Prevalence And Phylogenetic Analysis of Babesia Parasites In Host Animals In Fujian Province, Southeast China

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Research Article

Keywords: Babesia, host animals, risk factor, phylogenetic tree, Southeast China

Posted Date: August 27th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-793814/v1

Abstract

Background: Babesiosis is a tick-borne infectious disease of socio-economic importance. The clinical manifestations of babesiosis are usually intermittent fever, headache, chills, sweats, and myalgia. *Babesia* infections in small mammals and ticks have been reported in at least five provinces in China. However, the host range and geographical distribution of this parasite in Fujian Province are unclear. The aim of this study was to investigate the prevalence and genetic characteristics of *Babesia* in Fujian Province, Southeast China, between 2015 and 2020.

Methods: Rodent blood samples were collected from 26 different surveillance sites across Fujian Province. Genomic DNA was extracted to screen for *Babesia* infection via PCR amplification based on 18S rRNA. The prevalence of *Babesia* was compared using the chi-square test or Fisher's exact test. A phylogenetic tree was constructed using MEGA 5.0 by gene sequence alignment. DNA samples from 316 domestic goats, 85 water buffalo, 56 domestic dogs, and 18 domestic pigs were also collected in this survey.

Results: *Babesia* infection was confirmed in 3.96% (43/1087) of rodents and in 1.26% (6/475) of other mammals. Multivariate logistic regression analysis revealed that irrigated cropland, shrub, and forest were risk factors for *Babesia microti* infection. The infection rates of domestic pigs, dogs, and goats were determined to be 5.56%, 1.79%, and 1.27%, respectively, and no infection was found in water buffalo. Sequencing using the 18S rRNA gene revealed that rodents were infected with *Babesia* (*sensu lato*) while other mammals were infected with *Babesia* (*sensu stricto*).

Conclusions: The results indicate that there is a broad geographical distribution and phylogenetic relationship of *Babesia* in Southeast China. This study suggests that mammals, especially wild rodents, are the main natural hosts of *Babesia* in Fujian. Our research provides new insights into the exposure risk of *Babesia* in humans and animals, laying a solid foundation for the development of babesiosis prevention and control measures.

Background

Human babesiosis is an emerging, tick-transmitted zoonotic disease worldwide [1]. It poses a serious threat to public health with great economic, veterinary, and medical significance worldwide [2]. Babesiosis is caused by intraerythrocytic sporozoites of the genus *Babesia*, which infect animals (both wild and domestic) and humans [3, 4].

The first human case was reported in 1957 in Zagreb, Croatia, and subsequently found in all continents, with the exception of Antarctica. Infections are primarily found in tropical and subtropical areas [5, 6]. During the past few decades, an increasing number of *Babesia microti* species have been reported in the upper midwestern and northeastern regions of the United States [7, 8], where babesiosis was prevalent. In Europe, *Babesia divergens* is responsible for most cases of babesiosis and the parasites are transmitted by *Ixodes ricinus* ticks and infected bovines [9, 10]. Endemic infections of *B. microti* in rodents and ticks

have recently been detected in European countries, including Slovakia [11], Finland [12] and Belgium [13]. Cases have also been recorded in China [14], South Africa, India, and Australia.

Babesia parasites have a wide range of vertebrate hosts including rodents, horses, goats, cattle, dogs, cats, and humans [15]. More than 100 different *Babesia* species have been discovered; however, only a few can infect humans, including *B. microti*, *B. divergens*, *B. venatorum*, and *B. duncani* [16]. As the main etiological agent of human babesiosis, the rodent parasite *B. microti* is maintained through an enzootic cycle in nature, which involves ixodid ticks and small mammals [9, 17]. Clinical characterization of babesiosis ranges from asymptomatic infection to severe morbidity and death, including fever, chills, headache, fatigue, anemia, jaundice, thrombocytopenia, hemolysis, hemoglobinuria, and even MODS [7]. The susceptibility to *Babesia* infection is usually related to the age and immunity of the hosts. Neonates, people of advanced age, those undergoing immunosuppressive therapy, and individuals with HIV/AIDS or cancer are more susceptible to infection [10, 18]. Due to a lack of medical awareness, effective diagnosis technologies, and the low incidence of the disease, babesiosis is often neglected in China [16, 19]. To date, *B. microti*-like organisms have been reported in humans from Taiwan [20] and Yunnan [21], and *B. microti*-like parasites have been found in small mammals and hard ticks in Yunnan [22], Beijing [1], Taiwan [17], Heilongjiang [23] and Henan [24].

Fujian Province, located on the southeast coast of China, belongs to the subtropical climate zone and covers 124,000 square kilometers of land and 136,000 square kilometers of ocean. With sufficient rainfall, abundant sunshine, and the best forest coverage of 65.95% in China, the natural and geographical environments of Fujian Province provide ideal habitats for *Babesia* and favorable conditions for the spread of tick-borne diseases. The aim of this study was to investigate the infection prevalence and phylogenetic relationship of *Babesia* in mammals in eight cities of Fujian Province, where the host species are abundant.

