DETECTION OF RICKETTSIA SIBIRICA IN TICKS AND SMALL MAMMALS COLLECTED IN THREE DIFFERENT REGIONS OF CHINA

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Summary. – The primers Rr 190.70p and Rr 190.602n were used to detect spotted fever group (SFG) rickettsiae by polymerase chain reaction (PCR) in ticks and small mammals collected in three different regions of China. The obtained results indicated that specific DNA fragments of SFG rickettsiae were amplified from *Dermacentor silvarum, D. sinicus, D. auratus, Haemaphysalis concinna, H. wellingtoni, H. yeni, Apodemus agrarius, Microtus fortis, Clethrionomys rufocanus, Ondatra zibethica, Rattus flavipectus* and hedgehog. The PCR product were digested with restriction endonucleases *Pst*I and *Rsa*I and the obtained electrophoretic profiles were compared with those of the prototype strains of SFG rickettsiae by the restriction fragment length polymorphism (RFLP) technique. The comparisons showed that the profiles were identical to those of *Rickettsia sibirica*. In addition, three new isolates of *R. sibirica* were obtained from *H. yeni, D. sinicus* and hedgehog, and designated NH-95, BJ-95 and BHJ-95, respectively. These results not only demonstrated a horizontal transmission of the rickettsiae between ticks and hosts but also suggested that *R. sibirica* is widely distributed in China and its hosts and vectors are various, all that indicating the existence of natural foci of North Asia tick-borne spotted fever specific to China.

Key words: *Rickettsia sibirica*; polymerase chain reaction; restriction analysis; natural foci

Introduction

Spotted fever is a group of diseases caused by pathogenic SFG rickettsiae and is widely distributed in the world. SFG rickettsiae consist of a number of species of obligate intracellular bacteria which coexist with arthropods and rodents. Pathogenic rickettsiae are transmitted to humans by the bite of infected ticks and mites. Anthropods play an important role in the preservation and transmission of SFG rickettsiae. Thus, to investigate the ecology, epidemiology and prevention of spotted fever, it is very important to map the rickettsiae of concern in arthropods and animal hosts. Recently, with the increase of practical activities and exploitation of natural resources, people have more opportunities to get in contact with vectors and animal hosts of SFG rickettsiae, and the infection rate of spotted fever is increasing in China (Lin *et al.*, 1992; He *et al.*, 1993; Wu *et al.*, 1997; Li *et al.*, 1997). First reports on spotted fever in China date to 1958. It has been demonstrated that the natural foci of North Asia tick-borne spotted fever existed in a large region between 40° – 50° of north latitude and 80° – 135° of east longitude (Fan *et al.*, 1992). There have been also isolated SFG rickettsiae strains in Heilongjiang Province, Inner Mongolia and Beijing which were antigenically and genetically different from the prototype and reference strains (Lou *et al.*, 1985; Jin *et al.*, 1993; Yu *et al.*, 1991; Zhang *et al.*, 1996). The ecology, epidemiology and identification of the new strains attracted the attention of rickettsiologists (Yu *et al.*, 1993; Zhang *et al.*, 1997b). Recently, antibodies to spotted fever were also found in sera of humans and rodents south of 26 of north latitude (He *et al.*, 1993).

Since a large-scale investigation of spotted fever was recommended by Beati *et al.* (1992), increasing number of authors has used their scheme in epidemiological studies (Beati *et al.*, 1994; Gage *et al.*, 1994). In China, Zhang *et*

Abbreviations: PCR = polymerase chain reaction; RFLP = restriction fragment length polymorphism; SFG = spotted fever group

al. (1995) amplified DNA fragments specific to SFG rickettsiae in ticks, tick ova and tick faeces, which was important to the epidemiology of spotted fever.

To investigate the spotted fever infection in vectors and hosts, ticks and small mammals were collected in three different regions of China (Fig. 1) and examined directly by PCR/RFLP technique. Simultaneously, attempts were made to isolate rickettsiae from the field samples in yolk sacs.

Materials and Methods

Ticks described in this report were identified by standard taxonomic keys. In May 1992, 200 *H. concinna* and 500 *D. silvarum* ticks were collected by flagging the vegetation along the bank of Heilongjiang river. In May 1994, 99 *H. wellingtoni,* 22 *D. auratus*, 191 *H. formosensis* and 385 *H. yeni* ticks were collected in Ninhua County, Fujian Province. Three *D. sinicus*, 2,000 *H. longicornis* and 3 *D. nuttalli* ticks were collected in suburbs of Beijing in 1995 and 1996 (Table 1).

