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

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Research Article

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Prevalence and Phylogenetic analysis of *Babesia* in host animals in Fujian Province, Southeast China

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Abstract

Background: Babesiosis is a socioeconomically typical tick-borne infectious disease. *Babesia* infections in small mammals and ticks were reported in at least five provinces in China. However, the host range and geographical distribution of this parasite in Fujian province was unclear. The aim of this work was to investigate the prevalence and genetic diversity of *Babesia* in Fujian province, Southeast China, between 2015 and 2020.

Methods: Blood samples of rodents were collected from 26 various types of surveillance sites accross Fujian province. Genomic DNA was extracted for screening the *Babesia* infection via PCR amplification based on 18S rRNA. The prevalences of *Babesia* were compared by the Chi-square test or Fisher exact test. Phylogenetic tree was constructed using MEGA 5.0 by gene sequence alignment. DNA samples of 316 *Capra Caprinae*, 85 *Bos Bovine*, 56 *Canis lupus familiaris*, 27 *Lepus sinensis* and 18 *Sus scrofa domesticus* were also collected in this survey.

Results: A total of 1123 rodents were trapped and tested. *Babesia* infection was confirmed in 3.83% (43/1123) of the rodents and in 1.20% (6/502) of other trapped mammals. Multivariate logistic

regression analysis revealed that irrigated cropland, reeds, shrub and hills were risk factors for *Babesia* infection. The infection rate of *S.scrofa domesticus*, *C.lupus familiaris* and *C.Caprinae* were determine to be 5.56%, 1.79% and 1.27%, and no infection were found in *B.Bovine* and *L.sinensis*. There was a significant difference in the prevalence of *Babesia* between rodents and other host animals.

Conclusions: The results indicated that there exits a broad geographical distribution and genetic diversity of *Babesia* in Southeast China. This study suggested that the mammals, especially wild rodents were the main natural hosts of *Babesia* in Fujian. Our research provided new insights on the exposure risk of *Babesia* in humans and animals, laying a solid foundation for the development of babesiosis prevention and control measures.

Keywords: *Babesia*, mammals, Epidemiological characteristics, Phylogenetic tree, Southeast China

Background

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

The first human case was reported in 1957 in Zagreb, Croatia and subsequently found in all continents but Antarctica, primarily in tropical and subtropical areas [5, 6]. During the past few decades, increasing numbers of species of *Babesia microti* were reported in the upper midwestern and northeastern regions of United States [7, 8], where babesiosis were prevalent. In Europe, most cases of babesiosis were caused by *Babesia divergens*, which were transmitted by *I.ricinus* ticks and infected bovines [9, 10]. Endemic infections of *Babesia microti* in rodents and ticks were also found

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

Having a wide range of vertebrate hosts, *Babesia* can infect rodents, horses, goats, cattles, dogs, cats and humans [15]. More than 100 different *Babesia* species have been discovered, however only a few can infect humans, mainly including *Babesia microti*, *Babesia divergens*, *Babesia venatorum*, *Babesia duncani* and *Babesia* sp. MO1 [16]. As the main etiological agent of human *Babesia*, rodent parasite *Babesia microti* is maintained through an enzootic cycle in nature, which involves ixodid ticks and small mammals [9, 17]. Clinical characterizations of babesiosis range 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 usually relates to age and immunity of the hosts. Neonates, people with advanced ages, HIV/AIDS, cancer, and people underwent with immunosuppressive therapy are more susceptible to the infection [10, 18]. Due to the lack of medical awareness, effective diagnosis technologies and the low incidence of the disease, babesiosis is often neglected in China [16, 19]. To date, *Babesia microti*-like organisms have been reported from Taiwan [20] and Yunnan [21] in human beings , and *Babesia microti*-like parasites have been found in Yunnan [22], Beijing [1], Taiwan [17], Heilongjiang [23] and Henan [24] in small mammals and hard ticks.

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 its sufficient rainfall, abundant sunshine, and the best forest coverage of 65.95% in China, the special natural and geographical environments of Fujian province provide superior habitats for *Babesia* and favorable conditions for the spread of tick-borne diseases. The aim of this work was to investigate the infection prevalence and genetic diversity of *Babesia* in mammals in eight cities of Fujian province, where the host species are in abundance.

