcatus* tick in this study. In the current study, only *A. ovis* was identified from one *I. persulcatus*, *Ba. venatorum* in *I. persulcatus* and *Ha. concinna*, *Bo. garinii* in *I. persulcatus*, consistent with the previous studies [3,45].

Candidatus R. principis, a *Rickettsia* species of unknown pathogenicity, was first identified in *H. japonica* in Russian Far East [46].

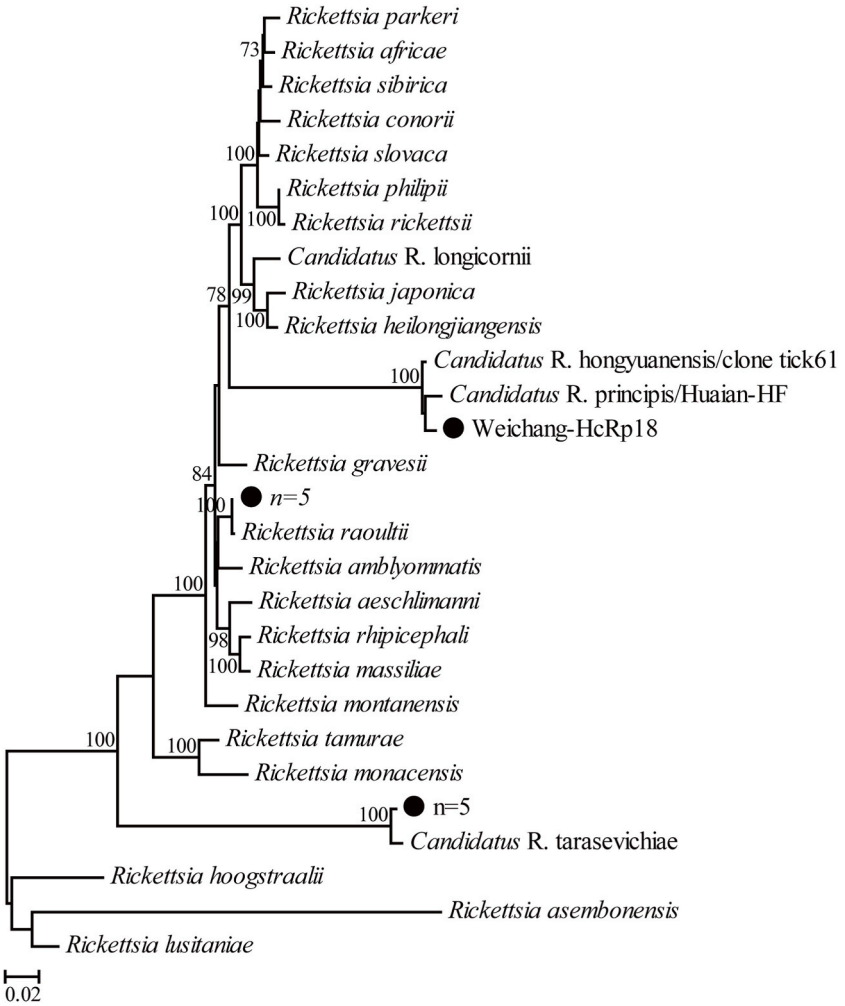

Fig. 2. Phylogenetic tree based on the polymorphisms from the *ompA* and *gltA* genes sequences of the genus *Rickettsia*. The legend follows that of Fig. 1.

Since then, this candidate *Rickettsia* species was found in *H. concinna* and *H. japonica* ticks again [44]. In China, it was identified in *H. qinghaiensis* in Gansu [47], *H. danieli* in Xinjiang Uygur Autonomous Region [48] and *H. flava* in Jiangsu [49]. In this study, *Candidatus* R. principis was identified in *H. concinna* in Hebei province. In 2022, *Candidatus* R. hongyuanensis, a novel *Rickettsia* species was identified in *H. qinghaiensis* in Sichuan, Southwest China [35]. Based on the criteria suggested by Fournier et al. [50] and nucleotide identity of the *gltA* and *ompA* genes between *Candidatus* R. hongyuanensis and *Candidatus* R. principis, these two candidate *Rickettsia* species are actually the same species. Totally, *Candidatus* R. principis has been identified in northern and southwest China, consistent with the distribution of the tick vectors. In addition, the above-mentioned *Haemaphysalis* spp., the vectors of *Candidatus* R. principis, are widely distributed in northern China other than Hebei, Gansu, Jiangsu provinces and Xinjiang Uygur Autonomous Region [51], hence, this candidate *Rickettsia* species may be widely distributed in northern China than it has been identified. Moreover, although *Candidatus* R. principis is not associated with human cases, it belongs to Spotted fever group Rickettsiae (SFGR) in genus *Rickettsia*, most of which are pathogenic to humans. Therefore, its pathogenicity should be further assessed.