Methods

Sample Collection

A total of 1087 rodents were captured using animal snap traps from eight cities in Fujian Province between 2015 and 2020. The sampling sites included four different habitat environments: residential area, irrigated cropland, shrub, and forest. Traps were placed for three continuous nights at locations where rodent activities were detected and then retrieved the following morning. Chinese monographs were used to identify the species of trapped rodents according to their morphology [25, 26]. The sex, developmental stage, and ecological habitat of the mammals were also recorded. Some rare rodent species have been identified using DNA barcoding technology [27]. After ether inhalation anesthesia and disinfection, the blood of the rodents was collected through cardiac puncture and stored at -80°C for further tests. Blood samples from 316 domestic goats, 85 water buffalo, 56 domestic dogs, and 18 domestic pigs were collected in this survey.

DNA extraction

Genomic DNA was extracted from the blood of the animals described above using the Blood Genomic DNA Kit (Tagene Biotechnology, Xiamen, China), according to the manufacturer's instructions for animal blood. The genomic DNA was dissolved in 100 µl elution buffer and stored at -20℃ for further use.

Detection of *Babesia* infection by polymerase chain reaction

Conventional PCR amplification of the specific fragment of the *Babesia* 18S rRNA gene region was performed using the following primers: PIRO-A, 5'-AATACCCAATCCTGACACAGGG-3', PIRO-B, 5'-TTAAATACGAATGCCCCCAAC-3' [28]. Target DNA amplification was carried out under the following conditions: 94 °C for 5 min; 40 cycles of 94 °C for 45 s, 55 °C for 45 s, and 72 °C for 45 s; followed by a final extension step at 72 °C for 5 min. The reaction used 5 µl of genomic DNA as template in 25 µl reaction mixture containing 12.5 µl Premix Taq (TaKaRa Taq Version 2.0 plus dye) and 1.0 µl of each primer (final concentration 0.4 µM). Negative and positive controls were added throughout the experiment to exclude the possibility of contamination. Amplification products were subjected to electrophoresis on a 1.5% agarose gel stained with gold and visualized under UV light. Positive PCR products were sequenced by Sangon Biotechnology Company.

Phylogenetic analysis

The sequences were assembled using the SeqMan program software 7.0.1 (DNASTAR, Inc.; Madison, WI, USA). All newly generated sequences were subjected to BLAST analysis against the GenBank database. Phylogenetic trees were constructed using the neighbor-joining (NJ) method, with 1000 replications for bootstrap tests. All other parameters were set at default.

Statistical analysis

A spatial map of the prevalence of *Babesia* in rodents was drawn using ArcGIS 10.3.1. The geographic data used the vector map of the administrative divisions of the county boundaries of Fujian province (1:1000000), and the latitude and longitude were retrieved from Google Maps. The association between rodent species, sex, developmental stage, habitat environments, sampling locations, and *Babesia* infection were analyzed using univariate analysis based on the Chi-square test or Fisher's exact test. Multivariate logistic regression was used to analyze the risk factors for *Babesia microti* infection. All analyses were conducted using SPSS software (version 20.0, SPSS Inc. Chicago, IL). The significance level for all results was set at $p < 0.05$.

Sequences Used for Phylogenetic analysis in the Study

To clarify the phylogenetic relationship of *Babesia* species detected in host animals collected in Fujian Province, sequences of the 18S rRNA gene fragments were used for alignment and comparison to the sequences from the GenBank database and are summarized in Additional file 2: Table S2, with additional sequence details if available (species, strains, hosts, years, countries, regions, and GenBank accession numbers).

Results

The prevalence of Babesia microti in rodents captured from different cities in Fujian province

A total of 1087 rodents were captured from 26 surveillance points in eight cities in Fujian Province (Figs. 1 and 2, Table 1, Additional file 1: Table S1). The captured rodents belonged to Rodentia, including two families, seven genera, and twelve species (Table 2). Sequencing analysis by blastn showed that 3.96% (43/1087) of rodents were infected by *B. microti* (Table 1).

Table 1
The prevalence of *B. microti* in rodents captured from eight cities in Fujian Province

Cities	No. of traps	No. of rodents tested	No. of positive for *B. microti*	Density (%)	Positive rate (%)	Odd ratio
Sanming	2307	171	17	7.41	9.94	5.96
Ningde	2549	209	15	8.20	7.18	4.18
Nanping	3922	330	8	8.41	2.42	1.34
Fuzhou	1790	165	3	9.22	1.82	1.00
Putian	355	30	0	8.45	0.00	
Quanzhou	1549	110	0	7.10	0.00	
Zhangzhou	439	41	0	9.34	0.00	
Longyan	584	31	0	5.31	0.00	
Total	13495	1087	43	8.05	3.96	

