Importance of Primer Specificity for PCR Detection of *Anaplasma phagocytophila* among *Ixodes scapularis* Ticks from Wisconsin

In a recent issue, Massung and Slater reported a comparative study of PCR assays used for detection of *Anaplasma phagocytophila*, the etiologic agent of human granulocytic ehrlichiosis (HGE) (2). They observed significant differences in the analytical sensitivity and specificity for 13 primer sets reported previously in the medical literature. In particular, the PCR assay employing primers ehr 521 and ehr 747 was found to be highly sensitive but had poor specificity, since it detected *Rickettsia rickettsii*, *Bartonella henselae*, and *Ehrlichia chaffeensis* in addition to *A. phagocytophila*. Although the authors did not include data to specifically address this point, they indicated that PCR with ehr 521 and ehr 747 should not be used for testing *Ixodes scapularis* ticks or rodents for the presence of *A. phagocytophila* due to possible interference with bacterial endosymbionts within ticks or the presence of closely related *Rickettsia* species within vectors and reservoir hosts (3). We report our experience with the use of the ehr 521-ehr 747 PCR assay to determine the prevalence of *A. phagocytophila* infection among *Ixodes scapularis* ticks from Wisconsin.

During May and June of 1998, 636 nymphal and adult *I. scapularis* were collected from four Wisconsin state parks and were tested for infection with *A. phagocytophila* by a PCR assay that used primers ehr 521 and ehr 747 followed by Southern hybridization with a 178-bp internal probe generated by primers ehr 552 and ehr 706 (4). Twenty-eight of 636 (4.4%) tick specimens had a visible PCR product by gel electrophoresis and had a positive Southern hybridization. All 28 PCR products were sequenced. A tick was considered positive when the PCR amplicon had >98% sequence homology with *A. phagocytophila* upon direct sequencing. Overall, 24 out of 636 (3.8%) ticks were positive by both PCR and sequencing (20 nymphs, 4 adults). Four of 28 (14%), positive by both PCR and Southern hybridization, had sequences homologous to those of *Ixodes* endosymbionts. An additional 67 out of 636 (10.5%) were negative for visible bands by gel electrophoresis but were positive by Southern hybridization. After reamplification with nested primers, 14 of the additional 67 were chosen randomly for sequencing. Only three exhibited >98% sequence homology with *A. phagocytophila*; the remaining 11 had closest sequence homology to *Ixodes* endosymbionts.

These findings are significant because they underscore the importance of DNA sequencing in confirming primer specific-

ity when using PCR to determine the prevalence of *A. phagocytophila* and other infections in ticks. It seems likely that at least some previously published PCR-based studies that did not incorporate sequencing could have significantly overestimated the prevalence of ehrlichial infections in ticks. The issue is complicated even further by the growing recognition of genetic variants of *A. phagocytophila* that differ by only a few base pairs in the 16S rRNA gene (1). This seemingly minor genetic variation may be associated with significant differences in host range and virulence. Future molecular studies investigating the prevalence and dynamics of HGE and other tick-borne pathogens should be carefully designed to maximize the specificity of amplification and detection methods.

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Sanjay K. Shukla
Mary F. Vandermause
Edward A. Belongia
Kurt D. Reed*
Marshfield Clinic Research Foundation
Marshfield, Wisconsin

Susan M. Paskewitz
Department of Entomology
University of Wisconsin
Madison, Wisconsin

James Kazmierczak
Bureau of Communicable Diseases
Madison, Wisconsin

*Phone: (715) 389-5478
Fax: (715) 389-3319
E-mail: reed.kurt@mcrf.mfldclin.edu