In China, *R. heilongjiangensis*, *R. sibirica*, *R. monacensis*, *A. phagocytophilum*, *A. capra*, *E. chaffeensis*, *Candidatus* N. mikurensis, *Bo. burgdorferi*, *Bo. afzelii*, *Bo. bavariensis*, *Bo. valaisiana, Bo. miyamotoi*, *Bo. bissettii*, *Ba. microti*, *Ba. divergens*, *Ba. crassa*-like, TBEV, SFSTV, JMTV, and ALSV have been identified in *I. persulcatus*; *R. heilongjiangensis*, *R. sibirica*, *R. slovaca*, *A. phagocytophilum*, *E. chaffeensis*, *Candidatus* N. mikurensis, *Bo. burgdorferi*, *Bo. afzelii*, *Bo. bavariensis*, SFSTV, and ALSV identified in *D. silvarum*; and *R. heilongjiangensis*, *A. phagocytophilum*, *E. chaffeensis*, *Candidatus* N. mikurensis, *Bo. burgdorferi*, *Bo. afzelii*, *Bo. bavariensis*, *Bo. miyamotoi*, *Ba. microti*, *Ba. divergens*, *Ba. crassa*-like, TBEV, SFSTV, and JMTV identified in *H. concinna* [2,3]. However, none of them were detected in this study probably due to the limited number of tick samples, which causes an incomplete picture of the human pathogens in local ticks and makes it impossible to analyze the prevalence elements of the identified pathogens. Hence, more tick specimens should be collected to determine their circulation. In this study, another limitation is that no sample was collected from humans bitten by ticks; therefore, further epidemiological studies in the human populations are needed to assess the scope of this threat to human health.

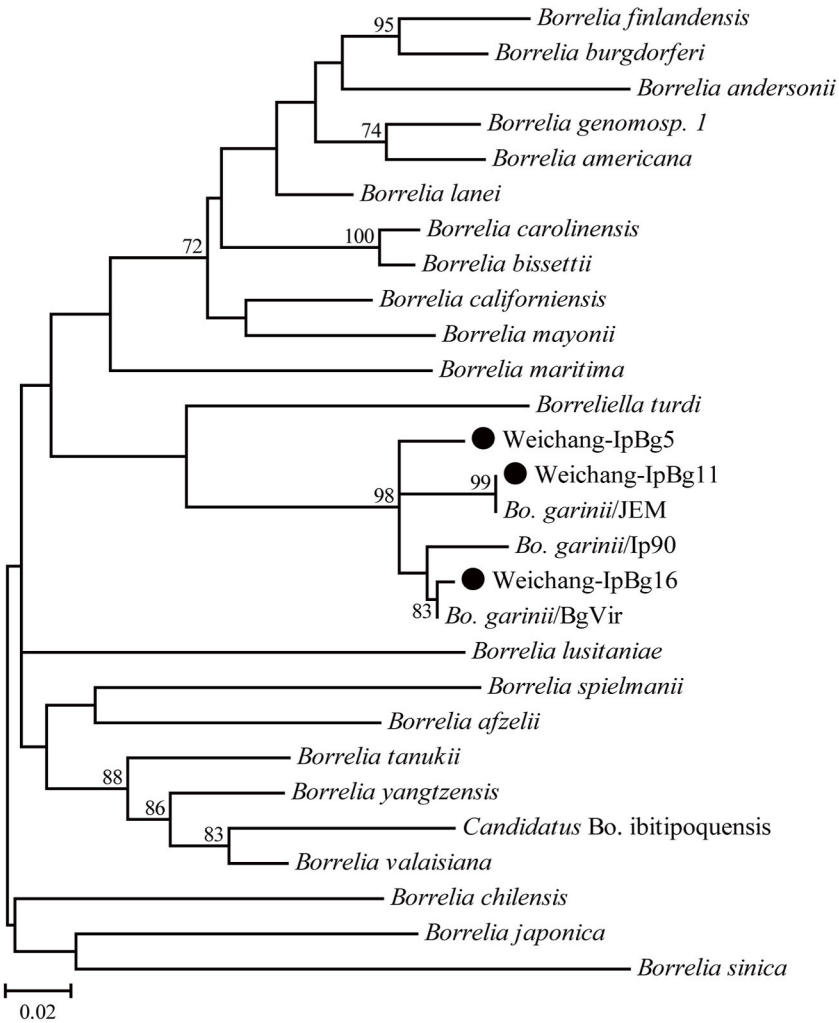

Fig. 3. Phylogenetic tree based on the *flagellin* gene sequences of the genus *Borrelia*. The legend follows that of Fig. 1.

