Detection of Spotted Fever Group Rickettsia in *Haemaphysalis longicornis* from Hebei Province, China

Author(s): Yaxue Zou, Qiuyue Wang, Zhixin Fu, Pengpeng Liu, Hongtao Jin, Huanhuan Yang, Hongwei Gao, Zhang Xi, Quan Liu, and Lifeng Chen
Source: Journal of Parasitology, 97(5):960-962.
Published By: American Society of Parasitologists
DOI: http://dx.doi.org/10.1645/GE-2751.1
URL: http://www.bioone.org/doi/full/10.1645/GE-2751.1

J. Parasitol., 97(5), 2011, pp. 960–962

Detection of Spotted Fever Group Rickettsia in *Haemaphysalis longicornis* From Hebei Province, China

Yaxue Zou, Qiuyue Wang, Zhixin Fu, Pengpeng Liu, Hongtao Jin*, Huanhuan Yang*, Hongwei Gao*, Zhang Xi†, Quan Liu*‡, and Lifeng Chen‡§, Key Laboratory of Preventive Veterinary Medicine in Hebei Province, Hebei Normal University of Science & Techology, 360 Hebei Street, Qinhuangdao, 066000, China; *Institute of Military Veterinary, Academy of Military Medical Sciences, Key Laboratory of Jilin Province for Zoonosis Prevention and Control, 666 Liuyung Xilu, Changchun 130122, Jilin Province, People's Republic of China; †National Research Center for Wildlife Borne Diseases, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China; §to whom correspondence should be addressed: *e-mail: Chen_lifeng@163.com*

ABSTRACT: DNA samples from 737 tick pools, representing 6,850 *Haemaphysalis longicornis* and 51 *Dermacentor nuttalli* collected from Hebei Province, China, were analyzed by polymerase chain reaction (PCR) for the presence of spotted fever group *Rickettsia*. Fifty (6.9%) of 724 *H. longicornis* in the tick pool were positive, but no positive samples were found in 13 *D. nuttalli*. Sequence analysis of the partial outer membrane protein A (*ompA*) genes from the 10 positive samples showed 97.4–99.8% identity, but were different from the homologous sequence of *Rickettsia* previously deposited in GenBank. Phylogenetic analysis of *ompA* genes indicated that the *Rickettsia* detected in this study belonged to a novel haplotype, and formed a clade distinct from *Rickettsia heilongjiangii*, *Rickettsia sibirica*, and *Rickettsia hulinii* in China. The new strain, named Candidatus *Rickettsia hebeii*, appears to represent a distinct lineage and could constitute a new species with a minimum prevalence of about 0.7% in *H. longicornis* from Hebei Province, China.

Tick-borne rickettsioses are caused by gram-negative, intracellular bacteria of the spotted fever group (SFG) in the genus *Rickettsia*, and are recognized as emerging vector-borne diseases worldwide (Rizzo et al., 2004; Walker et al., 2008). The clinical signs include fever, headache, eruption, and incidental eschar formation at the site of tick bites (Kaabia and Letaief, 2009). In China, many SFG *Rickettsiae* belong to *Rickettsia sibirica*, including 2 subspecies, i.e., *R. sibirica sibirica*, the agent of North Asian tick typhus detected in *Dermacentor silvarum* and *Dermacentor sinicus* in northern China, and *Rickettsia sibirica mongolotimonae*, the agent of lymphangitis-associated rickettsiosis isolated from *Hyalomma asiaticum* in Inner Mongolia (Yu et al., 1993; Zhang et al., 2006). *Rickettsia heilongjiangensis*, first isolated from *D. silvarum* ticks in Heilongjiang Province, can cause spotted fever in humans (Fournier et al., 2003; Jiao et al., 2005). *Rickettsia hulinii* was first isolated from *Haemaphysalis concinna* in Heilongjiang Province, but its pathogenic role in humans has not been demonstrated (Zhang et al., 2000). These rickettsiae are usually associated with ixodid ticks, which act as both vectors and reservoirs (Azad and Beard, 1998). Some SFG rickettsiae detected or isolated from ticks only, and initially considered potential pathogens, were later recognized as emerging pathogens, and some novel rickettsiae of unknown pathogenicity have also been documented in ixodid ticks (Shpynov et al., 2003; Rolain et al., 2006; Sreter-Lancz et al., 2006). The present study reports the presence of a novel *Rickettsia* species in *Haemaphysalis longicornis* in China.