Methods

Sample Collection

A total of 1123 rodents were captured using animal snap traps from eight cities in Fujian province between 2015 and 2020. The sampling sites included five different habitat environments: residential area, irrigated cropland, shrub, hill and reeds. Traps were placed for three continuous nights at locations where rodent activities were detected, and then retrieved on the next morning. According to the fur color, appearance morphology, gonad development observed after dissection, the species of trapped rodents were identified, while the sex, developmental stage, and ecological habitat were also recorded. Some rare rodents species were identified using the DNA barcoding technology [25]. After ether inhalation anesthesia and disinfection, blood of the rodents were collected through cardiac puncture and stored at -80℃ for further tests. The blood samples of 316 *Capra Caprinae*, 85 *Bos Bovine*, 56 *Canis lupus familiaris*, 27 *Lepus sinensis* and 18 *Sus scrofa domesticus* were also collected in this survey.

DNA extraction

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

Detection of *Babesia* infection by polymerase chain reaction

Conventional PCR amplification for the specific fragment of *Babesia* 18S rRNA gene region was performed using the following primers: PIRO-A: 5’-AATACCCAATCCTGACACAGGG-3’, PIRO-B: 5’-TTAAATACGAATGCCAAC-3’ [26] . Target DNA amplification was carried out under the following conditions: 94℃ for 5 min; 35 cycles of 94℃ for 30 s, 60℃ for 40 s and 72℃ for 40 s; followed by a final extension step at 72℃ for 5 min. The reaction used 5μl of genomic DNA as

template in 25μl reaction mixture containing 12.5μl Premix Taq (TaKaRa Taq Version 2.0 plus dye) and 1.0μl 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 goldview, and visualized under UV light. Positive PCR products were later sequenced by the Sangon Biotechnology Company.

Phylogenetic analysis

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

Statistical analysis

Spatial map of the prevalence of *Babesia* in rodents was drawn using ArcGIS 10.3.1 software. 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 prevalences of *Babesia* were compared with Chi-square test or Fisher exact test. All the analyses were conducted using SPSS software (version 20.0, SPSS Inc. Chicago, IL). Significance level for all results was set at P values <0.05.

Results

The prevalence of *Babesia* in host animals captured from different cities in Fujian province

A total of 1123 rodents were trapped from 26 various surveillance points in eight cities in Fujian province (Figs 1 and 2, Table 1). The captured rodents belonged to the two orders of Rodentia and

Insectivora, including four families, ten genera and fourteen species (Table 2). The results showed that an average of 3.83% (43/1123) rodents were infected by *Babesia* via sequence blastn (Table 1) .

Of all the fourteen species of trapped rodents, *Rattus norvegicus* accounted for the most (30.01%, n=337), followed by *Rattus losea* (19.15%, n=215), and *Sciuridae* were counted the least (0.09%, n=1). Except for *Suneus murinus*, *Sciuridae*, *Microtus fortis* and *Rattus Rattus*, ten of fourteen species were tested positive for *Babesia* infection. The positive infection rates of *Babesia* ranged from 0.61% (1/163) in *Rattus flavipectus* to 30.36% (17/56) in *Bandicota indica* (Table 2).

Infected rodents were caught from four cities including Sanming, Ningde, Nanping and Fuzhou (Table 1). Rodents collected from Sanming had the highest *Babesia* infection rate of 9.94% (17/171). *Babesia* infection rates in hosts from Sanming and Ningde were both significantly higher that in hosts captured in Fuzhou (odd ratios: 6.03, 4.21, respectively; *P* values both <0.05) (Table 1).

The blood samples of 316 *Capra Caprinae*, 85 *Bos Bovine*, 56 *Canis lupus familiaris*, 27 *Lepus sinensis* and 18 *Sus scrofa domesticus* were also collected for the detection of *Babesia* infection. Surprisingly, neither *Bos Bovine* nor *Lepus sinensis* were infected with *Babesia* (Table 3). There was no significant difference in the prevalence of *Babesia* between male and female *Capra Caprinae* (χ^2 = 1.296, P = 0.255) (Not shown in the table). There was a significant difference in the prevalence of *Babesia* between rodents and other mammals (χ^2 = 7.353, P = 0.007).

