Prevalence and phylogenetic analysis of *Babesia* parasites in reservoir host species in Fujian province, Southeast China

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**Abstract**

Babesiosis is a tick-borne disease that mainly affects small mammals and has been reported in at least five provinces in China. However, the host range and geographical distribution of the parasite in Fujian province are unclear. Therefore, we investigated the prevalence and genetic characteristics of *Babesia* in Fujian province, Southeast China, between 2015 and 2020. Rodent blood samples were collected from 26 different surveillance sites across Fujian province. Genomic DNA was extracted to screen for *Babesia* infection using polymerase chain reaction based on 18S rRNA. DNA samples from 316 domestic goats, 85 water buffalo, 56 domestic dogs and 18 domestic pigs were examined. The prevalence of *Babesia* was statistically analysed using the Chi-square test or Fisher’s exact test. *Babesia* infections were detected in 3.96% (43/1,087; 95%CI: 2.80%, 5.12%) of rodents and 1.26% (6/475; 95%CI: 0.26%, 2.26%) of other mammals. Multivariate logistic regression analysis revealed that irrigated cropland, shrubs and forests were risk factors for *Babesia microti* infections. The infection rates among domestic pigs, dogs and goats were 5.56%, 1.79% and 1.27%, respectively, with no infection found in water buffalo. The 18S rRNA gene sequencing revealed that rodents were infected with *Babesia* (sensu lato), whereas other mammals were infected with *Babesia* (sensu stricto). The geographical distribution and phylogenetic relationship of *Babesia* was determined in Southeast China. Mammals, particularly wild rodents, maybe the main natural hosts of *Babesia* in Fujian. Our findings provide a foundation for public health officials to develop prevention and control measures for *Babesia*.

**KEYWORDS**

*Babesia*, Babesiosis, mammal, phylogenetic tree, risk factor, Southeast China

1 | INTRODUCTION

Human babesiosis is a tick-borne zoonotic disease emerging globally (Krause, 2019; Vannier & Krause, 2012; Wei et al., 2020). It poses a serious threat to public health and shows global economic, veterinary and medical significance (Li et al., 2020; Schnittger et al., 2012; Vannier & Krause, 2012). Babesiosis is caused by intraerythrocytic sporozoites of the genus *Babesia*, infecting both animals (wild and domestic) and humans (Jalovecka et al., 2019; Schnittger et al., 2012). *Babesia* (sensu stricto) is distinguished from *Theileria* by the absence of schizont stages and is considered as the ‘true’ *Babesia* species. *Babesia* (sensu stricto) possesses the ability of transovarial transmission, which corresponds to their single-host ticks that persist on vertebrate hosts throughout the developmental stage (Jalovecka et al., 2019). However, *Babesia* (sensu lato) requires two or three host ticks to support their successful
dissemination to uninfected hosts due to its lack of ability for transovarial transmission.

The first human case of babesiosis was reported in 1957 in Zagreb, Croatia, after which this disease was detected in all continents except for Antarctica. Infections primarily occur in tropical and subtropical areas (Liu et al., 2007; Wang, Zhang, et al., 2019). More than 60 cases of human babesiosis were recently reported in China, including 48 patients infected with Babesia venatorum in Heilongjiang (Jiang et al., 2015), 12 infected with B. divergens in Shandong (Qi et al., 2011) and Gansu (Wang, Zhang, et al., 2019), 8 infected with B. microti in Yunnan (Zhou et al., 2013) and 1 case infected with Babesia sp. XXB/Hangzhou in Zhejiang (Man et al., 2016). Over the past few decades, an increasing number of B. microti species has been reported in the upper midwestern and north-eastern regions of the USA (Krause, 2019; Liu et al., 2019). In Europe, B. divergens is responsible for most babesiosis cases, with the parasites transmitted by Ixodes ricinus ticks and infected cows (Gray, 2006; Martinot et al., 2011). Endemic infections of B. microti transmitted by rodents and ticks were recently detected in many European countries, including Slovakia (Bláharová et al., 2016), Finland (Kallio et al., 2014), Belgium (Lemperre et al., 2011), Switzerland (Poppa et al., 2002), Poland (Sisinski et al., 2006; Tolkacz et al., 2017) and France (Jouglin et al., 2017). Cases have also been recorded in South Africa (Bush et al., 1990), India (Marathe et al., 2005) and Australia (Senanayake et al., 2012). Therefore, the recent emergence of babesiosis has become a worldwide public health concern.

