Molecular Detection of *Rickettsia felis* in *Haemaphysalis longicornis* Ticks in Anhui Province of China

Bo-Yu Liu
Anhui Medical University
Wan-Rong Luo
Anhui Medical University
Zhen Chen
Anhui Medical University
Cui-Ping Ren
Anhui Medical University
Ying Hong
Anhui Medical University
Dong-yang Yu
Anhui Medical University
Yue Gao
Anhui Medical University
Yu Zhu
Anhui Medical University
Li Yang
Anhui Medical University
Yan Liu (yanliu@ahmu.edu.cn)
Anhui Medical University

Research

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Abstract

Background

Rickettsia felis causes febrile illness in humans. There is no information on the organism in Anhui Province of China.

Methods

Questing ticks were collected from Anhui Province of China and tick DNA was used as template for nested PCR (or real-time PCR) to amplify *Rickettsia felis* with 16S rRNA gene (*rrs*), 17kDa protein gene and *orfB* gene.

Results

PCR results indicated that 0.22% (2/907) ticks were positive to *R. felis*. Sequence analysis of the partial *rrs* and 17 kDa protein genes showed *R. felis* in ticks was highly homologous with *R.felis* URRWXCal2 with similarity of 98.91% and 99%, respectively.

Conclusions

Our study demonstrated the first evidence of *R. felis* in *H. longicornis* ticks from Anhui Province of China, suggesting that a potential transmission of *R. felis* to humans through tick bite in China.

Introduction

Rickettsia felis, a Gram-negative obligate intracellular bacterium, is a human pathogen responsible for flea-borne spotted fever (FBSF), also known as cat flea typhus (CFT). It can infect arthropods (ticks, mites and mosquitoes) and mammals (humans, apes, gorillas, monkeys, rats, dogs, and cats) [1], and cause an acute febrile illness commonly involving headache, malaise, myalgia, rash, eschar and other symptoms[2], even to severe neurological and respiratory complications. These symptoms are very similar to those caused by related rickettsial species [3, 4]. *Rickettsia felis* was first discovered in fleas with worldwide distribution. Recently flea-borne rickettsiosis outbreak in the United States, where *R. felis* is more prevalent in arthropods and mammals in the area than *R. typhi* [3, 5, 6, 7]. In Asia, *R. felis* has been detected by PCR in fleas from hailand, South Korea, Afghanistan, Laos, Malaysia, and China), ticks from China and Japan, lice from China, mosquito from China, raccoons from Japan, dogs from China, cat from China and people from China and South Korea [8, 9]. In recent years, *R. felis* infection is increasingly reported in China, including Jiangsu, Yunnan, Gansu, Shaanxi and Hongkong [10, 11]. However, there is no report on *R. felis* infection in ticks from Anhui Province of China. The aim of this study is to investigate the infection of *R. felis* in ticks collected from Anhui Province.

Methods

Tick Samples collection

Questing ticks were collected from vegetation from April to August in 2018 in five counties and one district in Anhui Province including Jinzhai County, Huoshan County, Tongcheng County, Qianshan County, Mingguang County and Nanqiao District of Chuzhou City by flagging over vegetation. All study sites are mountainous areas. Ticks were frozen at −80°C until use. Tick species and developmental stages were determined morphologically by an experienced expert.

PCR amplification and DNA sequencing

The ticks were grouped according to their developmental stages, and each group contained 19 nymphs or 3 adult ticks. DNA was extracted from ticks with the QIAamp® DNA Blood Mini Kit (QIAgen, Germany) following the manufacturer's instruction. The DNA samples were amplified by nested PCR with PCR primers derived from rickettsial genes including 16S rRNA (*rrs*), 17 kDa protein genes. PCR primers were described previously and summarized in table 1. The PCR conditions for the two genes were the same as follows: 5 min at 95℃, followed by 40 cycles of 45 s at 94℃, 45 s at 50℃, 1 min at 72℃, and a final extension at 72℃ for 10 min. The nested PCR was conducted using 1 μL first-round PCR product as template under the following conditions: 5 min at 95℃, followed by 40 cycles of 45 s at 94℃, 45 s at 53℃, 1 min at 72℃, and a final extension at 72℃ for 10 min. The PCR products were electrophoresed in a 1.2% agarose gel and detected under UV light. Positive amplicons were purified using agarose gel DNA extraction kit (TaKaRa, Dalian, China). The purified PCR fragments were ligated into the pMD-18T plasmid by T-A cloning, and then transformed into competent *E.coli* DH5α, cultivated at 37℃ for filtration of blue and white clone. Single clones were sequenced using the Sanger sequencing technique (Sangon Biotech, Shanghai, China) after colony PCR identification on both strands. All sequences were compared with the known sequence in GenBank with BLAST program.