Table 2
The prevalence of *Babesia* in host animals of different species

Orders	Families	Genera	Species	No. of examined	No. of positive (%)
Rodentia	Muridae	*Rattus*	*R. norvegicus*	337	3(0.89)
			R. losea	215	3(1.40)
			R. tanezumi	163	1(0.61)
			R. edwardsi	17	1(5.88)
			R. rattus	4	0(0.00)
		Apodemus	*A. agrarius*	32	1(3.13)
		Mus	*M. musculus*	5	1(20.00)
		Niviventer	*N. confucianus*	47	8(17.02)
			N. fulvescens	152	7(4.61)
		Berylmys	*B. bowersi*	50	1(2.00)
		Bandicota	*B. indica*	56	17(30.36)
	Cricetidae	*Microtus*	*M. fortis*	9	0(0.00)
Artiodactyla	Bovidae	*Capra*	*C. a. hircus*	316	4(1.27)
		Bos	*B. bubalis*	85	0(0.00)
	Suidae	*Sus*	*S. s. domesticus*	18	1(5.56)
Carnivora	Canidae	*Canis*	*C. l. familiaris*	56	1(1.79)

Of the 12 species of trapped rodents, the brown rat (*Rattus norvegicus*) accounted for the most (30.00%, n = 337), followed by *Rattus losea* (19.78%, n = 215), whilst *Rattus rattus* accounted for the least (0.37%, n = 4). With the exception of *Microtus fortis* and *R. rattus*, 10 of 12 species tested positive for *B. microti* infection. The positive infection rates of *B. microti* ranged from 0.61% (1/163) in *Rattus tanezumi* to 30.36% (17/56) in *Bandicota indica* (Table 2). There was no significant difference in the prevalence of *B. microti* in the common domestic rats from residential areas (*R. norvegicus* and *R. tanezumi* ($P = 0.739$)). Within the wild rodents, (from irrigated cropland, shrub, and forest), the sum of *B. microti* infection rates in the rats *B. indica* and *Niviventer confucianus* was 24.27%, which was significantly higher than that of other species of rodents ($\chi^2 = 66.003$, $P = 0.000$).

Infected rodents were caught from four cities, including Sanming, Ningde, Nanping, and Fuzhou (Table 1). Rodents collected from Sanming had the highest *B. microti* infection rate of 9.94% (17/171). *B. microti* infection rates in hosts from Sanming and Ningde were both significantly higher than in hosts captured in Fuzhou (odds ratios: 5.96, 4.18, respectively; $P < 0.05$) (Table 1).

The prevalence of Babesia in domestic animals in Fujian

Blood samples from 316 domestic goats, 85 water buffalo, 56 domestic dogs, and 18 domestic pigs were also collected for the detection of *Babesia* infection. Surprisingly, no water buffaloes were infected with *Babesia* (Table 2). There was no significant difference in the prevalence of *Babesia* between male and female domestic goats ($P = 0.129$) (not shown in the table).

The risk factors associated with B. microti infection

Risk factors related to *B. microti* infection in rodents were analyzed with respect to sex, age, and ecological habitat (Table 3). There was no significant difference in the prevalence of *B. microti* between male and female rodents ($\chi^2 = 0.466$, $P = 0.495$). However, the prevalence of *B. microti* in adult rodents (4.53%) was significantly higher ($\chi^2 = 4.645$, $P = 0.031$) than in pubertal rodents (1.10%). It is worth noting that the prevalence of *B. microti* in mammals from irrigated cropland, shrub, and forest, which were 4.70%, 11.18%, and 4.55%, respectively, were all significantly higher than those in rodents from residential areas ($P < 0.05$, Tables 3 and 4). Furthermore, the multivariate logistic regression analysis suggested that irrigated cropland, shrub, and forest were risk factors for *B. microti* infection (Table 4).

Table 3
Risk factors related to *Babesia microti* based on univariate analyses

Variable	Simple size		*Babesia microti* infection		
	cases	constituent ratio (%)	positive rate (%)	χ^2	P-value
gender					
male	561	51.61	3.57	0.466	0.495
female	526	48.39	4.37		
age					
pubertal	181	16.65	1.10	4.645	0.031
adult	906	83.35	4.53		
habitat					
residential areas	504	46.36	0.99	35.438	0.000
irrigated cropland	149	13.71	4.70		
shrub	170	15.64	11.18		
forest	264	24.29	4.55		

Table 4
Risk factors related to *Babesia microti* infection based on multivariate logistic regression

Variable	OR(95% *CI*)	*P*-value
gender		
male	1	
female	0.728 (0.390–1.360)	0.319
age		
pubertal	1	
adult	0.307(0.072–1.304)	0.110
habitat		
residential areas	1	
irrigated cropland	0.198 (0.061–0.635)	0.006
shrub	0.084(0.031–0.231)	0.000
forest	0.200(0.070–0.576)	0.003

Genetic and phylogenetic analysis of Babesia **species**

Gene sequencing of the 18S rRNA gene from the positive samples found 43 samples containing *B. microti*, five containing *Babesia sp.*, and one containing *Babesia canis vogeli*. In order to construct the phylogenetic tree, the 18S rRNA gene sequences of another 18 isolates *of B. microti* from other regions were included for comparison. *Babesia sp. venatorum* from Heilongjiang, *Babesia sp.* XXB/Hangzhou from Zhejiang and *B. divergens* from Ireland were used as the outgroup. All *B. microti* sequences from infected rodents shared 100% homology with sequences from Japan (AB032434.1). The sequence was deposited in GenBank with accession number MZ619064. Phylogenetic analyzes revealed that MZ619064 belonged to Kobe-type (Fig. 3).