The risk factors associated with *Babesia* infection

Risk factors relating to the *Babesia* infection in rodents were analyzed with respect to different genders, ages and ecological habitats (Table 3). There was no significant difference in the prevalence of *Babesia* between male and female rodents (χ^2 = 0.350, P = 0.554). However, there were significant differences in the prevalences of *Babesia* among host animals with different ages, in

which the prevalences were 1.10%, 4.62% and 1.30% in child, adult and old hosts, respectively (χ^2 = 6.472, P = 0.039). It was worth noting that the prevalences of *Babesia* in mammals from irrigated cropland, reeds, shrub and hills, which were 4.64%, 10.98%, 16.67% and 4.51%, respectively, were all significantly higher than that in animals from residential areas ($P < 0.05$, Table 3 and 4). Furthermore, multivariate logistic regression analysis suggested that irrigated cropland, reeds, shrub and hills were risk factors for *Babesia* infection (Table 4).

Genetic and phylogenetic analysis of *Babesia*

In order to clarify the genetic diversity of *Babesia* detected in host animals collected in Fujian province, sequences of the near full-length 18S rRNA gene fragments were used for PCR amplification, aligning and comparison to the sequences from GenBank database, with additional sequence details if available (species, strains, years, countries, regions and GenBank accession numbers). *Babesia* sequences from three infected animal species, including *Mustela sibirica*, *Capra Caprinae*, and *Canis lupus familiaris*, were used for phylogenetic analyses (Fig 3). All the *Babesia microti* sequences from infected rodents shared 99% homology with sequences from Wuyi Mountain (AB241633) and Zhejiang (JQ609304). The *Babesia* sequences from the infected *Canis lupus familiaris* shared 99% homology with sequences from Brazil (KU662365) and India (KT246307). Interestingly, *Capra Caprinae* and *Sus scrofa domesticus* in Fujian were infected with *Babesia sp.*, the sequence of which shared 99% homology with sequence from Japan (AB649052).

Discussion

Our research systematically illustrated the wide prevalence and genetic diversities of *Babesia* in host animals in Fujian province, Southeast China. The infections of the *Babesia* parasites were seen in four cities and eight sampling sites in Fujian province (Figs 1 and 2, Table 1). It was reported that *Babesia* existed in small mammals in Beijing [1], Henan [24], Yunnan [22] and Taiwan [17]. *Babesia*

microti infection was also previously reported in Wuyi Mountain area, Fujian [27], however, the epidemiological features of *Babesia* remained unclear in other cities in Fujian. The high prevalences of *Babesia* infection in rodents in Ningde and Sanming in this survey strongly verified the hypothesis that these surveillance points were major natural foci for human babesiosis. Furthermore, the results called for closely monitoring of the *Babesia* transmissions in Ningde and Sanming, while the epidemic of *Babesia* in other cities shall not be ignored. It should be noted that the *Babesia* 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, it was not always of similar prevalences of *Babesia* infection in animals from neighboring districts. Although both Fuzhou and Quanzhou are adjacent to Sanming, their *Babesia* infection rate were both lower than 5.00%, while Sanming had the highest infection rate. This may be attributed to the distributions and densities of host animals.

Interestingly, the infection rate of *Babesia* in Xiapu district, Ningde city was 15.79%, which could provide a novel clue to the first human babesiosis in Fujian [28]. The patient diagnosed with the *Babesia* infection lived and worked in a village in Xiapu, Ningde, which was surrounded by abundant shrub and forest. Our work revealed that the prevalences of *Babesia* in rodents from irrigated cropland, reeds, shrub and hills were significantly higher than the residential areas, suggesting that the ecological habitat types played an important role in the spread of *Babesia* (Table 4 and 5). It has been reported that forest was a essential risk factor for *Babesia* infection in Thailand, Cambodia, Lao PDR and China (Yunnan and Heilongjiang) [19, 22, 23]. Human babesiosis is a tick-borne zoonotic infectious disease. Considering that forest areas are burdened with tick-transmitted pathogens, people who work in or travel to the forests, should take appropriate protective measures. The fact that *Babesia* and *Plasmodium* are both intraerythrocytic protozoas and elicit similar inflammatory responses with similar clinical symptoms renders them 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* were *Babesia microti* in rodents in Fujian province, which can be concluded based on the plenty of samples detected, similar to the findings in Yunnan [16, 22], Taiwan [17] and Beijing [1]. Phylogenetic analysis suggested that *Babesia microti* in this survey shared high homology with that in Zhejiang province, where a confirmed human babesiosis was reported in Hangzhou after kidney transplantation in 2002 [27]. Unexpectedly, both *Capra Caprinae* and *Sus scrofa domesticus* were infected with *Babesia sp.*. In addition, neither *Bos Bovine* nor *Lepus sinensis* were infected with *Babesia*. Meanwhile, because of the insufficient samples from a single sampling location, hosts such as cattle should be investigated further. We detected the *Babesia canis* from the blood of *Canis lupus familiaris* for the first time, the sequence of which shared high homology with the *Babesia canis* from India (GenBank KT246307). The prevalence of *Babesia canis* in dogs had also been documented in Henan province previously [29].