To confirm positive results, real-time PCR targeting the *orf*B gene was performed using *R.felis*-specific primers [12] (table 1).

Phylogenetic analysis:

The DNA sequences of *rrs* and 17 kDa protein genes of the *Rickettsia species* were aligned and compared by MEGA software (Version 7). Phylogenetic trees were constructed using the Neighbor-joining method in MEGA software with 1000 bootstrap replications. A dendrogram was obtained based on genetic distances. All reference sequences were downloaded from NCBI.

Results

We collected 907 ticks from vegetation and goats, including 190 nymphs and 717 adult ticks, in five counties and one district of Anhui Province (Fig.1). All the ticks were identified as *Haemaphysalis longicornis* based on the morphological characters, implying *H. longicornis* is a predominated tick species in Anhui Province of China. The prevalence (minimum infection rate) of *R. felis* in each stage of

the tick was determined by the assumption that a positive pool of ticks contained one *R. felis*-infected tick. The prevalence of *R. felis* was 0 among 190 nymphs and 0.28% (2/717) among adult ticks. To further confirmation, we amplified the 17 kDa protein and *orfB* genes of *R. felis* and the results were the same as the *rrs* gene PCR.

DNA sequence analysis of *rrs* gene indicated that rickettsial sequences from ticks were highly homologous with that of *R. felis* URRWXCal2 with 98.91% identity (Fig.2A). The 17 kDa protein gene of the Anhui strains were 99% homologous with those of *R. felis* isolates (N1422-1, N1466-1, N1486-1, MF491767.1 and AF195118.1) (Fig.2B).

Discussion

R. felis has been traditionally grouped as a member of the spotted fever group (SFG) rickettsia [15]. It was first described in the cat flea, *Ctenocephalides felis,* in the United States [16]. Since then, the pathogen has been detected in mosquitoes, fleas, ticks, mites and lice [3, 10, 17, 18]. To date, thirty-nine species of arthropods have been associated with *R. felis* [3]. Ticks is considered one of the most important vectors of *R. felis*. Tick plays a key role in the epidemiology of zoonotic diseases, such as human granulocytic anaplasmosis[19]. Previous studies showed that *R. felis* was found to infect *H. longicornis* in regions of Jiangsu, China, and to infect *Leptotrombidium scutellare* mites in Huangdao District of Qingdao City, China [20]. The phenomenon may be attributed to the different vectors of *R. felis* in different ecological environments.

Anhui province located in the eastern part of China, it's an offshore inland province between east longitude 114°54 '~ 119° and northern latitude 37°41 '29 ~ 34°38' roughly, and it can be roughly divided into five regions: Huaibei Plain, Jianghuai Hill, Western Dabie Mountainous Area, Wannan Mountainous Area and the Plain along the river. In this study, we collected all the *H. longicornis* ticks from the mountainous areas owing to almost no ticks in the plain areas. We detected *R. felis* from these ticks with nested PCR (or real-time PCR) based on 16S rRNA, 17kDa and orfB genes. As we all known, sequence comparison of the 16S rRNA gene is considered one of the most powerful and precise molecular methods for analyzing the phylogenetic relationships of bacteria. In this study, the 16S rRNA gene sequences of the positive tick samples from Anhui Province were closely related to *R. felis* (Fig.1). Additionally, differences in sequencing results indicate that there may be a few of genetic variants in Anhui Province, even simultaneously in the same region. As can be seen from Figure 1, the two 16S RNA sequences from Anhui Province were all identical to *R. felis* URRWXCal2 (CP000053.1). However, phylogenetic analysis of 17kDa protein gene indicated that *R. felis* strain Anhui-1 detected in ticks from Anhui Province, was more similar with *R.felis* isolates (MF491767.1 and AF195118.1), while *R. felis* strain Anhui-2 was more similar with these isolates (MF491774.1, MF491775.1 and MF491778.1), implying *R. felis* in China is genetically diverse (Fig. 2). For further confirmation, qPCR was carried out using specific *R. felis* primers targeting *orfB* gene to detect the positive samples. Through sequence analysis, all the positive samples were *R. felis*, and the nucleotide sequences of *orfB* from the ticks were identical to each other.