The sequences of 18S rRNA genes with different *Babesia* species were used to reveal the phylogenetic relationship of *Babesia* identified in this study. *Toxoplasma gondii* (L24381.1) from Australia was used as an outgroup. *Babesia canis vogeli* detected in the domestic dog was identical to the sequences from Cote d' Ivoire (MK495837.1) and Brazil (KU662365.1). Both domestic pigs and domestic goats in Fujian were infected with *Babesia sp.*, and their homology was 98.17%. The sequences of *B. canis vogeli* from *Canis lupus familiaris*, *Babesia sp.* from *Sus scrofa domesticus*, and *Babesia sp.* from *Capra aegagrus hircus* in this survey were deposited in GenBank with accession numbers MZ618690, MZ619045, and MZ619046, respectively. Phylogenetic analyzes suggested that MZ618690, MZ619045, and MZ619046 belonged to *Babesia* (*sensu stricto*), while MZ619064 belonged to *Babesia* (*sensu lato*) (Fig. 4).

Discussion

Our research systematically illustrated the wide prevalence and phylogenetic relationship of *Babesia* in host animals in Fujian Province, Southeast China. Infections of *B. microti* parasites were observed in four cities and eight sampling sites in Fujian Province (Figs. 1, 2, Table 1). *Babesia microti* has been reported in small mammals in Beijing [1], Henan [24], Yunnan [22] and Taiwan [17]. *B. microti* infection was also previously reported in the Wuyi Mountain area, Fujian [29]; however, the epidemiological features of *Babesia* remain unclear in other cities in Fujian. The high prevalence of *B. microti* infection in rodents in Ningde and Sanming in this survey strongly supports the hypothesis that these surveillance points are major natural foci for human babesiosis. Furthermore, the results call for close monitoring of the *B. microti* transmissions in Ningde and Sanming, while the epidemic of *B. microti* in other cities should not be ignored. It should be noted that the *B. microti* infection rates in Putian, Quanzhou, Zhangzhou, and Longyan were zero, which may be attributed to the insufficient samples and the habitat of trapped rodents (Table 4). Obviously, the prevalence of *B. microti* was vary from district to district. Although both Fuzhou and Quanzhou are adjacent to Sanming, their *B. microti* infection rates were both lower than 5.00%, while Sanming had the highest infection rate. This may be attributed to the distribution and density of the host animals.

Interestingly, the infection rate of *Babesia* in Xiapu District, Ningde City, was 15.79%, which may provide a novel clue to the first human case of babesiosis in Fujian [30]. The patient who was diagnosed with *B. microti* infection lived and worked in a village in Xiapu, Ningde, which was surrounded by abundant shrubs and forests. Our study revealed that the prevalence of *B. microti* in rodents from irrigated croplands, shrubs, and forests was significantly higher than in the residential areas, suggesting that the ecological habitat types played an important role in the spread of *B. microti* (Tables 4 and 5). It has been reported that forest is an essential risk factor for *Babesia* infection in Thailand, Cambodia, Lao PDR, and China (Yunnan and Heilongjiang) [19, 22, 23]. Considering that forest areas are burdened with tick-transmitted pathogens, people who work in or travel to the forests should take appropriate protective measures. *Babesia* and *Plasmodium* are both intraerythrocytic protozoans and elicit similar inflammatory responses with similar clinical symptoms, which renders them to be easily misdiagnosed [14]. In summary, doctors should pay attention to human babesiosis, while public health agencies should formulate prevention and control measures urgently.

Our study revealed that all the collected *Babesia* parasites were *B. microti* in rodents in Fujian Province. This can be concluded based on the abundance of samples detected, similar to previous findings in Yunnan [16, 22], Taiwan [17] and Beijing [1]. Phylogenetic analysis suggested that *B. microti* in this survey shared high homology with those in Zhejiang province, where a confirmed human babesiosis case was reported in Hangzhou in 2002 following a kidney transplantation [29]. Surprisingly, no water buffaloes were infected with *Babesia bovis* or *Babesia bigemina*. In addition, both domestic goats and domestic pigs were infected with *Babesia sp.*. Meanwhile, because of insufficient samples from a single sampling location, hosts such as cattle should be investigated further. We detected *B. canis vogeli* from the blood of domestic dogs for the first time and the sequence shares high homology with *B. canis vogeli* from Cote

d' Ivoire (GenBank MK495837.1). The prevalence of *Babesia canis* in dogs has been previously documented in Henan province [31].