Small mammals. One *Microtus fortis*, 2 *Clethrionomys rufocanus*, 13 *Apodemus agrarius* and 1 *Ondatra zibethica* were collected along the bank of Heilongjiang river in May 1992. Twenty *Rattus flavipectus*, 11 *R. norvegicus*, 2 *Mus musculus* and 1 *R. coxingi* were collected in Zhangzhou, Fujian Province in 1994. Two hedgehogs were collected in suburbs of Beijing in May 1995 (Table 1).

Isolation of rickettsiae. The field samples were grouped according to the species and the places of collection. The isolation was carried out directly from the field samples by using embryonated hen's eggs (Bi *et al.*, 1997).

Rickettsiae. R. sibirica 246 strain and *R. rickettsii* R strain were kept at the Academy of Millitary Medical Sciences, Beijing, *R. conorii* Malish 7 strain was supplied by the University of Texas Medical Branch, Galveston, TX, USA. *R. parkeri* and *R. australis* were provided by the Department of Health Laboratory, Galveston, TX, USA. *R. akari* Kaplan strain was supplied by the ATCC, Rockville, MD, USA. The new isolates of *R. sibirica* from *D. sinicus, H. yeni* and hedgehog were designated BJ-95, NH-95 and BJH-95, respectively.

PCR/RFLP. Samples of smal mammals and rickettsiae for PCR/RFLP were prepared by methods described previously (Gage *et al.*, 1994; Silhavy *et al.*, 1989). The primers Rr 190.70p (5'-ATGGCGAATATTTCTCCAAAA-3') and Rr 190.602n (5'-AGTGCAGCATTCGCTCCCCCT-3') originally designed for amplification of the 190K antigen gene of *R. rickettsii* by PCR (Anderson *et al.*, 1990; Regnery *et al.*, 1991) were synthesised at the Chinese Academy of Sciences, Beijing. The amplification reaction was carried out in 100 µl volume in a 90-Aptype thermal cycler in 30 cycles (94°C for 1 min, 48°C for 40 secs, 66°C for 90 secs, the last cycle extended to 5 mins). Each PCR assay included one positive (*R. sibirica*) and one negative control (no template). The PCR products (10 µl samples) were visualised in 1.2% agarose gel after electrophoresis. *Hinf*I-digested pBR322 was employed as size marker. The PCR products were digested with *Pst*I and *Rsa*I and the obtained fragments were separated on 8% polyacrylamide gels and silver-stained. *Hae*III-digested pGEM7z was used as size marker.

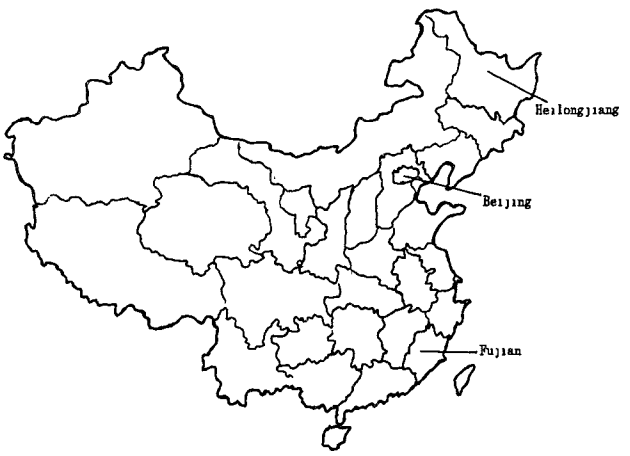

Fig. 1
Map of P.R. of China with localities of field sample collection in this study

Results and Discussion

In this study, we detected and identified *R. sibirica* in *H. concinna, D. silvarum, M. fortis, C. rufocanus, A. agrarius,* and *O. zibethica* collected along the bank of Heilongjiang river (Fig. 2). Previous studies (Lou *et al.*, 1985; Zhang *et al.*, 1996) have indicated that there existed new species of SFG rickettsiae in Heilongjiang Province, but we were not able to identify them in ticks and rodents collected there, perhaps because of relatively limited number of the samples.

H. longicornis is dominant in Beijing suburbs, however, we could not detected *R. sibirica* in it, perhaps because it was not infected with *R. sibirica* or the amount of *R. sibirica* was below the limit of the PCR assay. *D. sinicus* often parasites on wild animals, such as hedgehog, as well as on domestic animals, e.g. cows, sheep, horses etc. Their activities peak in May. The fact that *R. sibirica* was isolated from *D. sinicus* several times demonstrated that the infection rate in this particular tick species was very high (Yu *et al.*, 1991; Zhang *et al.*, 1997a; Chen *et al.*, 1997a). In the investigation of Beijing suburbs, *R. sibirica* was detected and identified in *D. sinicus* and its host – hedgehog (data not shown), an observation suggesting horizontal transmission between ticks and small mammals. Although the main host of *D. sinicus*, hedgehog does not have many chances to contact humans, its activities are very extensive and it might have an important role in the maintenance of SFG rickettsiae.