To our knowledge, this is the first report of *R. felis* in *H. longicornis* in Anhui Province of China. Their vectorial capacity should be further experimentally validated. Additionally, this study further supports the potential role of ticks as vectors of *R. felis* and suggests that this pathogen has a wide geographical distribution in China, including Anhui Province. Further studies are needed to evaluate the potential risk of humans to acquire *R. felis* infection and investigate the epidemiology (for example, in mosquitoes, lice and mice) and transmission mechanisms of *R. felis*, because there is no doubt that humans live in close association with *R. felis*-infected vectors throughout Anhui Province of China.

Abbreviations

PCR: polymerase chain reaction; CFT: cat flea typhus; FBSF: flea-borne spotted fever.

Declarations

Author Contributions

Conceived and designed the experiments: Yan Liu, contributed materials/reagents/analysis tools and performed experiments: Bo-Yu Liu and Wan-Rong Luo; analyzed the data and wrote the main manuscript text: Bo-Yu Liu ; All authors reviewed the manuscript.

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Availability of data and materials

Data supporting the conclusions of this article are included within the article. The raw data obtained during the present study are available upon request.

Ethics approval

This study received ethical approval from the human bioethics committee of Anhui Medical University, China.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

References

1. Moonga LC, Hayashida K, Nakao R, Lisulo M, Kaneko C, Nakamura I, et al. Molecular detection of *Rickettsia felis* in dogs, rodents and cat fleas in Zambia. Parasites vectors. 2019;12:168.
2. Hii SF, Kopp SR, Abdad MY, Thompson MF, Oleary C, Rees R, et al. Molecular Evidence Supports the Role of Dogs as Potential Reservoirs for *Rickettsia felis*. Vector Borne Zoonotic Dis. 2011;11:1007-12.
3. Legendre KP, Macaluso KR. *Rickettsia felis*: A Review of Transmission Mechanisms of an Emerging Pathogen. Trop Med Infect Dis. 2017; 2: 64.
4. Blanton LS, Walker DH. Flea-Borne Rickettsioses and Rickettsiae. Am J Trop Med Hyg. 2017; 96:53-6.
5. Boostrom A, Beier MS, Macaluso JA, Macaluso KR, Sprenger D, Hayes J, et al. Geographic Association of *Rickettsia felis*-Infected Opossums with Human Murine Typhus, Texas. Emerg Infect Dis. 2002; 8: 549-54.
6. Maina AN, Fogarty C, Krueger L, Macaluso KR, Odhiambo A, Nguyen K, et al. Rickettsial Infections among *Ctenocephalides felis* and Host Animals during a Flea-Borne Rickettsioses Outbreak in Orange County, California. PLoS One .2016;11: e0160604.
7. Eremeeva ME, Warashina WR, Sturgeon MM, Buchholz AE, Olmsted GK, Park SY, et al. *Rickettsia typhi* and *R. felis* in Rat Fleas (*Xenopsylla cheopis*), Oahu, Hawaii. Emerg Infect Dis. 2008;14:1613-15.
8. Parola P. *Rickettsia felis*: from a rare disease in the USA to a common cause of fever in sub-Saharan Africa. Clin Microbiol Infect 2011;17: 996-1000.
9. Sashika M, Abe G, Matsumoto K, Inokuma H. Molecular survey of rickettsial agents in feral raccoons (Procyon lotor) in Hokkaido, Japan. Japanese journal of infectious diseases. 2010;63:353-354.
10. Zhang J, Lu G, Kelly P, Zhang Z, Wei L, Yu D, et al. First report of *Rickettsia felis* in China. BMC Infect Dis. 2014;14: 682.
11. Slapeta J, Lawrence A, Reichel MP. Cat fleas (*Ctenocephalides felis*) carrying Rickettsia felis and Bartonella species in Hong Kong. Parasitol Int. 2018;67:209-12;
12. Socolovschi C, Pages F, Raoult D. *Rickettsia felis* in Aedes albopictus mosquitoes, Libreville, Gabon. Emerg Infect Dis. 2012;18:1687-89.
13. Wenzel RP, Hayden FG, Groschel DH, Salata RA, Young WS, Greenlee JE, et al. Acute febrile cerebrovasculitis: a syndrome of unknown, perhaps rickettsial, cause. Ann Intern Med. 1986;104:606-15.
14. Webb L, Carl M, Malloy DC, Dasch GA, Azad AF. Detection of murine typhus infection in fleas by using the polymerase chain reaction. J Clin Microbiol. 1990; 28:530-34.
15. Bouyer DH, Stenos J, Crocquet-Valdes P, Moron CG, Popov VL, Zavala-Velazquez JE, et al. *Rickettsia felis*: molecular characterization of a new member of the spotted fever group. International journal of systematic and evolutionary microbiology Int J Syst Evol Microbiol. 2001;51:339-47.