The study was performed in rural areas of Qinghuangdao (39°56′N, 119°36′E), Zhangjiakou (40°46′N, 114°56′E), Tangshan (39°37′N, 118°11′E), Baoding (38°10′N, 113°40′E), and Chengde (40°11′N, 115°54′E) in Hebei Province, China from April 2008 to May 2010. Ticks were collected by dragging vegetation or directly removed from sheep or hedgehogs. All the specimens were stored in 95% ethanol and later identified to the species level by standard guides (Chen et al., 2010).

Ticks were pooled prior to DNA extraction. The pools consisted of 2–10 ticks of 1 species collected from the same site. DNA extraction was performed as described elsewhere (Fyumagwa et al., 2009). Briefly, the ticks were disinfected in 70% ethanol for 10 min, rinsed with sterilized distilled water, placed in a microtube, and mechanically disrupted with sterile scissors in 50 µl DNA extract buffer (10 mM Tris pH 8.0, 2 mM ethylenediaminetetraacetic acid [EDTA], 0.1% sodium dodecyl sulfate, and 500 µg of proteinase K per milliliter). The sample was incubated at 56 C for 4 hr, then boiled at 100 C for 10 min to inactivate proteinase K. After centrifugation, the supernatant was transferred to a fresh microtube and DNA was purified by extracting twice with an equal volume of phenol-chloroform, which was then stored at −20 C until use.

A polymerase chain reaction (PCR) was performed with the use of primers (5′-ATGGCGAATATTTCTCCAAAA-3′; 5′-GTTCCGTTA-ATGGCAGCATCT-3′) designed to amplify the outer membrane protein A (*ompA*) gene of *Rickettsia* sp. as described previously (Matsumoto et al., 2007). Distilled water instead of tick DNA template was used as a negative control, and DNA from *H. longicornis* ticks infected with SFG *Rickettsia* was used as a positive control.

PCR products were purified and sequenced. The sequences obtained were compared with previously published sequences deposited in GenBank with the use of BLAST, and phylogenetically analyzed. A phylogenetic tree was constructed with the use of the neighbor-joining algorithm of Phylip 3.69 software with Kimura 2-parameter model.

A chi-square test was used to compare spotted-fever group *Rickettsia* prevalences in ticks from different sampling sites. The difference was considered statistically significant when $P < 0.05$. Statistical analysis was performed with the use of SPSS v. 11.0 software (SPSS, Chicago, Illinois).

In the 8,454 ticks collected, 2 species, *H. longicornis* and *D. nuttalli*, were identified based on morphological characters; *H. longicornis* predominated in Hebei Province, accounting for 98.9% of the ticks collected. Species and regional distribution of ticks are shown in Table I.

The DNA samples from 737 tick pools, representing 6,850 *H. longicornis* and 51 *D. nuttalli* collected from Zhangjiakou, Tangshan, Qinhuangdao, and Baoding Cities of Hebei Province, were analyzed by PCR for the presence of SFG *Rickettsia*. There were 50 (6.9%) of 724 *H. longicornis* pools, which came from Zhangjiakou (15), Tangshan (25), and Qinhuangdao (10) that tested positive, but no positive samples were found in 13 *D. nuttalli* (Table I). No significant difference was found among prevalences of spotted fever group *Rickettsia* in Zhangjiakou, Tangshan, and Qinhuangdao ($P > 0.05$).

Sequence analysis of the 10 positive samples, including 5 from Tangshan (TS-1, TS-2, TS-3, TS-4, and TS-5), 3 from Qinhuangdao (QHD-1, QHD-2, and QHD-3), and 2 from Zhangjiakou (ZJK-1 and ZJK-2) showed 97.4–99.8% identity, and the predicted amino acid sequences

TABLE I. Tick samples and infection rates of spotted fever group *Rickettsia* in Hebei Province, China.

Tick species	Region	No. of collected ticks	No. of examined ticks	No. of pools	No. of positive pools (%)
Haemaphysalis longicornis	Zhangjiakou	2,575	2,025	225	15 (6.7)
	Tangshan	2,987	2,520	315	25 (7.9)
	Qinghuangdao	1,934	1,515	130	10 (7.7)
	Baoding	523	510	34	0
	Chengde	348	280	20	0
	Subtotal	8,367	6,850	724	50 (6.9)
Dermacentor nuttalli	Zhangjiakou	6	3	1	0
	Qinghuangdao	81	48	12	0
	Subtotal	87	51	13	0