Ovine babesiosis is a tick-borne disease in goats, sheep, and cattle, posing a huge threat to the livestock industry [32, 33]. Although the infection rate of ovine babesiosis is extremely low in this study, relevant institutions should pay more attention and strengthen quarantine for early detection and treatment. *Babesia* infection can also be transmitted through blood transfusion when the infected is asymptomatic or in the latent period of the infection [18]. Therefore, it is necessary to test *Babesia* infection in donors when evaluating the risk of blood transfusion. In the future, we will investigate the prevalence of *Babesia* in different ticks and blood donors to provide scientific evidence for preventing and controlling babesiosis epidemics.

There are some limitations in our study. Nested PCR approach was used to investigate the rates of *Babesia* parasites in mammals in some reports [16, 19]. Because of we had a large number of samples (a total of 1562 samples in this survey), with a high risk of contaminations during nested PCR, we did not use nested PCR method. In addition, there was no significant difference between conventional PCR and nested PCR in sensitivity and specificity[34]. It was reported that the altitude was a risk factor association with *Babesia* infection [1, 22]. But the altitude was neglected to record in our study. With the development of economy and the improvement of living standards, the number of pet dogs and cats have increased. There were some cases of *B. vogeli* found in dogs and cats in China [2, 15, 31]. So it is necessary to monitor the epidemics of *Babesia* infection in pet dogs and cats further.

Conclusions

Our study suggests a wide distribution and phylogenetic relationship of *Babesia* in mammals in Fujian Province, Southeast China. This research provided basic data to support public health authorities in developing prevention measures and other control measures. Due to insufficient samples from a single sampling surveillance, hosts such as livestock animals should be investigated further. Human babesiosis is a tick-borne disease that is transmitted through blood transfusions. Therefore, it is necessary to survey the prevalence of *Babesia* in different tick and donor populations.

Abbreviations

DNA: deoxyribonucleic acid; 18S rRNA: 18 Svedberg ribosomal RNA; PCR: polymerase chain reaction;

CI: confidence interval; MODS: multiple organ dysfunction syndrome; HIV: human immunodeficiency virus; AIDS: acquired immune deficiency syndrome; UV: ultraviolet tray.

Declarations

Acknowledgements

We would like to thank all the participants for their commitment. We would like to thank Editage (www.editage.cn) for English language editing. We also thank the staff of the Centers for Disease Control and Prevention of Fuzhou District, Putian District, Quanzhou District, Zhangzhou District, Longyan District, Sanming District and Nanping District, Ningde District, Fujian, China for their assistance in collecting samples.

Authors' contributions

FZX and YQD designed the study and contributed to reviewing the manuscript. ZWZ drafted the manuscript, performed the statistical analysis, and participated in the sampling collection. SHZ, WJL, TWH, GYX, JL, and JXW conducted the molecular biological detection and sampling acquisition and identified the host animal species. All authors read and approved the final manuscript.

Funding

This work was sponsored by the National Science and Technology Major Project of China (No.2018ZX10734401-007), Fujian Provincial Science and Technology Innovation Platform Construction Project (2019Y2001), and Fujian Provincial Health Technology Project (2019-ZQN-27). The funders had no role in the design of the study, collection, analysis, and interpretation of data, or in writing the manuscript.

Availability of data and materials

The data collected and analyzed during the current study are available from the corresponding author upon reasonable request. Please contact the author for the data requests.

Ethics approval and consent to participate

The research protocol, which involved trapping wild and domestic animals, was approved by the Laboratory Animal Welfare Ethical Review Committee of Fujian Provincial Center for Disease Control and Prevention (FJCDC) (permission number: FJCDCNT1811-2015). All animal experiments were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals.

Consent for publication

Not applicable.

Competing interests

The authors have declared that no competing interests exist.

