10.1111/j.1469-0691.2008.02140.x

Detection of *Rickettsia felis* and *Rickettsia typhi* in an area of California endemic for murine typhus

S. E. Karpathy[1], *E. K. Hayes*[1], *A. M. Williams*[1], *R. Hu*[2], *L. Krueger*[3], *S. Bennett*[3], *A. Tilzer*[3], *R. K. Velten*[3], *N. Kerr*[4], *W. Moore*[5] *and M. E. Eremeeva*[1]

[1]Rickettsial Zoonoses Branch, National Center for Zoonotic, Vector-Borne and Enteric Diseases, Centers for Disease Control and Prevention, Atlanta, GA, [2]California Department of Public Health, Vector-Borne Disease Section, Ontario, CA, [3]Orange County Vector Control District, Garden Grove, CA, USA, [4]Long Beach Department of Health and Human Services, Vector Management Program, Long Beach, CA, USA, and [5]Long Beach Department of Health and Human Services, Animal Control Division, Long Beach, CA, USA

INTRODUCTION

Murine typhus is a mild febrile illness caused by the Gram-negative bacterium *Rickettsia typhi*. It has usually been associated with a transmission cycle involving rats, *Rattus norvegicus* and *Rattus rattus*, and the oriental rat flea, *Xenopsylla cheopis* [1]. A second transmission cycle has been described involving peridomestic opossums, *Didelphis virginiana*, and their associated flea, *Ctenocephalides felis* [2]. Another rickettsial agent, *Rickettsia felis*, has also been associated with *C. felis* [3], and has been implicated in a murine typhus-like disease of humans, cat flea rickettsiosis [4]. Current serological diagnostic assays are unable to differentiate between these two infections, partly due to the limited availability of *R. felis* antigens, suggesting that some human cases of cat flea rickettsiosis are being misdiagnosed as murine typhus. We have developed a multiplex TaqMan based PCR assay that facilitates the concurrent detection and differentiation of *R. typhi* and *R. felis* DNA for vector surveillance and for an acute-stage diagnosis of the disease.

Corresponding author and reprint requests: Marina E. Eremeeva, Rickettsial Zoonoses Branch, Mail Stop G-13, National Center for Zoonotic, Vector-borne and Enteric Diseases, Centers for Disease Control and Prevention, 1600 Clifton Road NE, Atlanta, GA 30333, USA
E-mail: meremeeva@cdc.gov

The findings and conclusions described in this manuscript are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention and the Department of Health and Human Services.

No conflicts of interest declared.

METHODS

The PCR primers gltAF.287 (5′-GAT TTT TTA GAA GTG GCA TAT TTG-3′) and gltAR.552 (5′-GGK ATY TTA GCW ATC ATT CTA ATA GC-3′) were selected to amplify a 265-bp fragment of the citrate synthase gene, *glt*A, from all rickettsial species. The primers were tested using genomic DNA from 14 isolates of *Rickettsia prowazekii*, eight isolates of *R. typhi*, and 57 isolates of spotted fever group *Rickettsia*. A single band of the expected size was amplified from all the DNA samples.

Specific species TaqMan probes were designed to permit the discrimination of *R. typhi* and *R. felis* from all other *Rickettsia*. Locked nucleic acid bases, shown in parentheses, were incorporated into the probes to raise the melting temperatures and to increase specificity [5] (*R. typhi glt*A, 5′-CalRed 610-TT (T)A (C)TA (C)A (A)AG (A)T (T)G (C)T (C) A-BHQ2; *R. felis glt*A, 5′-Cy-5-CTA (C)GG A(G)A ATT (G)C-C A-BHQ3). The specificity of the TaqMan probes was tested using two isolates of *R. prowazekii*, two isolates of *R. typhi*, and 19 spotted fever group rickettsial isolates.