DOI: 10.1645/GE-2751.1

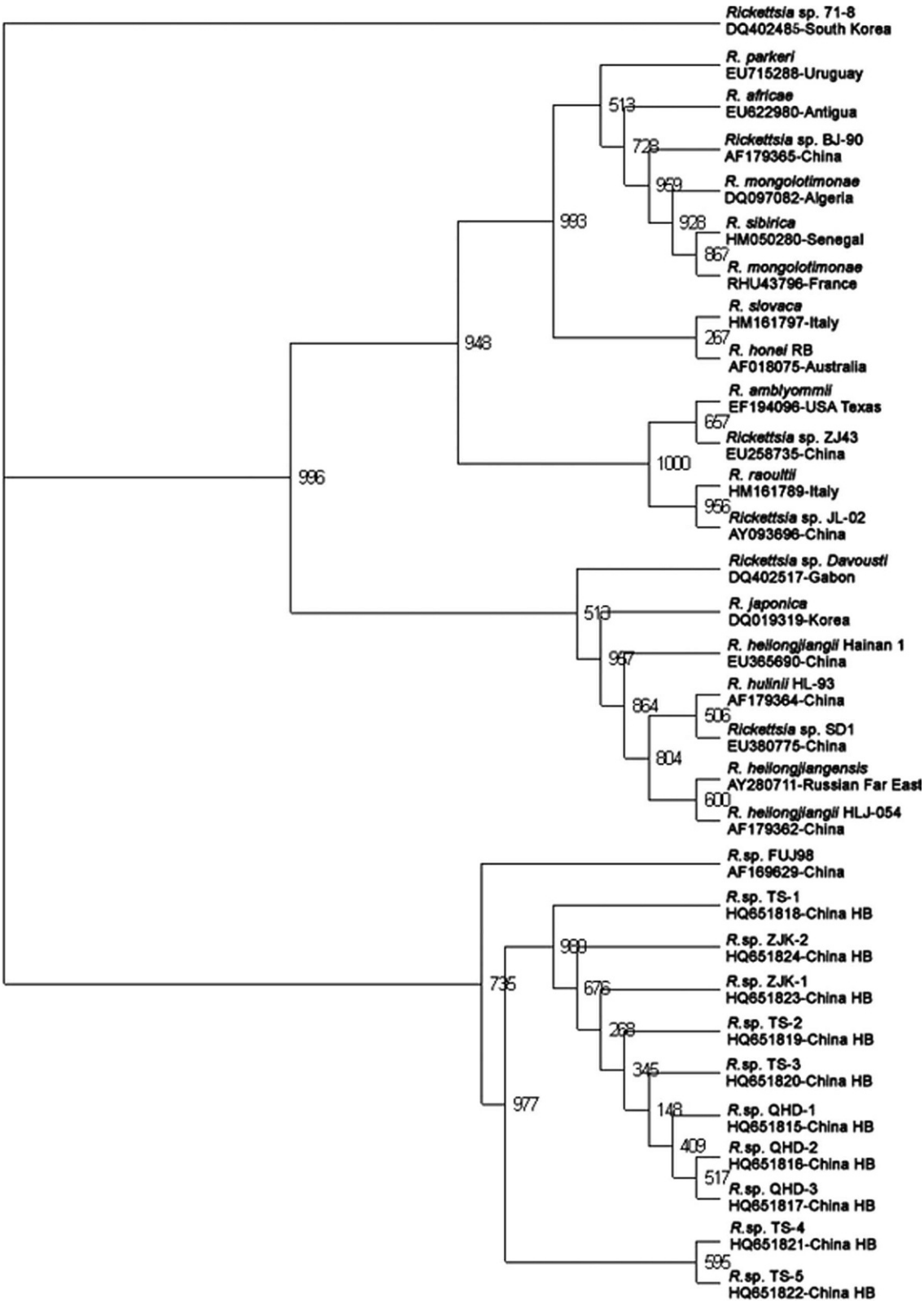

FIGURE 1. Phylogenetic tree for partial OmpA gene sequences of *Rickettsia* spp. constructed by the neighbor-joining method with the use of the Phylip program. The neighbor-joining consensus tree used 1,000 bootstrap replicates. The number represents bootstrap values. The *Rickettsia* species, GenBank accession number, and the country are included.

showed 95.4–99.5% identity. The sequences were different from the predicted amino acid sequences for rickettsiae previously deposited in GenBank, but closest to that of *Rickettsia* sp. from Fujian Province, China (AF169629). The DNA sequences of this study were submitted to GenBank, where they received the following accession numbers: HQ651815, HQ651816, HQ651817, HQ651818, HQ651819, HQ651820, HQ651821, HQ651822, HQ651823, and HQ651824. Phylogenetic analysis indicated that *Rickettsia* sp. detected in ticks from Hebei Province, China belonged to a novel haplotype. They and the Fujiang strain (AF169629) formed a clade distinct from *Rickettsia heilongjiangii* in China. Based on the phylogenetic analysis, the new agent, named Candidatus *Rickettsia hebeiii*, appears to represent a distinct lineage and could constitute a new species (Fig. 1). As there were 50 positive pools, there must have been at least 50 infected ticks among the 6,850 investigated, giving a minimum prevalence of about 0.7% for Candidatus *R. hebeiii* in *H. longicornis*.