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Table

Table 5 is not available with this version

Figures

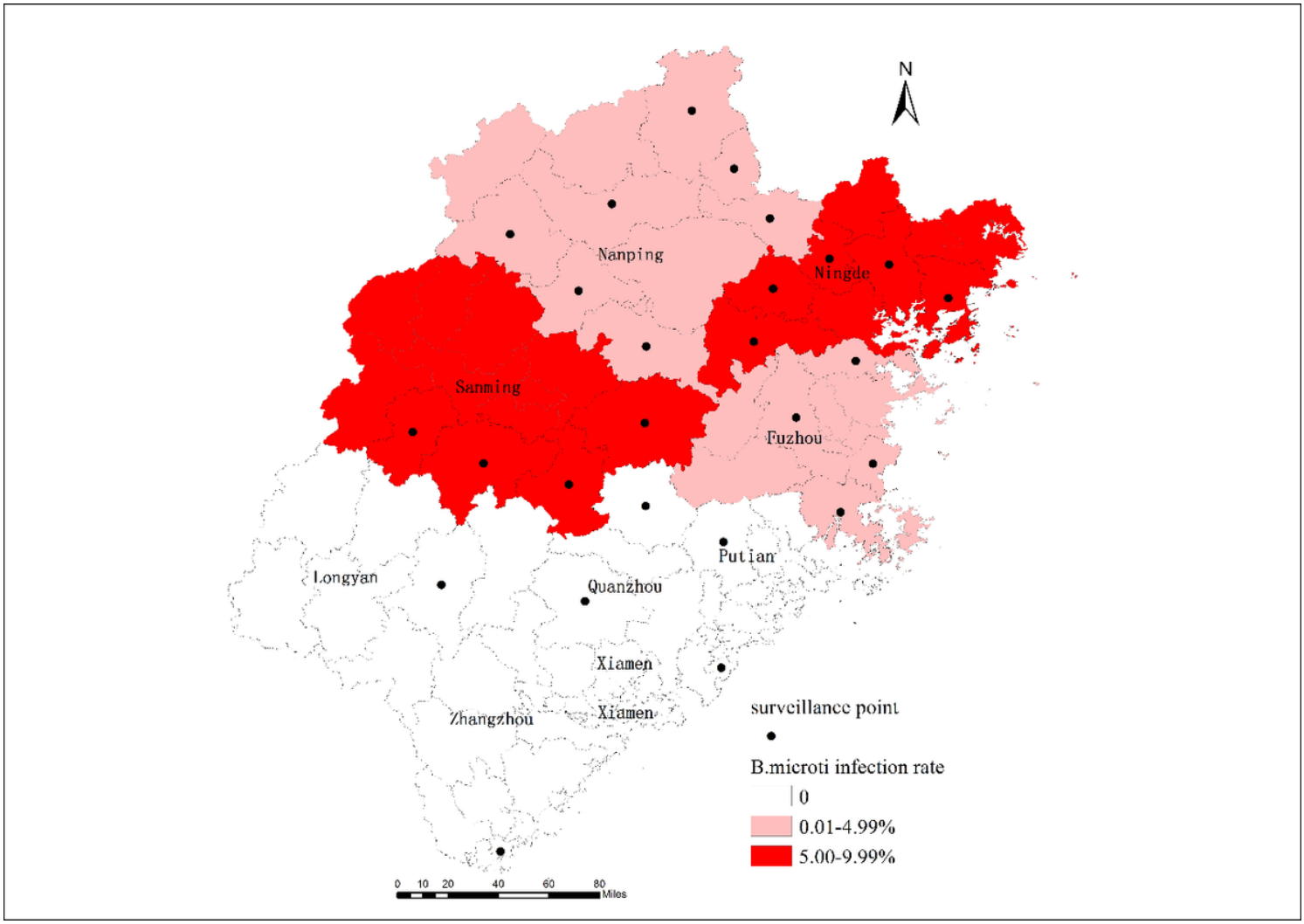

Figure 1

The map showed the prevalence of Babesia microti in rodents at 26 various surveillance points in eight cities in Fujian province, Southeast China. The prevalence of Babesia microti infection in each city is indicated by different colors, and special infection rate is provided in the legend at the lower right corner. The geographic location of each surveillance point is labeled with black dots for easy recognition on the map.