Babesia parasites have a wide range of vertebrate hosts, including rodents, horses, goats, cattle, dogs, cats and humans (Schnittger et al., 2012). More than 100 different Babesia species have been discovered; however, only a few can infect humans, including B. microti (Goethert et al., 2018), B. divergens (Wang, Zhang, et al., 2019), B. venatorum (Sun et al., 2014) and B. duncanii (Conrad et al., 2006). As the main aetiological agent of human babesiosis, the rodent parasite B. microti is maintained in nature through an enzootic cycle that involves ixodid ticks and small mammals (Chao et al., 2017; Gray, 2006). The clinical characteristics of babesiosis range from asymptomatic infection to severe morbidity (fever, chills, headache, fatigue, anaemia, jaundice, thrombocytopenia, haemolysis, haemoglobinuria and even multiple organ dysfunction syndromes) and may result in death (Krause, 2019). Susceptibility to Babesia infection is typically related to age and the immune status of the host. Neonates, people of advanced age, those undergoing immunosuppressive therapy and individuals with acquired immune deficiency syndrome or cancer are more susceptible to Babesia infection (Bloch et al., 2018; Martinot et al., 2011). Babesiosis is frequently overlooked in China because of the lack of medical awareness, effective diagnostic techniques and low incidence of the disease (Chen et al., 2017; Karrchhabanathoeng et al., 2018). To date, B. microti-like organisms have been reported in humans from Taiwan (Shih et al., 1997) and Yunnan (Zhou, Li, et al., 2014), and B. microti-like parasites have been found in small mammals and hard ticks in Yunnan (Gao et al., 2017), Beijing (Wei et al., 2020), Taiwan (Chao et al., 2017), Heilongjiang (Sun et al., 2008) and Henan (Zhao et al., 2013).

Fujian province is on the Southeast coast of China, has a subtropical climate and encompasses 124,000 km2 of land and 136,000 km2 of ocean. The natural and geographical environments in Fujian provide an ideal habitat for Babesia and favourable conditions for the spread of tick-borne diseases, with an average annual rainfall of 1,400–2,000 mm, abundant sunshine and 65.95% forest coverage. This study was performed to investigate the infection prevalence and phylogenetic relationship of Babesia in mammals across eight cities of Fujian province, where its host species are abundant.

2  |  MATERIAL AND METHODS

2.1  |  Sample collection

A total of 1,087 rodents were captured with live animal traps from eight cities in Fujian province between 2015 and 2020. The sampling sites included four different habitats: residential areas, irrigated cropland, shrubland and forests. Live traps were placed every night at each surveillance point for three consecutive nights in locations where rodent activities were detected, and the trapped rodents were retrieved the following morning. Chinese monographs were used to identify the species of trapped rodents according to their morphology (Huang, 1995; Zhang, 1999). The sex, age class and ecological habitat were recorded. Any rare rodent species captured were identified using DNA barcoding technology (Liu et al., 2020). All animal experiments were performed according to the Guide for the Care and Use of Laboratory Animal (Committee, N. R. C. U., 2011). After the rodents were anesthetized and disinfected, 1ml of blood from each rodent was collected through cardiac puncture and stored at −80°C until further tests. In addition, blood samples (2 ml) from 316 domestic goats, 85 water buffalo, 56 domestic dogs and 18 domestic pigs in Fujian province were collected, and the animals were returned to their farms. Both groups of animals...
used in this study were part of larger surveillance projects investigating haemorrhagic fever with renal syndrome (rodents) (Liu et al., 2021), pathogenic Leptospira (rodents) (Xu et al., 2022) and severe fever with thrombocytopenic syndrome (domestic animals).

