# GENOTYPIC IDENTIFICATION OF SEVEN *RICKETTSIA CONORII* STRAINS

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*Received April 8, 1993; revised June 15, 1993*

**Summary.** – Restriction endonuclease fragment length polymorphism (RFLP) analysis and polymerase chain reaction (PCR) were used to characterize the genotypic diversity of 7 strains of *Rickettsia conorii* from South Africa, Ethiopia, Morocco, India and Russia. The strains of *R. conorii* were divided into four genotypes by *Rsa* I or *Pst* I endonuclease digestion of PCR-amplified rickettsial DNA using primers derived from the *R. rickettsii* 190 K antigen gene. M-1 and Barbash strains were genotypically identical, but different from Indian, Ethiopian and $S_7$ strains, which formed another group. Simko and Moroccan strains were genotypically different from each other and also from other strains of *R. conorii*. We conclude that there exist a genotypic diversity among intraspecies of *R. conorii*.

**Key words:** *Rickettsia conorii*; genotypic identification; PCR; RFLP analysis

## Introduction

Rickettsia conorii, a member of the spotted fever group (SFG) rickettsiae, is the aetilogic agent of boutonneuse fever that is a tick transmitted rickettsiosis endemic in Mediterranean countries, Africa and Asia (Walker *et al.*, 1989). Antigenic and genetic diversity of different strains of *R. conorii* became one of many studies. Goldwasser *et al.* (1974) demonstrated serological differences between Indian and Casablanca strains of *R. conorii* using cross-inhibition indirect fluorescent antibody assay. Later, Gear *et al.* (1983) reported antigenic differences in rickettsial strains from South Africa. Kirkman *et al.* (1985) found a genetic diversity between $S_7$ and Indian strains of *R. conorri* by the method of DNA restriction endonuclease analysis. The genotypic differences between the Moroccan and Israel tick typhus strains (Regnery *et al.*, 1991) were noted by PCR/RFLP analysis.

The purpose of this study aimed at determining genotypic differences among 7 strains of *R. connorii* from different geographic distribution.

## Materials and Methods

*Rickettsial strains.* Malish 7 ($S_7$), Ethiopian and Moroccan strains were provided by the University of Texas Medical Branch, Galveston, USA. Simko and Barbash strains were obtained from the Chinese Military Academy for Medical Research; M-1 and India strains were provided by the Gamaleya Research Institute of Epidemiology and Microbiology, Moscow, Russia.

*Rickettsial cultivation.* All the rickettsiae were cultivated in specific pathogen-free embryonated hen's eggs (Stoenner *et al.*, 1962). The rickettsiae were purified by renografin density gradient centrifugation in a 30 % – 36 % – 42 % discontinuous gradient (Hanson *et al.*, 1981). The light and heavy bands were collected and pelleted at 22 500 × g for 20 mins. The pellets were resuspended in PBS pH 7.5. The purified rickettsiae were stored at –70 °C (Fan *et al.*, 1988).

*DNA extraction.* Purified rickettsial cells were lysed in a solution containing 10 % SDS and 0.2 mg/ml proteinase K. The lysate was extracted with phenol-chloroform repeadly (Sambrook *et al.*, 1989). After ethanol precipitation the DNA pellet was dissolved in TE (10 mmol/l Tris, 0.1 mmol/l EDTA pH 8.0) at 4 °C.

*PCR amplification.* A pair of oligonucleotide primers (primer 1: ATTGCAAAAAGTACAGTGAAGA; primer 2: ATGGCGA-ATATTTCTCCAAAA) (Promega Biotec, USA) was synthesized according to the DNA sequence of the gene encoding the 190 K antigen of *R. rickettsii* (Anderson *et al.*, 1990; Regnery *et al.*, 1991). PCR amplification was accomplished in 100 µl volumes according to the Promega Biotec protocol. 35 cycles of the PCR were performed in a Gene ATAQ Controller (Pharmacia) at 91 °C for 30 secs, at 62 °C for 30 secs, and at 72 °C for 1 min. PCR amplification included a negative control (no template DNA), a

positive control (*R. conorii* strain DNA), and control material from non-infected yolk sac. PCR product was verified by agarose electrophoresis.

*DNA digestion and electrophoresis.* PCR products were digested with *Pst* I and *Rsa* I restriction endonucleases (Promega) according to the standard techniques (Sambrook *et al.*, 1989). The digested products were separated on 8 % polyacrylamide vertical gels. The gels were then stained with ethidium bromide. *Hind* III – digested pBR322 DNA (Promega) served as size standards.

## Results

Nucleotide primers corresponding to *R. rickettsii* 190 K antigen gene primed the synthesis of DNA products from all strains of *R. conorii*. Agarose gel electrophoresis of uncut PCR products demonstrated that the size of the PCR products of tested strains of *R. conorii* were different from each other (Fig. 1). The size of PCR products was determined after agarose gel electrophoresis as 427 bp for M-1 and Barbash strains, 550 bp for Simko and Moroccan strains, and 563 bp for S7, Ethiopian and Indian strains, respectively. Only minimal amounts of products were obtained in two negative controls (data not shown).