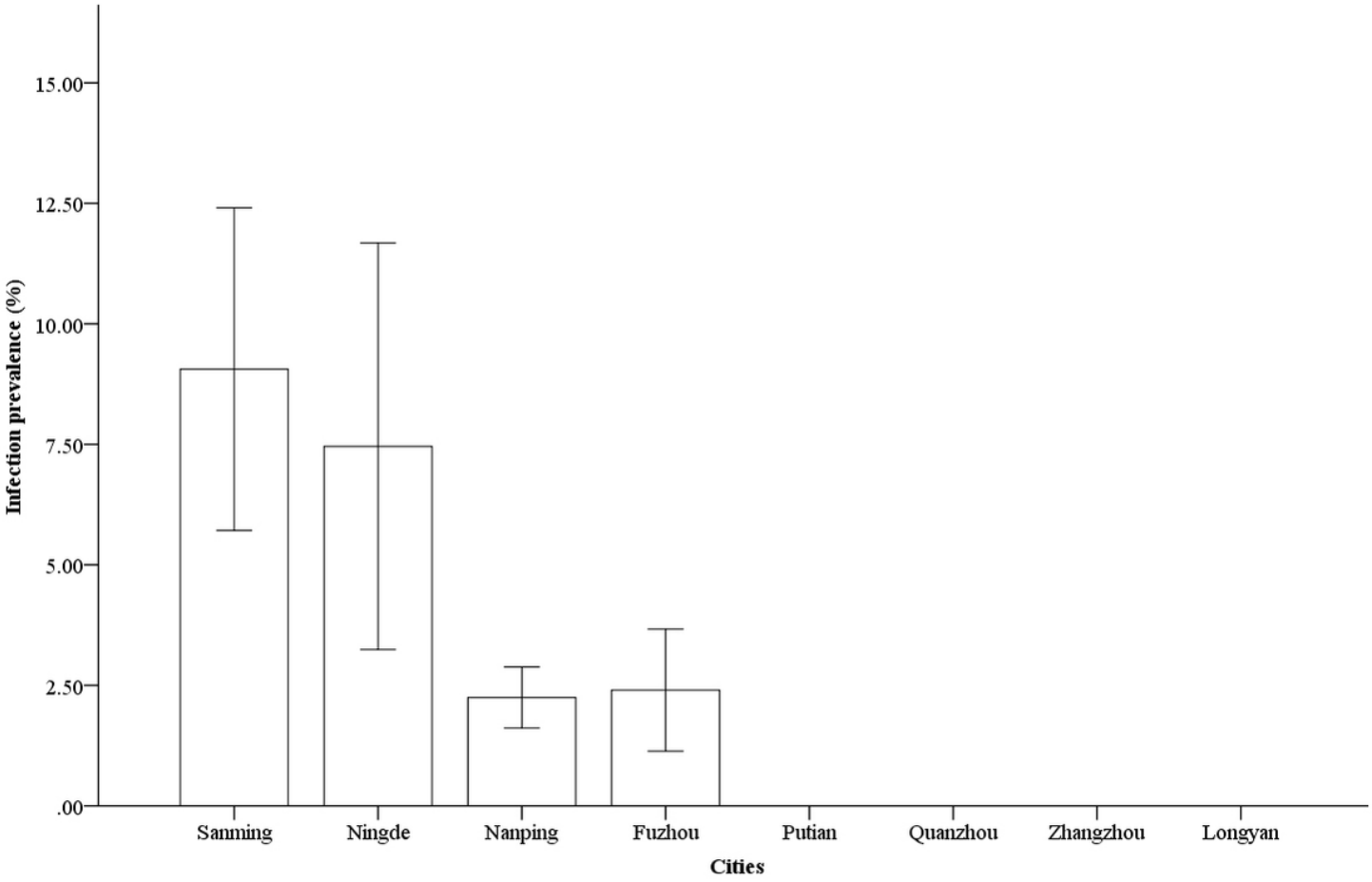

Figure 2

The prevalence of Babesia microti in rodents from eight cities in Fujian province. Legend: Error bar indicate ±1.0 standard deviation