2.2 | DNA extraction

A Blood Genomic DNA Kit (Tagene Biotechnology) was used to extract genomic DNA from the animal blood samples according to the manufacturer’s instructions. The genomic DNA was dissolved in 100 μl elution buffer, and the 260/280 absorbance ratio was measured to confirm that the DNA purity was between 1.7 and 1.9 using a spectrophotometer (DS-11, DeNovix). Samples were stored at −20°C until use.

2.3 | Detection of Babesia infection using polymerase chain reaction

Amplification of a variable section of Babesia 18S rRNA gene region was performed using polymerase chain reaction with the primers PIRO-A, 5′-AATACCAATCCTGACACAGG-3′ and PIRO-B, 5′-TTAAATACGAATGCCCCCAAC-3′, described in previous studies (Olmeda et al., 1997; Stahl et al., 2018). Target DNA amplification was performed under the following conditions: 94°C for 5 min; followed by 40 cycles of 94°C for 45 s, 55°C for 45 s and 72°C for 45 s; and a final extension step at 72°C for 5 min. A concentration of 20–50 ng/μl of genomic DNA (5 μl) was used as a template in a 25 μl reaction, which contained 12.5 μl Premix Taq (Taq Version 2.0 plus dye, TaKaRa) and 1.0 μl of each primer (final concentration 0.4 μM). Negative (nuclease-free water) and positive controls (confirmed Babesia sample from the first human babesiosis case in Fujian province) were also tested to eliminate the possibility of contamination. Amplified products were electrophoresed on a 1.5% agarose gel stained with SYBR gold (Invitrogen) and visualized under ultraviolet light. About 408bp positive PCR products were purified and sent for Sanger sequencing at Sangon Biotechnology Company using the primers listed above.

2.4 | Phylogenetic analysis

The sequences were assembled using the SeqMan software 7.0.1 (DNASTAR, Inc.). All newly generated sequences were compared to those of the strains of validated Babesia species in the GenBank database using NCBI BLAST. Sequences of the 18S rRNA gene fragments were aligned by the Clustal W. The Molecular Evolutionary Genetics Analysis (MEGA) version 5.05 software was used for phylogenetic analyses. Neighbour-joining phylogenetic trees with 1,000 bootstrap replications were constructed using a maximum composite likelihood method. All other parameters were set to default parameters.

2.5 | 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 (1:1,000,000), and the latitude and longitude were retrieved from Google Maps. The association among rodent species, sex, age class, habitat environments, sampling locations and Babesia infection was analysed using univariate analysis based on Chi-square ($\chi^2$) test or Fisher’s exact test. Multivariate logistic regression was used to analyse the risk factors for B. microti infection. All analyses were conducted using ssps software (version 20.0, SPSS, Inc.). The significance level for all results was set at $p<.05$.

2.6 | Sequences used for phylogenetic analysis

The sequences of Babesia 18S rRNA gene fragments were used for alignment and comparison to sequences from the GenBank.
database and are summarized in Table S1, with additional sequence details, if available (species, strains, hosts, years, countries, regions, and GenBank accession numbers).

3 | RESULTS

3.1 | Prevalence of Babesia microti in rodents captured from different cities in Fujian province

We captured 1,087 rodents from 26 surveillance points in eight cities in Fujian province (Figure 1, Table 1, Table S2). The rodents belonged to the order Rodentia and were divided into two families, 7 genera and 12 species (Table 2). Sequencing analysis using the Nucleotide Basic Local Alignment Tool revealed that 3.96% (43/1,087) of the rodents were infected by B. microti (Table 1).