Previous studies have demonstrated the existence of natural foci of spotted fever in the north of China. Recently, antibodies to SFG rickettsiae have been found in healthy persons and rodents in Fujian Province (Bi *et al.*, 1996), but

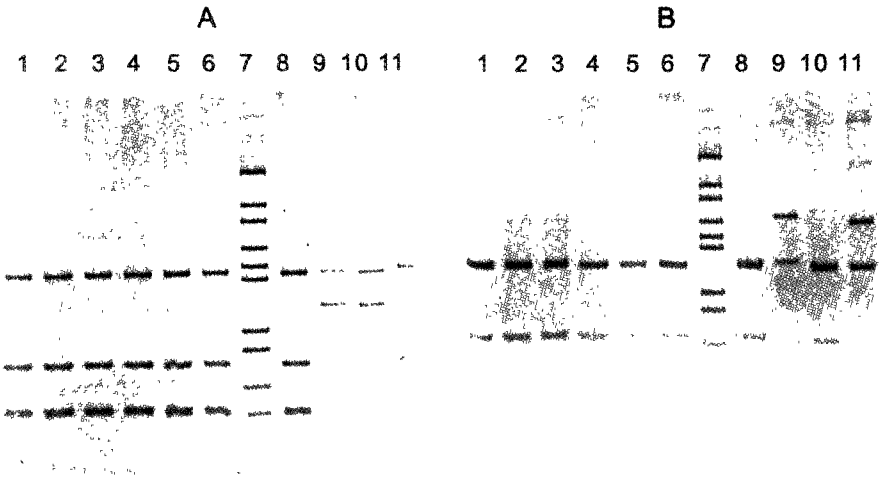

Fig. 2
RFLP analysis of PCR products of field samples from the bank of Heilongjiang river
*Pst*I (A) and *Rsa*I (B) digestion. *H. concinna* (lane 1), *D. silvarum* (lane 2), *A. agrarius* (lane 3), *O. zibethica* (lane 4), *M. fortis* (lane 5), *C. rufocanus* (lane 6), pGEM7z/*Hae*III markers (lane 7), *R. sibirica* (lane 8), *R. conori* (lane 9), *R. rickettsii* (lane 10), and *R. parkeri* (lane 11).

Table 1. Results of field sample collection and detection by PCR/RFLP and isolation of SFG rickettsiae

Locality, year		Field sample	PCR	Isolation
Heilongjiang province.	1992	*Haemaphysalis concinna*	+	–
		Dermacentor silvarum	+	–
		Microtus fortis	+	–
		Clethrionomys rufocanus	+	–
		Apodemus agrarius	+	–
		Ondatra zibethica	+	–
Beijing suburbs,	1995	*Dermacentor sinicus*	+	+
		hedgehog	+	+
	1994	*Haemaphysalis longicornis*	–	–
		Dermacentor nuttalli	–	–
Fujian Province,	1994	*Haemaphysalis wellingtoni*	+	–
		Dermacentor auratus	+	–
		Haemaphysalis formosensis	–	–
		Haemaphysalis yeni	+	+
		Rattus flavipectus	+	–
		Rattus norvegicus	–	–
		Mus musculus	–	–
		Rattus coxingi	–	–

(+) = positive; (–) = negative.

there are so far no reports on typical cases of spotted fever. The results of an investigation in Fujian Province showed that *H. wellingtoni, H. yeni, D. auratus* and *R. flavipectus* were all infected with *R. sibirica* (data not shown). It might suggest that natural foci of the North Asia tick-borne spotted fever exist in the south of China.

Three isolates of SFG rickettsiae, BJ-95, NH-95 and BJH-95, which had been found in *D. sinicus, H. yeni* and hedgehog respectively, were identified as *R. sibirica* (Chen *et al.*, 1997a,b). As the results of isolation and identification of SFG rickettsiae were identical to those of direct detection in field samples, it may indicate the PCR/RFLP technique not only increases the sensitivity, accelerates and facilities the isolation of SFG rickettsiae, but it is also helpful in defining the character of natural foci of spotted fever. Though *R. sibirica* is known to be widely distributed in whole Chi-

na, the natural foci of North Asia tick-borne spotted fever in the south of China should be further investigated.

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