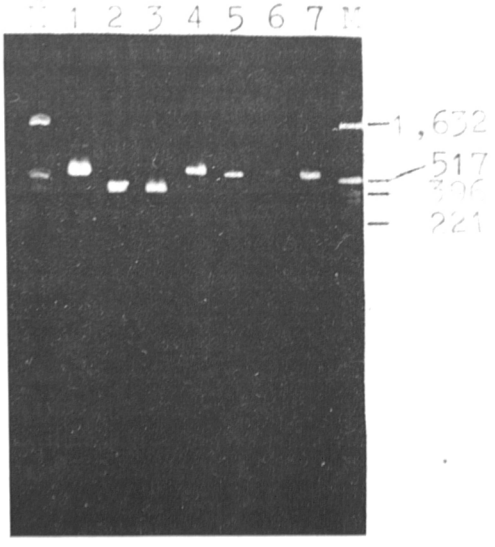


**Fig. 1**
**Agarose gel electrophoresis of PCR-amplified products**
Simko strain (lane 1), Ethiopian strain (lane 2), Moroccan strain (lane 3), S7 strain (lane 4), M-1 strain (lane 7). Lane M: *Hind* III – digested pBR322 DNA (1632, 517, 506, 396, 344, 298, 224, and 154 bp).

*Rsa* I endonuclease digestion of PCR-amplified rickettsial DNA divided all tested strains of *R. conorii* into 4 genotypes (Fig. 2). M-1 and Barbash strains shared identical patterns with two bands of 224 bp and 200 bp, respectively. Indian, Ethiopian and S7 strains had identical patterns with

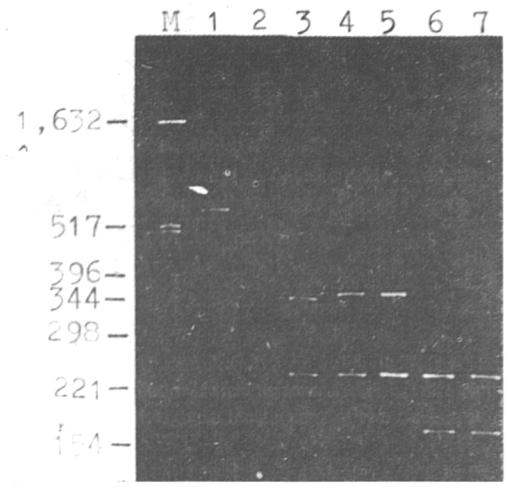


**Fig. 2**
**Polyacrylamide gel electrophoresis of Rsa I digested PCR-amplified products**
Simko strain (lane 1), Ethiopian strain (lane 2), Moroccan strain (lane 3), S7 strain (lane 4), Indian strain (lane 5), M-1 (lane 6), Barbash strain (lan). Lane M: the same as in Fig. 1.

two bands of 330 bp and 224 bp. The pattern of Moroccan strain was slightly different from those of Indian, Ethiopian and Malish 7 (two bands of 347 bp and 224 bp). Simko strain showed only one band of the same size as uncut PCR products.

PCR-amplified rickettsial DNA digested with *Pst* I demonstrated the same genotypic differences among the strains of *R. conorii* as compared to *Rsa* I. *Pst* I patterns of PCR-amplified rickettsial DNA demonstrated that (1) M-1 and Barbash strains were identical with 3 bands of 204 bp, 190 bp and 40 bp; (2) Indian, Ethiopian and S7 strains represented another pattern with 3 bands of 258 bp, 204 bp and 98 bp; (3) Moroccan and Simko strain were different from each other and from the other strains with 3 bands of 254 bp, 204 bp and 98 bp for Moroccan strain, and with 2 bands of 297 bp and 258 bp for Simko strain (data not shown).

## Discussion

The DNA from 7 *R. conorii* strains tested, after PCR amplification with primers derived from the 190 K antigen gene of *R. rickettsii*, could be readily divided into four different genotypes. In the *Rsa* I and *Pst* I digestion patterns, a marked difference (approximately 130 bp) between Russian strains (M-1 and Barbash) and other strains (S7, Simko, Ethiopian, Indian and Moroccan) was noted in this study. Moroccan strain differed slightly (approximately 10 bp) from S7, Indian and Ethiopian strains, which had the same

PCR/RFLP pattern. Simko, although having the same PCR products in size, had no *Rsa* I recognition site and lacked one *Pst* I recognition site as compared to other 6 strains tested. These results obviously confirms that some genotypic diversity exists among intraspecies of *R. conorii.*

Comparing the PCR/RFLP patterns of two Asian strains (Indian and Barbash), one European strain (M-1) and four African strains (Moroccan, Simko, Ethiopian and $S_7$), we found that the genotypic diversity among the strains of *R. conorii* were not significantly dependent upon their geographic distribution.

**Acknowledgements.** We are grateful to Xuejie Yu for critical review of the manuscript and to Zhongxing Liang for the photographic technical assistance.

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