16. Schriefer ME, Sacci JB Jr, Dumler JS, Bullen MG, Azad AF. Identification of a novel rickettsial infection in a patient diagnosed with murine typhus. J Clin Microbiol. 1994; 32: 949-54.
17. Teoh YT, Hii SF, Graves S, Rees R, Stenos J, Traub RJ. The epidemiology of *Rickettsia felis* infecting fleas of companion animals in eastern Australia. Parasites vectors. 2018;11:138.
18. Brown LD, Macaluso KR. *Rickettsia felis*, an Emerging Flea-Borne Rickettsiosis. Curr Trop Med Rep. 2016;3:27-39.
19. Ohashi N, Inayoshi M, Kitamura K, Kawamori F, Kawaguchi D, Nishimura Y, et al. Anaplasma phagocytophilum-infected ticks, Japan. Emerg Infect Dis. 2005;11 11:1780-83.
20. Huang Y, Zhao L, Zhang Z, Liu M, Xue Z, Ma D, et al. Detection of a Novel *Rickettsia* From *Leptotrombidium scutellare* Mites (Acari: Trombiculidae) From Shandong of China. J Med Entomol. 2017;54:544-49.

Table

Table 1. Primers for amplification of *Rickettsia felis*

Gene	Primer	Nucleotide sequence (5′-3′)	Reference
rrs	fD1out-f	AGA GTT TGA TCC TGG CTC AG	[13]
	357f	TAC GGG AGG CAG CAG	
	800r	CTA CCA GGG TAT CTA AT	
	1050r	CAC GAG CTG ACG ACA	
	rP2-out-r	ACG GCT ACC TTG TTA CGA CTT	
17 kDa protein gene	17K-5	GCTCTTGCAACTTCTATGTT	[14]
	17K-3	CATTGTTCGTCAGGTTGGCG	
	17kD1	GCTCTTGCAACTTCTATGTT	
	17kD2	CATTGTTCGTCAGGTTGGCG	
*orf*B	*orf*B-F	CCCTTTTCGTAACGCTTTGCT	[12]
	*orf*B-R	GGGCTAAACCAGGGAAACCT	
	*orf*B-probe	6-FAM-TGTTCCGGTTTTAACGGCAGATACCCA-TAMRA	

Figures

Figure 1

We collected 907 ticks from vegetation and goats, including 190 nymphs and 717 adult ticks, in five counties and one district of Anhui Province. 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.