All quantitative PCR reactions were conducted in a 20-μL final reaction volume using the Brilliant qPCR kit (Stratagene, La Jolla, CA, USA) in an iCycler iQ (BioRad, Hercules, CA, USA). Each reaction consisted of 2 μL of 10× core reaction buffer, 4.5 mM $MgCl_2$, 300 nM each primer, 400 nM *R. typhi glt*A probe, 600 nM *R. felis glt*A probe, 800 μM combined dNTPs, 1.25 U of SureStart Taq DNA polymerase, and 4 μL of the DNA sample. All reactions were run with a non-template control in which 4 μL of sterile water was substituted for the DNA sample. The cycle parameters consisted of 10 min of enzyme activation at 95°C followed by 50 cycles of a 30 s denaturation at 95°C, 30 s at 62°C to allow the primers and probes to anneal to the target DNA, and 1 min at 72°C for extension. The reaction was concluded with a final extension at 72°C for 10 min.

Positive control plasmids were constructed by using primers gltAF.287 and gltAR.552 to amplify the target fragments from *R. typhi* strain Wilmington and *R. felis* strain LSU. These fragments were cloned into pCR2.1 using the TA cloning kit (Invitrogen, Carlsbad, CA, USA), following the manufacturer's instructions. The construction of the clone was confirmed via sequencing.

RESULTS

Using control plasmid DNA, the detection limit for this assay was verified using the control

Table 1. PCR detection of *Rickettsia* in California flea pools

Flea species	Total fleas tested	Flea pools tested	Minimal infection rate (%)
Ctenocephalides felis	2222	1200	21.6
Pulex irritans	106	55	11.3
Xenopsylla cheopis	4	4	50
Diamanus montanus	4	3	50
Echidnophaga gallinacea	15	8	26.7
Leptosylla segnis	1	1	0
Total	2352	1271	21.3

plasmids and was determined to be between 1 and 2.5 copies/reaction for both *R. typhi* and *R. felis*.

This assay was applied to detect the presence of *R. typhi* and *R. felis* DNA in fleas from California (Table 1). Fleas of the same species and from the same animal were combined into pools of two fleas, and minimal infection rates were established. In total, 1271 pools were screened; 463 pools (36.4%) were positive for *R. felis*, 17 pools (1.3%) were positive for *R. typhi*, and 21 pools (1.7%) were positive for both agents. This assay was also used to screen fleas from Hawaii (*X. cheopis*) and from the Democratic Republic of Congo (*Pulex irritans*, *Xenopsylla brasiliensis*, *Tunga penetrans*, *C. felis*, and *Echidnophaga gallinacea*).

The Taqman assay has also been used to screen opossum tissues and opossum and rat blood samples from California. No evidence of rickettsiemia was obtained in 133 animals tested; however, *R. felis* DNA was detected in liver and/or kidney samples from three of 16 opossums tested. Selected amplicons from each collection site were sequenced to confirm the identity of the DNA species detected, and respective amplicons were >98% identical to either *R. typhi* Wilmington or *R. felis* URRWXCal2.

ACKNOWLEDGEMENTS

We wish to thank K. McCaustland for advice concerning primer and probe design, and G. Dasch for thoughtful discussions. This research was supported in part by an appointment of S. Karpathy to the Research Participation Program at the Centers for Disease Control and Prevention administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the US Department of Energy and CDC.

REFERENCES

1. Azad AF. Epidemiology of murine typhus. *Annu Rev Entomol* 1990; **35:** 553–569.
2. Williams SG, Sacci JB Jr, Schriefer ME *et al.* Typhus and typhuslike rickettsiae associated with opossums and their fleas in Los Angeles County, California. *J Clin Microbiol* 1992; **30:** 1758–1762.
3. Adams JR, Schmidtmann ET, Azad AF. Infection of colonized cat fleas, *Ctenocephalides felis* (Bouché), with a rickettsia-like microorganism. *Am J Trop Med Hyg* 1990; **43:** 400–409.
4. 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:** 949–954.
5. Kumar R, Singh SK, Koshkin AA, Rajwanshi VK, Meldgaard M, Wengel J. The first analogues of lna (locked nucleic acids): phosphorothioate-lna and 2′-thio-lna. *Bioorg Med Chem Lett* 1998; **8:** 2219–2222.