Of the 12 rodent species, Rattus norvegicus (brown rat) had the highest number of captured (30.00%, n = 337), followed by Rattus losea (19.78%, n = 215). In contrast, Rattus rattus made up the smallest proportion, representing only 0.37% (n = 4). Except for Microtus fortis and R. rattus, 10 of the 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). Within the wild rodents (from irrigated cropland, shrubs and forests), the total infection rate of B. indica and Niviventer confucianus was 24.27%, which was significantly higher than that in other rodent species (χ² = 66.003, p < .001).

In addition, infected rodents were captured in four cities: Sanming, Ningde, Nanping and Fuzhou (Table 1). Rodents collected from Sanming showed the highest B. microti infection rate of 9.94% (17/171). Babesia microti infection rates in rodents from Sanming and Ningde were both significantly higher than that in rodents captured in Fuzhou (odds ratios: 5.96, 4.18, respectively; p < .05; Table 1).

3.2 | Prevalence of Babesia in domestic animals in Fujian

The positive infection rates of Babesia in domestic pigs, domestic dogs and domestic goats were 5.56%, 1.79% and 1.27% respectively. Water buffaloes were not infected with Babesia (Table 2), and no significant difference in the prevalence of Babesia between male and female domestic goats was observed (p = .129; data not shown).

3.3 | Risk factors associated with Babesia microti infection

Risk factors related to B. microti infection in rodents, including sex, age and ecological habitat, were analysed (Table 3). There was no significant difference in the prevalence of B. microti between male and female rodents (χ² = 0.466, p = .495). However, the prevalence of B. microti in adult rodents (4.53%) was significantly higher (χ² = 4.645, p = .031) than that in pubertal rodents (1.10%). Notably, the prevalence of B. microti in mammals from irrigated cropland, shrubs and forests were 4.70%, 11.18% and 4.55% respectively; these values were significantly higher than those in rodents from residential areas (p < .05, Tables 3 and 4). Furthermore, multivariate logistic regression analysis suggested that irrigated cropland, shrubs and forests were risk factors for B. microti infection (Table 4).

3.4 | Genetic and phylogenetic analysis of Babesia species

Sequencing the 18S rRNA gene from the positive samples revealed that 43 samples contained B. microti, 5 contained Babesia spp. and 1 contained Babesia canis vogeli. The 18S rRNA gene sequences of another 18 B. microti isolates from other regions were included for comparison and construction of a phylogenetic tree. Babesia

### TABLE 1 Prevalence of Babesia microti in rodents in Fujian province

| Cities      | No. of traps | No. of rodents tested | No. of positive samples for B. microti | Density (%) | Positive rate (%) | Odds ratio |
|-------------|--------------|-----------------------|----------------------------------------|-------------|-------------------|------------|
| Sanming     | 2,307        | 171                   | 17                                     | 7.41        | 9.94              | 5.96       |
| Ningde      | 2,549        | 209                   | 15                                     | 8.20        | 7.18              | 4.18       |
| Nanping     | 3,922        | 330                   | 8                                      | 8.41        | 2.42              | 1.34       |
| Fuzhou      | 1,790        | 165                   | 3                                      | 9.22        | 1.82              | 1.00       |
| Putian      | 355          | 30                    | 0                                      | 8.45        | 0.00              |            |
| Quanzhou    | 1,549        | 110                   | 0                                      | 7.10        | 0.00              |            |
| Zhangzhou   | 439          | 41                    | 0                                      | 9.34        | 0.00              |            |
| Longyan     | 584          | 31                    | 0                                      | 5.31        | 0.00              |            |
| Total       | 13,495       | 1,087                 | 43                                     | 8.05        | 3.96              |            |
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 those from Japan (AB032434.1). All sequences from this study were deposited in GenBank with accession number MZ619064. Phylogenetic analysis revealed that MZ619064 belonged to Kobe-type *B. microti* (Figure 2).

