Ehrlichia chaffeensis, a New Species Associated with Human Ehrlichiosis

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The bacterial 16S RNA genes from blood samples of two patients with human ehrlichiosis and from an isolate recovered from one of the patients were amplified by using the polymerase chain reaction. The amplimers were then cloned and sequenced. The 16S rRNA gene sequence was also determined for Ehrlichia canis (two strains), E. equi, phagocytophila (two strains), and E. sennetsu (two strains). These sequences, along with a previously published 16S rRNA gene sequence of E. risticii, were compared. The 16S rRNA gene sequences were identical for all three sources of the human ehrlichiosis agent. The sequence comparisons indicate that the human ehrlichiosis agent is a new species most closely related to E. canis (98.2%) and more distantly related to other Ehrlichia spp. We propose that this species be named Ehrlichia chaffeensis sp. nov., with the Arkansas strain as the type strain.

The genus Ehrlichia contains five species of small, pleomorphic, obligately intracellular bacteria that parasitize the cytoplasmic phagosomes of leukocytes. Until recently, Ehrlichia sennetsu (formerly Rickettsia sennetsu) was the only species known to infect humans, causing “sennetsu rickettsiosis” (11). However, more recently, an ehrlichial agent has been implicated in human disease clinically similar to Rocky Mountain spotted fever (4, 10). The diagnosis of this newly described human ehrlichiosis (HE) was based on morphological characteristics of the organism in infected leukocytes and the presence of serum antibodies that reacted with E. canis (10). Subsequent serologic studies have relied on a fourfold rise in antibody titer to E. canis for a diagnosis of HE (4), and it was assumed that E. canis, or a closely related organism, was the etiologic agent of this disease.

Since members of the genus Ehrlichia are difficult, or in some cases impossible, to cultivate and purify in sufficient quantities to allow exhaustive taxonomic comparisons by traditional microbiological and biochemical means, we utilized a method based on amplification and sequencing of the 16S rRNA gene (rDNA). This method allowed us to obtain 16S rDNA sequences from cultured ehrlichiae as well as from ehrlichiae circulating in the blood of infected hosts. We applied this method to human blood specimens from two patients diagnosed with ehrlichiosis, as well as to an isolate of the HE agent, made in our laboratory, from one of these patients (1b). We report the 16S rDNA sequence for the HE agent and the corresponding sequences from four other currently recognized Ehrlichia species and show that the HE agent is a new species. We also describe the phylogenetic relationship among all members of the genus Ehrlichia.

MATERIALS AND METHODS

Amplification of ehrlichial rDNA. The source of material for each of the strains used for polymerase chain reaction (PCR) amplification is shown in Table 1 and includes infected blood specimens from the host animal and tissue culture-propagated ehrlichiae. A description of the isolation of the HE agent is published in this issue (1b). The two patients from whom rDNA was taken and used as a PCR template were diagnosed as possible ehrlichiosis cases on the basis of clinical observations consistent with this disease (history of tick bite, fever, headache, and absence of a rash) (3a). These patients were subsequently confirmed as having ehrlichiosis by indirect immunofluorescent antibody assay as previously described (2). DNA was extracted from blood samples or tissue culture-derived material as previously described (16), except that the final concentration of DNA was performed by using a Centricon 30 concentrator (Du- pont, Beverly, Mass.) rather than by ethanol precipitation.

PCR amplification was performed with a thermal cycler and GeneAmp reagents (Perkin-Elmer Cetus, Norwalk, Conn.). Two pairs of “universal” primers, known to amplify >90% of the 16S rRNA gene as two separate PCR products from most if not all eubacteria (19), were used to prime the amplification. These universal primers allow the amplification of all nine regions that have been reported as variable areas of eubacterial rDNA (6). The 5' end of each primer was modified to contain either an XbaI or a BamHI restriction endonuclease site to facilitate directional cloning subsequent to amplification. These modified versions of the universal primer pairs are listed in Table 2 (pair EC9 and EC10 and pair EC11 and EC12). In addition to universal primers, a third pair of more specific primers derived as the 16S rDNA sequencing progressed was also used for amplification, cloning, and sequencing (Table 2, EC19 and EC20). Each sample was amplified for three cycles at 94°C (1 min), 48°C (2 min), and 66°C (1 min and 30 s), followed by either 37 cycles (for infected blood) or 27 cycles (for tissue culture-extracted templates) at 88°C (1 min), 52°C (2 min), and 68°C (1 min and 30 s). Products were electrophoresed through a 1.0% agarose gel to assess amplification efficiency. The resulting PCR products were digested with BamHI and XbaI, isolated from a 1.0% agarose gel and cloned into BamHI- and XbaI-digested, alkaline phosphatase-treated pUC19. The ligation mixture was used to transform Escherichia coli DH5α, and the colonies were screened for the correct size insert by preparing rapid alkaline lysis preparations digested with BamHI and XbaI.