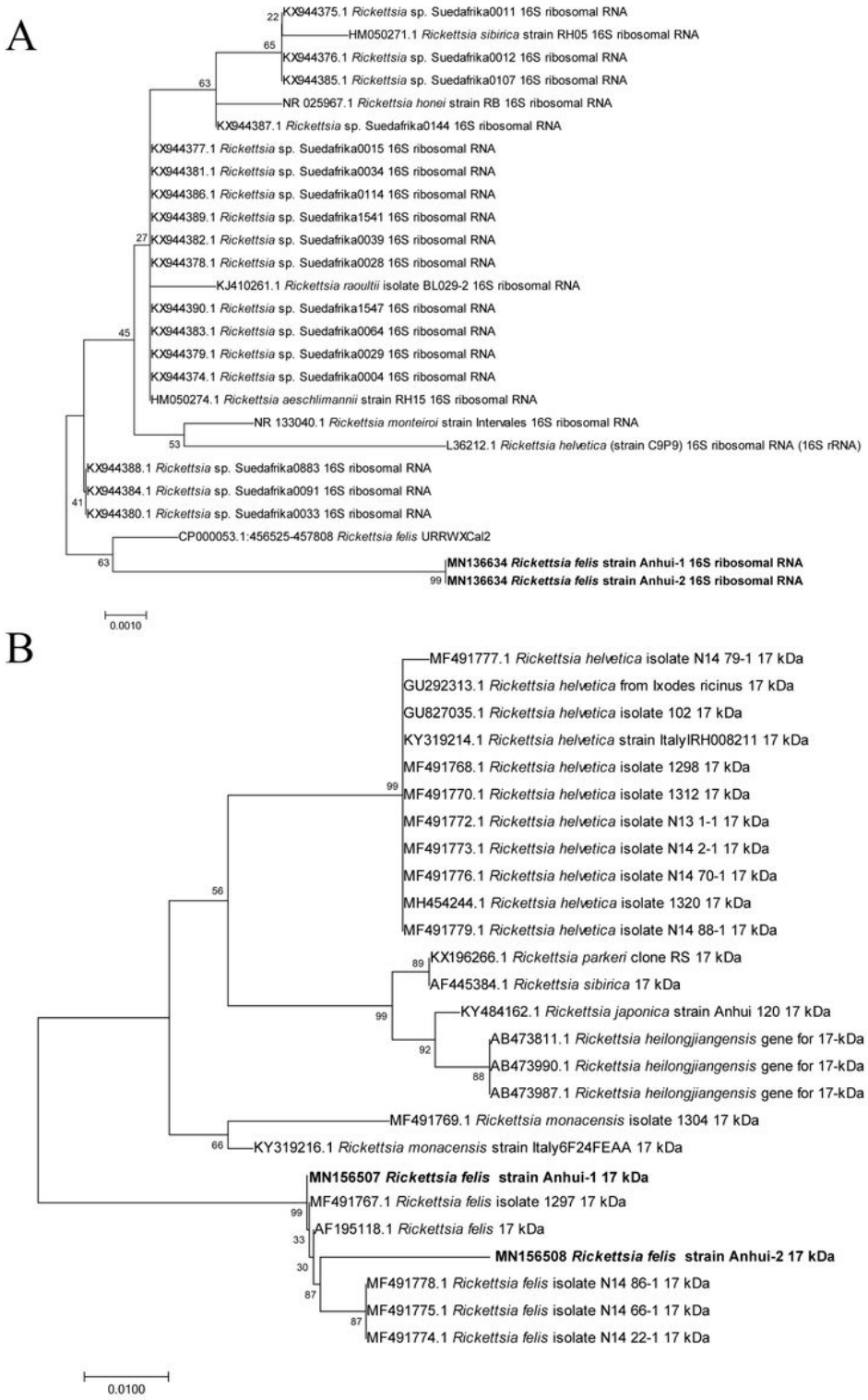

Figure 2

A. DNA sequence analysis of rrs gene indicated that rickettsial sequences from ticks were highly homologous with that of R. felis URRWXCal2 with 98.91% identity. B. The 17 kDa protein gene of the Anhui strains were 99% homologous with those of R. felis isolates (N1422-1, N1466-1, N1486-1, MF491767.1 and AF195118.1).