The sequences of 18S rRNA genes from different *Babesia* species were used to examine 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 *Canis lupus familiaris* were identical to the samples from Côte d’Ivoire (MK495837.1) and Brazil (KU662365.1). Both domestic pigs and goats in Fujian were infected with *Babesia* sp., showing a homology of 98.17%. In this survey, the sequences of *B. canis vogeli* from *C. lupus familiaris*, *Babesia* sp. from *Sus scrofa domesticus* and *Babesia* sp. from *Capra aegagrus* were deposited in GenBank with accession numbers MZ618690, MZ619045 and MZ619046 respectively. Phylogenetic analyses suggested that MZ618690, MZ619045 and MZ619046 belonged to *Babesia* (sensu stricto), whereas MZ619064 belonged to *Babesia* (sensu lato; Figure 3).

### DISCUSSION

In this study, we systematically illustrated the prevalence and phylogenetic relationship of *Babesia* species in reservoir host species in Fujian province, Southeast China. Infections of *B. microti* parasites

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**Table 2** Prevalence of *Babesia* in different reservoir host species

| Orders     | Families | Genera | Species              | No. of examined animals | No. of positive | Positive rate (%) |
|------------|----------|--------|----------------------|-------------------------|-----------------|------------------|
| Rodentia   | Muridae  | Rattus | Rattus norvegicus    | 337                     | 3               | 0.89             |
|            |          |        | Rattus losea         | 215                     | 3               | 1.40             |
|            |          |        | Rattus tanezumi      | 163                     | 1               | 0.61             |
|            |          |        | Rattus edwardsi      | 17                      | 1               | 5.88             |
|            |          |        | Rattus rattus        | 4                       | 0               | 0.00             |
| Apodemus   |          |        | Apodemus agrarius    | 32                      | 1               | 3.13             |
| Mus        |          |        | Mus musculus         | 5                       | 1               | 20.00            |
| Niviventer |          |        | Niviventer confucianus| 47                      | 8               | 17.02            |
|            |          |        | Niviventer fulvescens| 152                     | 7               | 4.61             |
| Berylmys   |          |        | Berylmys broversi    | 50                      | 1               | 2.00             |
| Bandicota  |          |        | Bandicota indica     | 56                      | 17              | 30.36            |
| Cricetida  |          |        | Microtus fortis      | 9                       | 0               | 0.00             |
| Artiodactyla |        | Capra | Capra aegagrus       | 316                     | 4               | 1.27             |
| Bovidae    |          |        | Bos bubalis          | 85                      | 0               | 0.00             |
| Suidae     |          | Sus    | Sus scrofa domesticus| 18                      | 1               | 5.56             |
| Carnivora  | Canidae  | Canis  | Canis lupus familiaris| 56                      | 1               | 1.79             |

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

| Variable       | Sample size | Positive rate (%) | \( \chi^2 \) | \( p \)-value |
|----------------|-------------|-------------------|--------------|--------------|
| Sex            | Cases       | Positive rate (%) | \( \chi^2 \) | \( p \)-value |
| Male           | 561         | 51.61             | 3.57         | .466         |
| Female         | 526         | 48.39             | 4.37         | .495         |
| Age            |             |                   |              |              |
| Pubertal       | 181         | 16.65             | 1.10         | .465         |
| Adult          | 906         | 83.35             | 4.53         | .031         |
| Habitat        |             |                   |              |              |
| Residential areas | 504     | 46.36             | 0.99         | 35.438       |
| Irrigated cropland | 149      | 13.71             | 4.70         | .495         |
| Shrub          | 170         | 15.64             | 11.18        | .031         |
| Forest         | 264         | 24.29             | 4.55         | .495         |