5. Conclusion

In summary, molecular identification of human pathogens in the limited number of ticks collected from humans was performed and the results reveal the presence of four validated tick-borne causative agents, namely *R. roaultii*, *Candidatus* R. tarasevichiae, *Ba. venatorum* and *Bo. garinii*, and *A. ovis* with potential zoonotic, in Hebei, China. Our results suggest that all these causative agents could have posed a public health threat in the human population in Hebei Province, China, and would be helpful for both the diagnosis and prevention of tick-borne disease.

Author contribution statement

Jing Xue: Performed the experiments; Analyzed and interpreted the data; wrote the paper.
Qing Ren: Performed the experiments.
Xiu-Li Yang: Performed the experiments.
Jiangli Wang: Performed the experiments.
Guangcheng Xie: Analyzed and interpreted the data.
Luanying Du: Analyzed and interpreted the data.
Wen-Ping Guo: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

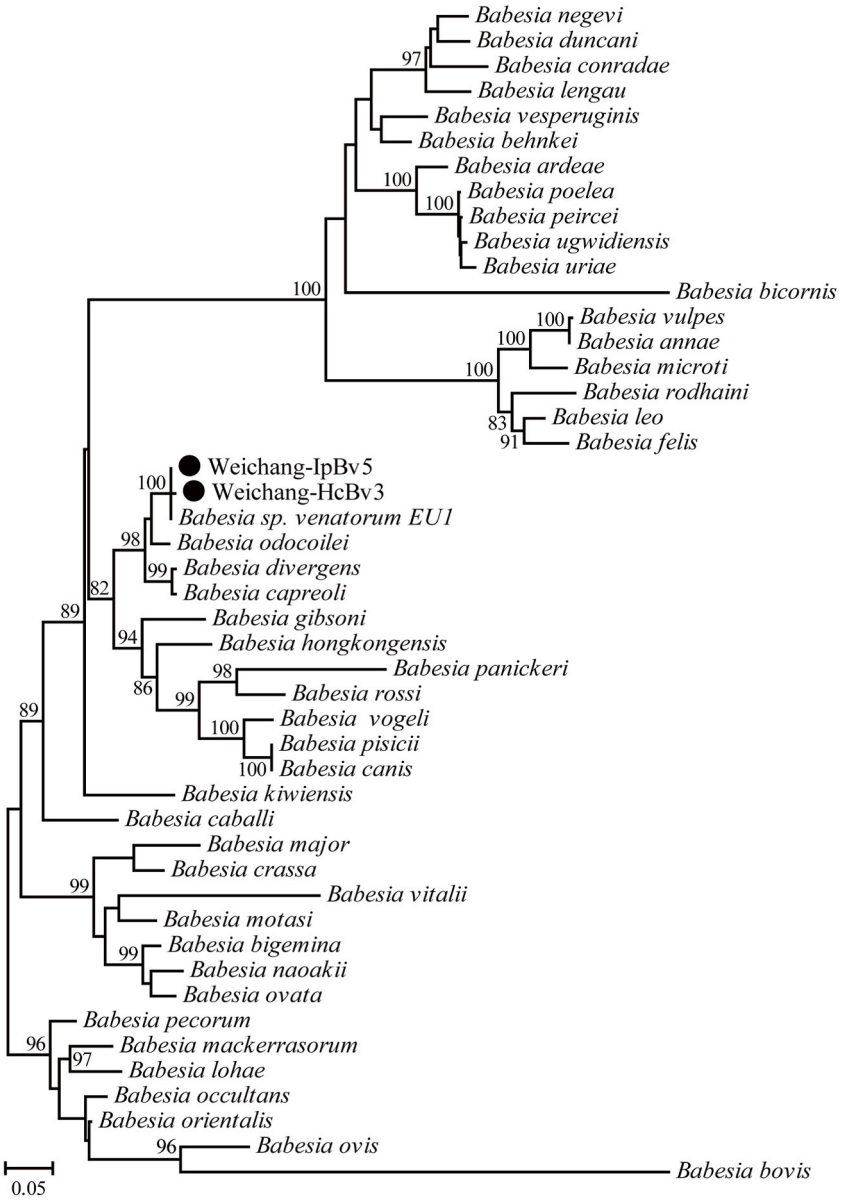

Fig. 4. Phylogenetic tree based on the 18S gene sequences of the genus *Babesia*. The legend follows that of Fig. 1.

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Data availability statement

Data associated with this study has been deposited at GenBank under the accession numbers OP363198-OP363200, OP363203-OP363204, OP382364-OP382390.

Declaration of interest's statement

The authors declare no competing interests.

Additional information

No additional information is available for this paper.

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