Ticks and Tick-borne Diseases 8 (2017) 574–580

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

# Extensive diversity of rickettsiales bacteria in ticks from Wuhan, China

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ARTICLE INFO

*Keywords:*
Rickettsiales bacteria
Ticks
Evolution
Phylogeny
Emerging disease
China

ABSTRACT

Rickettsiales bacteria are important agents of (re)emerging infectious diseases, with ticks playing a key role in their evolution and transmission. We collected 1079 hard ticks belonging to five species (*Ixodes sinensis, Rhipicephalus microplus, Haemaphysalis flava, Haemaphysalis hystricis* and *Haemaphysalis longicornis*) from cattle and goats in Wuhan city, Hubei province, China. The dominant tick species was *H. longicornis* (578, 53.57%), followed by *R. microplus* (354, 32.81%), *H. hystricis* (62, 5.75%), *H. flava* (57, 5.28%), and *I. sinensis* (28, 2.59%). Rickettsiales bacteria were identified in these ticks by amplifying the Rickettsiales 16S rRNA (*rrs*), citrate synthase (*gltA*), and heat shock protein (*groEL*) genes. The *rrs* gene of Rickettsiales was positive in 32 (2.97%) ticks, including 2 cases of co-infection, with 4 (0.69%) in *H. longicornis*, 15 (4.24%) in *R. microplus,* 7 (12.28%) in *H. flava*, 1 (1.61%) in *H. hystricis,* and 5 (17.86%) in *I. sinensis* ticks. Phylogenetic analysis revealed the presence of six recognized and seven *Candidatus* species of Rickettsiaceae, Anaplasmataceae and *Candidatus* Midichloriaceae. Notably, one lineage within both *Ehrlichia* and *Candidatus* Midichloriaceae was distinct from any known Rickettsiales, suggesting the presence of potentially novel species of Rickettsiales bacteria. In sum, these data reveal an extensive diversity of Rickettsiales in ticks from Wuhan, highlighting the need to understand Rickettsiales infection in local animals and humans.

## 1. Introduction

Rickettsiales bacteria are obligate intracellular parasites of eukaryotes. At present, the order comprises three established families (Rickettsiaceae, Anaplasmataceae and Holosporaceae) and one proposed family (*Candidatus* Midichloriaceae) (Driscoll et al., 2013; Montagna et al., 2013). Additionally, on the base of their position in phylogenetic trees based on partial or whole-genome sequences, some newly discovered Rickettsiales may represent novel members within this order (Driscoll et al., 2013; Gillespie et al., 2012; Hess et al., 2015; Guo et al., 2016). Most of the rickettsiales described are well known as zoonotic (re)emerging pathogens, and some can cause severe human diseases, including rickettsioses, anaplasmosis, ehrlichiosis and scrub typhus (Raoult and Parola, 2007). In addition, for bacteria such as *Wolbachia*, which were not considered to be human pathogens, recent evidence for human infection has also been provided (Chen et al., 2015). Indeed, as well as the increasing identification of new rickettsiales species worldwide from arthropods and mammals, the number of newly-discovered human pathogens has also increased rapidly during the last two decades (Pritt et al., 2011; Rar and Golovljova, 2011; Parola et al., 2013). Hence, the ongoing (re)emergence of known and unknown rickettsiales bacteria in humans means that they will remain a major threat to public health for the foreseeable future.

Despite the identification of Rickettsiales bacteria in a diverse range of hosts including protists, hydra, annelids, arthropods, vertebrates, and even plants (Darby et al., 2007; Guo et al., 2016; Kang et al., 2014; Merhej and Raoult, 2011; Sicard et al., 2014; Weinert et al., 2009), ticks (mainly hard ticks, Ixodidae) have been found to play an important role in their maintenance and transmission in nature, especially to humans (Eremeeva and Dasch, 2015; Kang et al., 2014; Raoult and Parola, 2007; Rar and Golovljova, 2011). The infection rates of rickettsiales in nature varies substantially with respect to vectors, hosts and geographic region (Eremeeva and Dasch, 2015; Rar and Golovljova, 2011; Stuen et al., 2013). Additionally, each tick species has preferred environmental conditions, which in turn determine the geographic distribution of the ticks and, consequently, the risk areas for tick-borne disease

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http://dx.doi.org/10.1016/j.ttbdis.2017.03.006
Received 31 August 2016; Received in revised form 1 March 2017; Accepted 22 March 2017
Available online 24 March 2017
1877-959X/ 