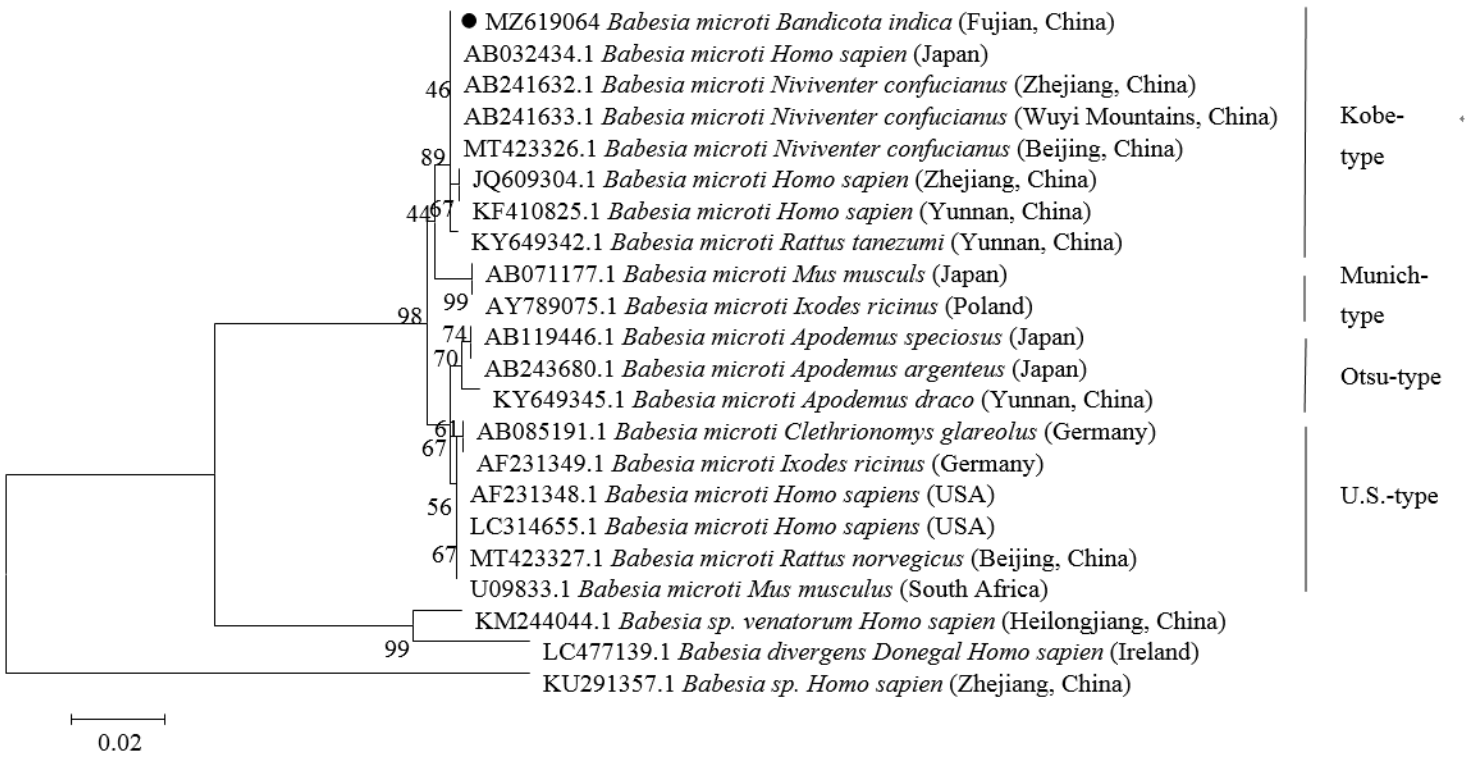

Figure 3

Neighbor-joining phylogenetic tree based on Babesia microti 18S rRNA partial sequence data from Fujian isolates with Babesia microti reference strains. Babesia divergens, Babesia sp. XXB/Hangzhou and Babesia sp. venatorum were used as the outgroup. For reference, taxon names include the corresponding GenBank accession number, Babesia species, hosts and regions of isolation. The number on each branch indicates the percent occurrence in 1,000 bootstrap replicates. The black circle stands for novel sequences identified in this work.

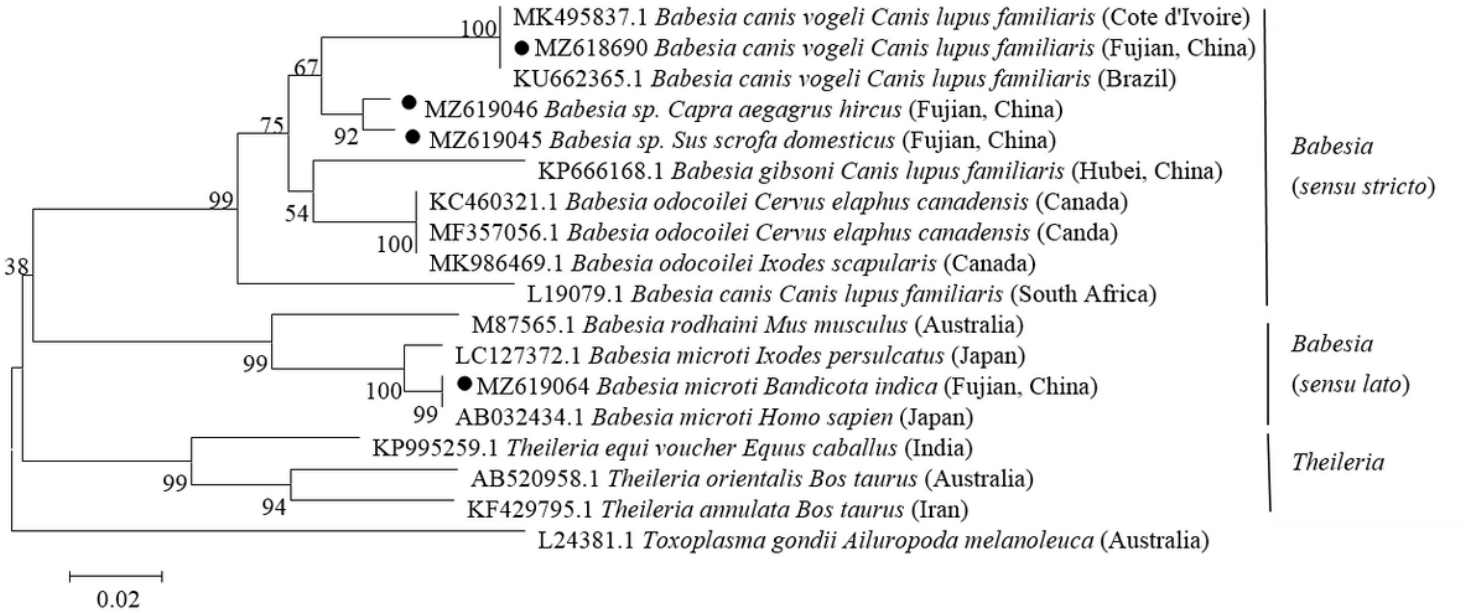

Figure 4

Neighbor-joining phylogenetic tree based on Babesia species 18S rRNA partial sequence data from Fujian isolates with Babesia species reference strains. Toxoplasma gondii (L24381.1) was used as the outgroup. For reference, taxon names include the corresponding GenBank accession number, Babesia species, hosts and regions of isolation. The number on each branch indicates the percent occurrence in 1,000 bootstrap replicates. The black circles stand for novel sequences identified in this work.

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