were observed in four cities and eight sampling sites in Fujian province (Figure 1, Table 1). *Babesia microti* infections were previously reported in the Wuyi Mountain area, Fujian (Saito-Ito et al., 2008); however, the epidemiological features of *Babesia* in other cities in Fujian remain unclear. In this study, the prevalence of *B. microti* in rodents (3.96%) followed the low prevalence described in Yunnan (4.31% (Chen et al., 2017) and 2.40% (Gao et al., 2017)) and the Dapan Mountains of Zhejiang (1.30%) (Wei et al., 2013). However, the positive infection rates of *B. microti* in *R. tanezumi* in Yunnan (2.70%) and the Dapan Mountains (5.56%) were higher than our results (0.61%). We found that the high prevalence of *B. microti* in *N. confucianus* in Fujian (17.02%) was similar to that in the Dapan Mountains of Zhejiang (20.0%), suggesting that *N. confucianus* is a superior reservoir host in Southeast China (Gao et al., 2017). Moreover, the high prevalence of *B. microti* infection in rodents in Ningde and Sanming in this study strongly supports that these surveillance points are major natural foci for human babesiosis. Furthermore, the results highlight the need for close monitoring of *B. microti* transmissions in Ningde and Sanming; however, *B. microti* epidemic in other cities should also be monitored. Notably, the *B. microti* infection rates were zero in Putian, Quanzhou, Zhangzhou and Longyan, possibly because of the lack of samples and rodent habitats in these regions (Table 4). Moreover, the prevalence of *B. microti* varied among districts. Although both Fuzhou and Quanzhou are adjacent to Sanming, the *B. microti* infection rates were lower than 5.00% in both areas. In contrast, Sanming had the highest infection rate, probably attributed to the distribution of the reservoir host species.

Interestingly, the infection rate of *Babesia* in Xiapu District, Ningde City, was 15.79% (Table S2), which may provide a novel link to the first human case of babesiosis in Fujian (Ou et al., 2018). The patient diagnosed with a *B. microti* infection lived and worked in a village in Xiapu, Ningde, surrounded by abundant shrubs and forests. Our study revealed that the prevalence of *B. microti* in rodents from shrubs (11.18%), irrigated cropland (4.70%) and forests (4.55%) was significantly higher than in the residential areas (0.99%), suggesting that ecological habitat plays an important role in the spread of *B. microti*. Furthermore, *B. microti* can live and reproduce in the wild rodents, which is likely related to the habitat and density of the tick vector (Gao et al., 2017). Similar results were reported in Yunnan (Gao et al., 2017) and Beijing (Wei et al., 2020). The prevalence of *B. microti* in small mammals from the forest (3.37%) and agricultural areas (1.79%) in Yunnan was significantly higher than in residential areas (0.93%). In Beijing, the positive rates of *B. microti* from different habitats including shrubs, broad-leaved forests, cropland, mixed forests and residential areas were 27.4%, 23%, 16%, 8.4% and 7.2% respectively. Forests are reported as an essential risk factor for *Babesia* infection in Thailand, Cambodia, Lao People’s Democratic Republic and China (Yunnan and Heilongjiang; Gao et al., 2017; Karnchanabanthoeng et al., 2018; Sun et al., 2008). Considering that forest areas are burdened with tick-transmitting pathogens, people who work in or travel to forests should take appropriate protective

![FIGURE 2 Neighbour-joining phylogenetic tree based on Babesia microti 18S rRNA partial sequence data from Fujian isolates, with B. microti reference strains. B. divergens, Babesia sp. XXB/Hangzhou and B. 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 percentage occurrence in 1,000 bootstrap replicates. Black circles represent novel sequences identified in this work.](image)
measures. Both Babesia and Plasmodium are intraerythrocytic protozoans and elicit similar inflammatory responses with similar clinical symptoms, easily leading to misdiagnosis (Zhou, Xia, et al., 2014). In summary, doctors should pay attention to human babesiosis, whereas public health agencies should urgently develop prevention and control measures. We found that the prevalence of B. microti in adult rodents (4.53%) was significantly higher than in pubertal rodents (1.10%), similar to the results of other studies in Yunnan (2.69% (adult) and 0.37% (pubertal); Gao et al., 2017) and Beijing (13.3% (adult) and 6.2% (pubertal); Wei et al., 2020).