Ovine babesiosis is a tick-borne disease of goat, sheep and cattle, posing a huge threat to the livestock industry [30, 31]. 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 transmit 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 the 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 the babesiosis epidemic.

Conclusions

Our study suggested that the wide distribution and genetic diversities of *Babesia* in mammals in Fujian province, Southeast China. The infection rates of *Babesia* in rodents were significantly higher

than in other host animals. This research provided basic data to support public health authorities in developing prevention 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, also transmitting through blood transfusion. So it is necessary to survey the prevalence of *Babesia* in different ticks and donor populations.

Supplementary information

Supplementary information accompanies this paper at: Additional file 1 Prevalence and Phylogenetic analysis of *Babesia* in host animals in Fujian Province, Southeast China. Table S1 The prevalence of *Babesia* in rodents captured from different sampling sites. Table S2 The 18S rRNA gene sequences of *Babesia* species used in this study.

Abbreviations

DNA: Deoxyribonucleic acid; rRNA: Ribosome ribonucleic acid; PCR: Polymerase chain reaction; *CI*: Confidence intervals; MODS: Multiple Organ Dysfunction Syndrome; HIV: Human Immunodeficiency Virus; AIDS: Acquired Immune Deficiency Syndrome; UV: Ultravioletray.

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Authors' contributions

F-ZX and Y-QD designed the whole study and contributed to reviewing the manuscript. Z-WZ drafted the manuscript, carried out statistical analysis, and participated in sampling collection. S-HZ, W-JL, T-WH, G-YX, JL and J-XW conducted the molecular biological detection, sampling acquisition and identified the host mammals species . All authors read and approved the final manuscript.

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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 author for 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). All animal experiments were performed in accordance 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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Figures