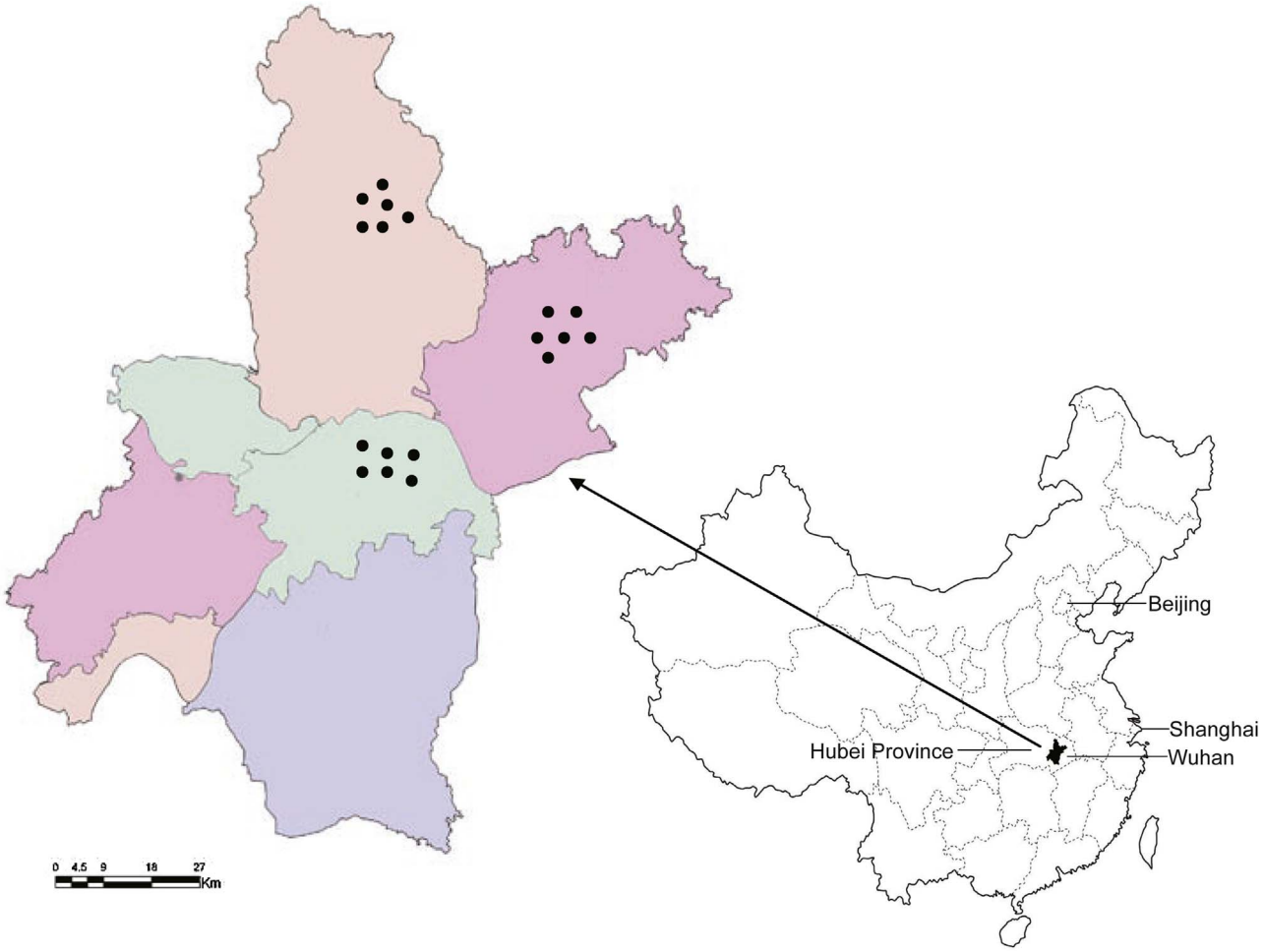


**Fig. 1.** Map showing the location of collection sites (•) in Wuhan city, Hubei Province, China.

(Parola and Raoult, 2001). Therefore, understanding the ecology of local tick species and identifying the Rickettsiales they carry is of public health importance.

Wuhan is the capital city of Hubei province (Fig. 1) and is one of the largest cities in China with a population of more than 7.8 million. Wuhan contains both urban and rural areas, and is situated at the intersection of the central reaches of the Yangtze and Hanshui rivers. The climate in Wuhan is a subtropical humid monsoon, with sufficient rainfall to support diverse flora and a high diversity of arthropods and mammals. Our previous studies revealed the co-existence of at least 13 species of hard ticks in Wuhan (Tian et al., 2014). More importantly, the surveillance of novel tick-transmitted diseases by the Wuhan Center for Disease Control and Prevention (CDC) identified a number of cases of severe fever and thrombocytopenia; as no viral agents were identified from those cases they could in theory be due to rickettsiales bacteria. To date, however, no molecular epidemiological investigation of rickettsiales in ticks has been carried out in Wuhan, although surveys of rickettsiales in ticks may assist the diagnosis of human disease and hence help in the prevention of the tick-borne disease both in Wuhan and other localities. In this study, we screened and characterized Rickettsiales bacteria in five tick species (*I. sinensis, R. microplus, H. flava, H. hystricis* and *H. longicornis*) collected from domestic animals in Wuhan.

## 2. Materials and methods

### 2.1. Collection and processing of ticks

During 2012, ticks were collected from Wuhan city (114.32°O, 30.52°N) in Hubei province, China (Fig. 1). Ticks were directly collected from domestic animals, including cattle and goats that were grazing on grassland or present in forested regions. All ticks were first identified morphologically by a trained technician, using light microscopy based on the differences in their capituli and body (Tian et al., 2014). Identification was confirmed by analyzing the mitochondrial 12S ribosomal RNA (12S rRNA) gene sequences recovered in this study and the reference sequences of each tick species from GenBank, using methods described in Burger et al. (2013), Lu et al. (2013), Li et al. (2015a) and Shi et al. (2016). The ticks collected were stored at −80 °C until DNA was extracted as described below.

### 2.2. Sample processing, DNA extraction, PCR and sequencing

Tick samples were processed as described previously (Kang et al., 2014). Briefly, individual ticks were washed 3 times with phosphate-buffered saline (PBS), and then were homogenized with a mortar and pestle on ice in 0.5 ml PBS solution. After homogenization, the