Positive PCR samples were sent for sequencing. And the sequence alignment and phylogenetic analysis revealed that all collected Babesia parasites from rodents in Fujian province were B. microti. This conclusion can be drawn based on the abundance of samples detected, similar to previous findings in Yunnan (Chen et al., 2017; Gao et al., 2017), Taiwan (Chao et al., 2017) and Beijing (Wei et al., 2020). Our phylogenetic analysis suggested that B. microti shared high homology with those in Zhejiang province, where a confirmed human babesiosis case was reported in Hangzhou in 2002 in a patient following kidney transplantation (Saito-Ito et al., 2008). Unexpectedly, water buffaloes were not infected with B. bovis or B. orientalis, which differs from other studies (He et al., 2012, 2017) and may be related to insufficient samples and analysis of a single sampling location. On the other hand, both domestic goats and domestic pigs were infected with Babesia spp. We detected B. canis vogeli in the blood of domestic dogs for the first time; the sequence shares high homology with B. canis vogeli from Côte d’Ivoire (GenBank MK495837.1). The prevalence of B. canis in dogs was previously documented in the Henan province (Wang, Liu, et al., 2019).

We also detected Babesia spp. in domestic goats for the first time. Ovine babesiosis is a great threat to the livestock industry as a tick-borne disease of goats and sheep in temperate, subtropical and tropical regions (Guswanto et al., 2017; He et al., 2021; Niu et al., 2016). Due to the underestimation and neglect of ovine babesiosis, the economic losses in the small ruminant industries may also be well underestimated. At least seven isolates causing ovine babesiosis have been isolated from sheep in China. Although the infection rate of ovine babesiosis was extremely low in this study, relevant institutions should pay more attention and strengthen quarantine measures for early detection and treatment. Moreover, the number of domesticated dogs and cats has greatly increased, with the development of the economy and improvements in living standards in China, and it resulted in the increase in B. vogeli cases in dogs and cats (Li et al., 2020; Wang, Liu, et al., 2019; Zhang et al., 2019). Therefore, monitoring Babesia infections in domestic dogs and cats is necessary.

There were limitations to this study. A nested PCR approach was used to investigate the incidence of Babesia parasites in mammals in some reports (Chen et al., 2017; Karnchanabanthoeng et al., 2018). A large number of samples (n = 1,562) with a high risk of contamination was included in this study, and therefore the nested PCR method was not used. We showed that the prevalence of B. microti was higher in northern Fujian (Sanming, Nanping, Ningde and Fuzhou) than in Southern Fujian (Putian, Quanzhou, Zhangzhou and Longyan), which may be attributed to altitude, as northern Fujian (495 m) has a higher average altitude than Southern Fujian (411 m). A study in Southern Norway showed that ticks exist at an altitude much higher than previously considered, leading to an increased risk of infection of mammals with tick-borne diseases (De Pelsmaeker et al., 2021). Altitude has been reported as a risk factor for Babesia infections (Gao et al., 2017; Wei et al., 2020); however, altitude was not considered in this study.

FIGURE 3 Neighbour-joining phylogenetic tree based on Babesia species using the 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 per cent occurrence in 1,000 bootstrap replicates. Black circles represent novel sequences identified in this work.
5 | CONCLUSIONS

Our study suggests the geographical distribution and phyloge netic relationship of Babesia in potential mammal reservoir hosts in Fujian province, Southeast China, and baseline data to help public health authorities develop prevention and control measures against Babesia infections. Because the number of samples from single sampling surveillance is insufficient, hosts such as livestock should be investigated. As human babesiosis is a tick-borne disease transmitted through blood transfusions, it is necessary to survey the prevalence of Babesia in tick species and donor populations.

AUTHOR CONTRIBUTIONS

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

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CONFLICT OF INTEREST

The authors have declared that no competing interests exist.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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 following the Guidelines for the Care and Use of Laboratory Animals.

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**SUPPORTING INFORMATION**

Additional supporting information can be found online in the Supporting Information section at the end of this article.