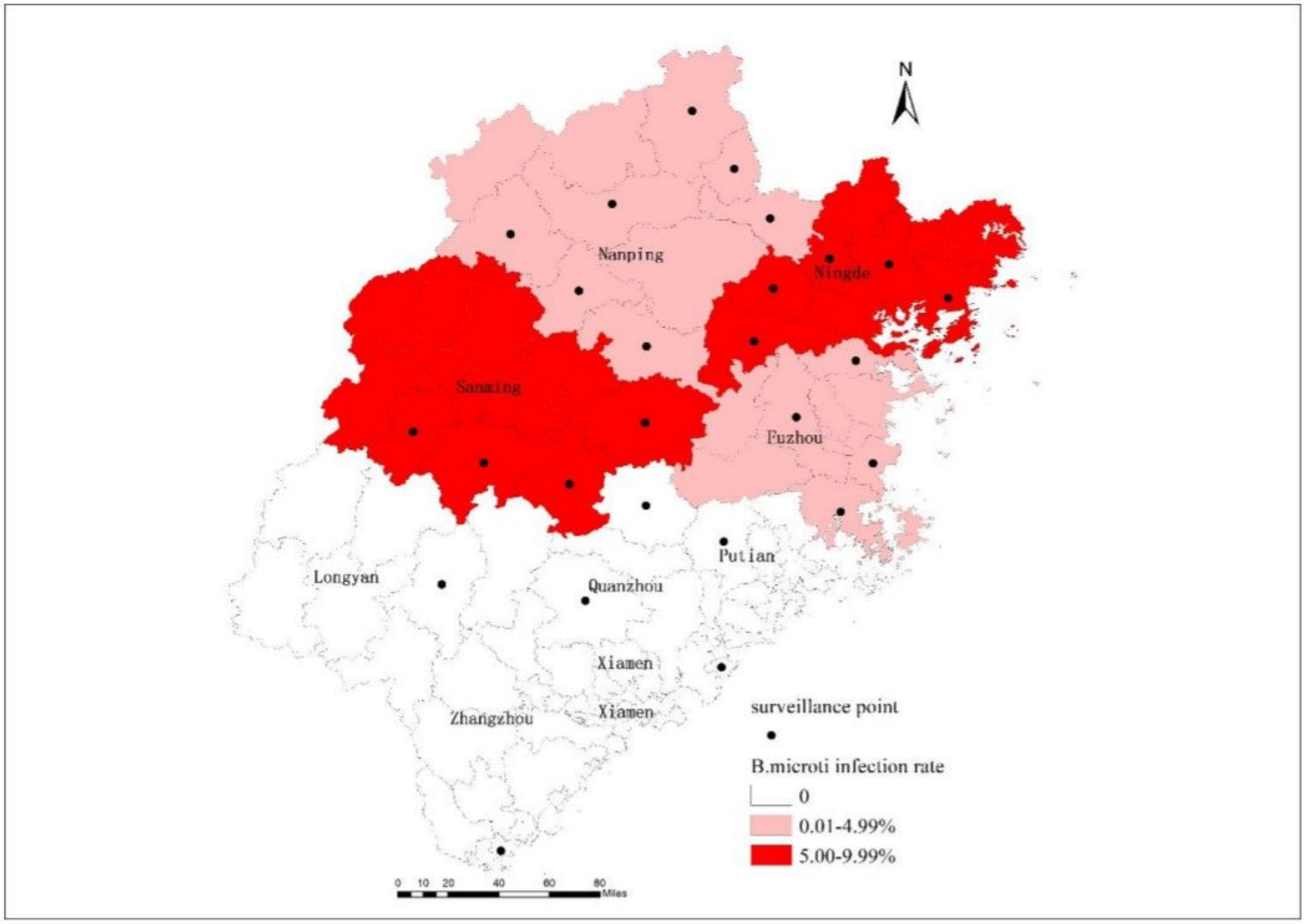

Figure 1

The map showed the prevalence of Babesia in rodents at 26 various surveillance points in eight cities in Fujian province, Southeast China. The prevalence of Babesia 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. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