This study was supported by Hebei Province Science and Technology Department in China (No.07276916), and "Gold Idea" Foundation of Institute of Military Veterinary, Academy of Military Medical Sciences (No. YCX0901), Changchun, Jilin Province, China.

LITERATURE CITED

Azad, A. F., and C. B. Beard. 1998. Rickettsial pathogens and their arthropod vectors. Emerging Infectious Diseases **4:** 179–186.

Chen, Z., X. Yang, F. Bu, X. Yang, X. Yang, and J. Liu. 2010. Ticks (Acari: Ixodoidea: Argasidae, Ixodidae) of China. Experimental and Applied Acarology **51:** 393–404.

Fournier, P. E., J. S. Dumler, G. Greub, J. Zhang, Y. Wu, and D. Raoult. 2003. Gene sequence-based criteria for identification of new rickettsia isolates and description of *Rickettsia heilongjiangensis* sp. nov. Journal of Clinical Microbiology **41:** 5456–5465.

Fyumagwa, R. D., P. Simmler, M. L. Meli, R. Hoare, R. Hofmann-Lehmann, and H. Lutz. 2009. Prevalence of *Anaplasma marginale* in different tick species from Ngorongoro Crater, Tanzania. Veterinary Parasitology **161:** 154–157.

Jiao, Y., B. Wen, M. Chen, D. Niu, J. Zhang, and L. Qiu. 2005. Analysis of immunoprotectivity of the recombinant OmpA of *Rickettsia heilongjiangensis*. Annals of the New York Academy of Sciences **1063:** 261–265.

Kaabia, N., and A. Letaief. 2009. Characterization of rickettsial diseases in a hospital-based population in central Tunisia. Annals of the New York Academy of Sciences **1166:** 167–171.

Matsumoto, K., P. Parola, J. M. Rolain, K. Jeffery, and D. Raoult. 2007. Detection of "*Rickettsia* sp. strain Uilenbergi" and "*Rickettsia* sp. strain Davousti" in *Amblyomma tholloni* ticks from elephants in Africa. BMC Microbiology **7:** 74–79.

Rizzo, M., P. Mansueto, G. Di Lorenzo, S. Morselli, S. Mansueto, and G. B. Rini. 2004. Rickettsial disease: Classical and modern aspects. New Microbiology **27:** 87–103.

Rolain, J. M., E. Mathai, H. Lepidi, H. R. Somashekar, L. G. Mathew, J. A. Prakash, and D. Raoult. 2006. "Candidatus *Rickettsia kellyi*," India. Emerging Infectious Diseases **12:** 483–485.

Shpynov, S., P. E. Fournier, N. Rudakov, and D. Raoult. 2003. "Candidatus *Rickettsia tarasevichiae*" in *Ixodes persulcatus* ticks collected in Russia. Annals of the New York Academy of Sciences **990:** 162–172.

Sreter-Lancz, Z., Z. Szell, G. Kovacs, L. Egyed, K. Marialigeti, and T. Sreter. 2006. Rickettsiae of the spotted-fever group in ixodid ticks from Hungary: Identification of a new genotype ('Candidatus *Rickettsia kotlani*'). Annals of Tropical Medicine and Parasitology **100:** 229–236.

Walker, D. H., C. D. Paddock, and J. S. Dumler. 2008. Emerging and re-emerging tick-transmitted rickettsial and ehrlichial infections. Medical Clinics of North America **92:** 1345–1361.

Yu, X., Y. Jin, M. Fan, G. Xu, Q. Liu, and D. Raoult. 1993. Genotypic and antigenic identification of two new strains of spotted fever group rickettsiae isolated from China. Journal of Clinical Microbiology **31:** 83–88.

Zhang, J. Z., M. Y. Fan, Y. M. Wu, P. E. Fournier, V. Roux, and D. Raoult. 2000. Genetic classification of "*Rickettsia heilongjiangii*" and "*Rickettsia hulinii*," two Chinese spotted fever group rickettsiae. Journal of Clinical Microbiology **38:** 3498–3501.

Zhang, L., J. Jin, X. Fu, D. Raoult, and P. E. Fournier. 2006. Genetic differentiation of Chinese isolates of *Rickettsia sibirica* by partial ompA gene sequencing and multispacer typing. Journal of Clinical Microbiology **44:** 2465–2467.

Date of Publication
Volume 97, No. 5, was mailed 3 October 2011