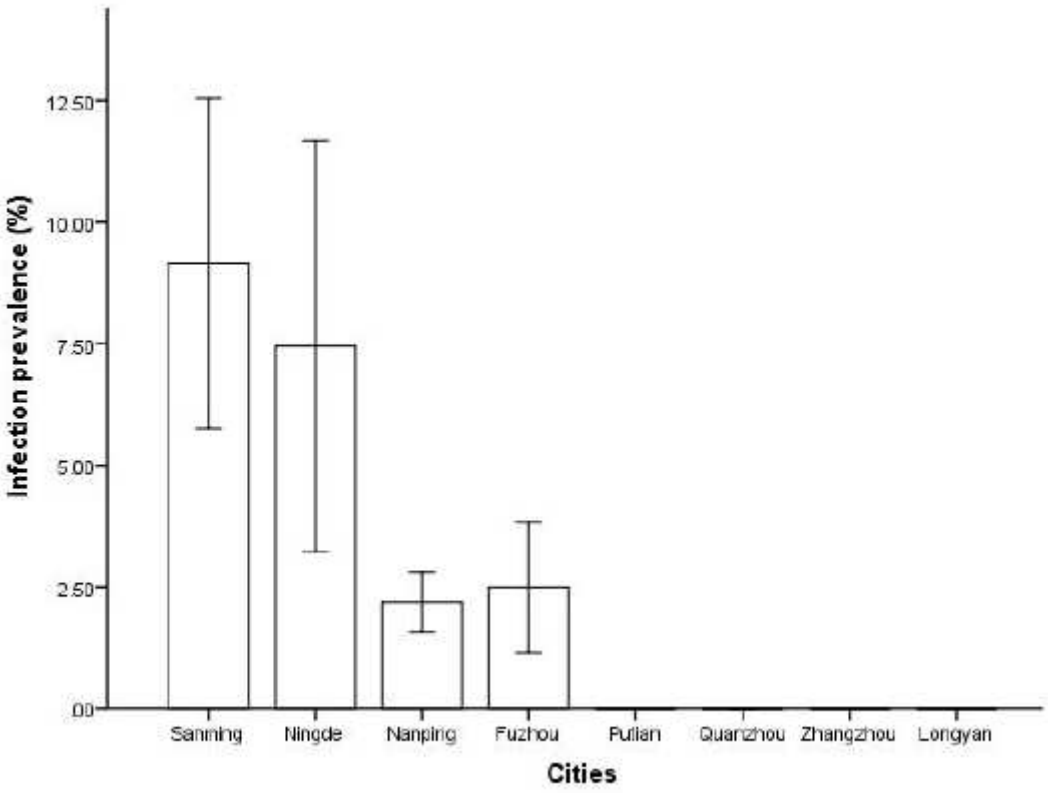

Figure 2

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

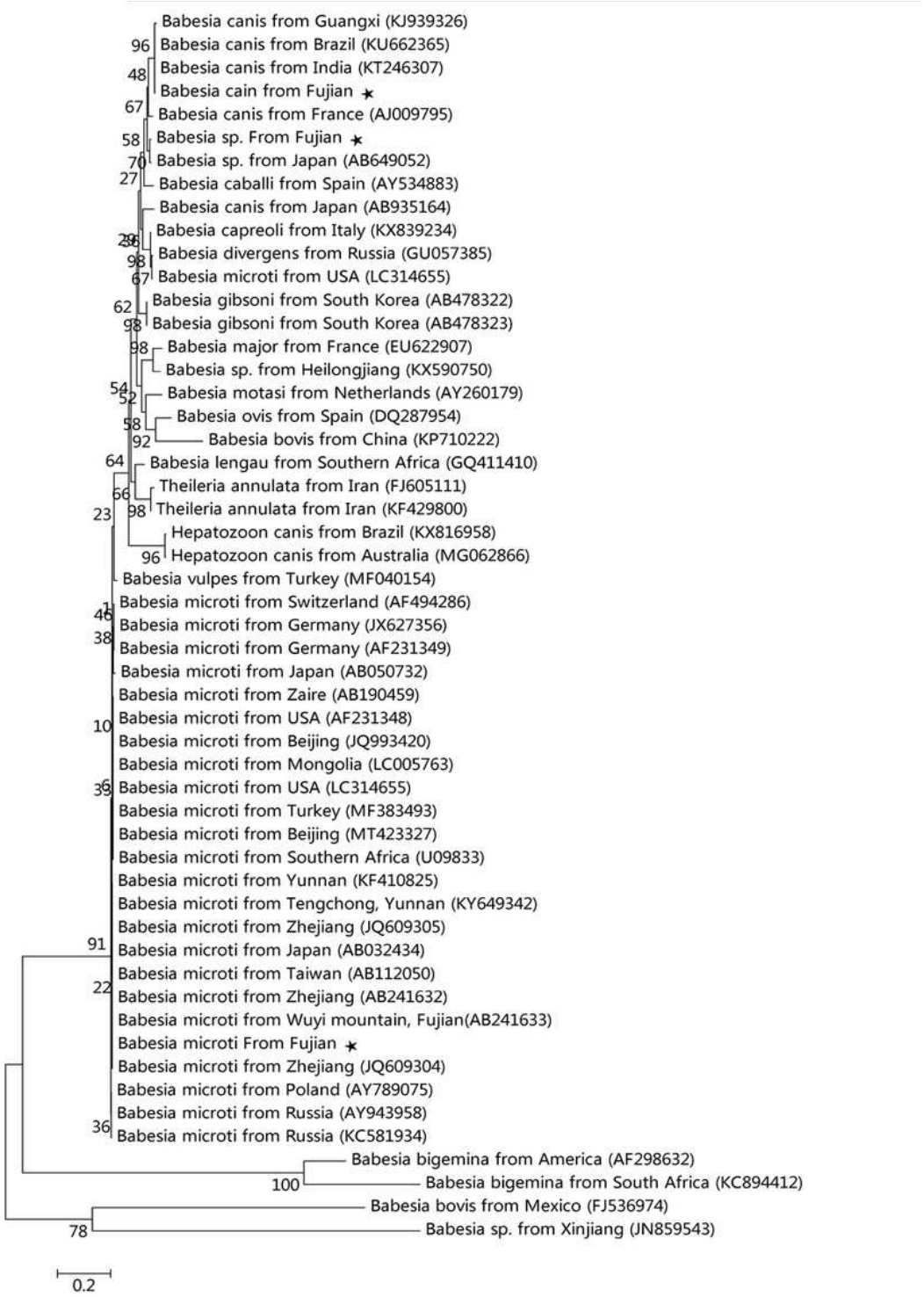

Figure 3

Phylogenetic relationships of Babesia protozoa were based on the open reading frame of 18S rRNA sequences obtained in this work. Additional Babesia protozoa genes from other countries and regions were retrieved from GenBank. For reference, taxon names include the species of Babesia, corresponding GenBank accession number and regions of isolation. The phylogenetic tree was constructed by the

Neighbor Joining method using the MEGA 5.0 program. The number on each branch indicates the percent occurrence in 1,000 bootstrap replicates. Black star stand for novel sequences identified in this work.

Supplementary Files

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- Additionalfile1.pdf