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TABLE 1. Sample information for material used for DNA extraction and subsequent PCR amplification and 16S rDNA sequencing

<table>
<thead>
<tr>
<th>Ehrlichia strain</th>
<th>Samplea</th>
<th>Place of origin</th>
<th>Source and reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. canis</em> Florida</td>
<td>TC</td>
<td>Florida</td>
<td>C. Holland (12)</td>
</tr>
<tr>
<td><em>E. canis</em> Oklahoma</td>
<td>TC</td>
<td>Oklahoma</td>
<td>J. Dawson (2)</td>
</tr>
<tr>
<td><em>E. equi</em></td>
<td>BL</td>
<td>California</td>
<td>R. Cortvret (9)</td>
</tr>
<tr>
<td><em>E. sennetsu</em> Miyayama</td>
<td>TC</td>
<td>Japan</td>
<td>ATCC® (11)</td>
</tr>
<tr>
<td><em>E. sennetsu</em> 11908</td>
<td>TC</td>
<td>Malaysia</td>
<td>G. Dasch (7)</td>
</tr>
<tr>
<td>HE agent, Arkansas</td>
<td>TC</td>
<td>Arkansas</td>
<td>J. Dawsond</td>
</tr>
<tr>
<td>HE agent</td>
<td>BL</td>
<td>Arkansas</td>
<td>U.S. Armyd</td>
</tr>
<tr>
<td>HE agent</td>
<td>BL</td>
<td>Oklahoma</td>
<td>This studye</td>
</tr>
<tr>
<td><em>E. phagocytophila</em> OS</td>
<td>BL</td>
<td>Scotland</td>
<td>G. Scott (5)</td>
</tr>
<tr>
<td><em>E. phagocytophila</em> FG</td>
<td>BL</td>
<td>Scotland</td>
<td>G. Scottf</td>
</tr>
</tbody>
</table>

* a TC, tissue culture-derived ehrlichiae; BL, blood from infected host animal.
* b ATCC, American Type Culture Collection.
* c For a description of the isolation of *E. chaffeensis* sp. nov., see reference 1b.
* d Blood sample collected by J. Sanchez, Fort Chaffee, Ark.
* e Blood sample collected by M. Rowland, Tulsa, Okla.
* f Strain originally obtained from the blood of a feral goat.

Sequencing of the rDNA clones. Clones were sequenced by using double-stranded sequencing with T7 DNA polymerase (Sequenase; U.S. Biochemicals, Cleveland, Ohio). Each source of template was amplified, cloned, and sequenced at least twice to prevent the reading of PCR incorporation errors; if the sequences were not in perfect agreement, a third, independent sequence was determined. Nucleotide sequences were assembled from the two PCR products sequenced (the product obtained with EC11 and EC12 and the product obtained with EC9 and EC10). The sequence obtained from the third PCR product (using primers EC19 and EC20) allowed sequencing across the junction of the other two PCR products, including the site of EC10 and EC11 primers. Sequences were aligned over the entire 16S rDNA for maximal homology by using the “gap” program of the genetics computer group package (3). Total sequence divergence (percent difference) was the criterion used to produce a dendrogram showing phylogenetic distance and illustrating branching order, with *Rickettsia rickettsii* used as a point of reference.

Nucleotide sequence accession number. The GenBank accession numbers for the 16S rDNAs of the strains of ehrlichiae studied are as follows: human agent (*E. chaffeensis*), M73222; *E. canis* Florida, M73226; *E. canis* Oklahoma M73221; *E. equi*, M73223; *E. sennetsu* Miyayama, M73219; *E. sennetsu* 11908, M73225; *E. phagocytophila* feral goat (FG) strain, M73224; *E. phagocytophila* Old Sourhope (OS) strain, M73220; and *E. risticii*, M21290.

RESULTS

Primers EC9 and EC10, which are modified versions of P3mod and PC5 (19), produced a 733-bp fragment upon PCR amplification with ehrlichial templates (Fig. 1). The two blood samples from HE patients (lanes B and D) showed an

FIG. 1. Agarose gel of PCR products that were obtained after the amplification of various *Ehrlichia* DNA templates with primer pair EC9 and EC10. The source of DNA templates used for amplification are as follows: lane B, blood from HE patient (Arkansas); lane C, isolate from HE patient (Arkansas); lane D, blood from HE patient (Oklahoma); lane E, *E. canis* isolate (Oklahoma); lane F, *E. canis* isolate (Florida); lane G, blood from *E. equi*-infected horse; lane H, *E. sennetsu* isolate (Miyayama strain); lane I, *E. sennetsu* (11908 strain); lane J, no DNA template (control). Lane A contains ΦX174 phage DNA digested with *Hae*III as molecular size standards (1,353, 1,078, 872, 603, 310, 281/271, 234, 194, 118, and 72 bp, top to bottom).

TABLE 2. Primers used for PCR amplification in this study

<table>
<thead>
<tr>
<th>Primer pair</th>
<th>Sequencea</th>
<th>Product size (bp)b</th>
<th>Sequence rangec</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC9</td>
<td>5' AAGGATCCTACCTTGTACGACTT 3'</td>
<td>733</td>
<td>787-1507</td>
</tr>
<tr>
<td>EC10</td>
<td>5' AATCTAGATATACCTTGTACGACTT 3'd</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EC11</td>
<td>5' AAGGATCCGGACTACAGGTATCTAAAT 3'e</td>
<td>767</td>
<td>18-806</td>
</tr>
<tr>
<td>EC12</td>
<td>5' AATCTAGATATGACTTCTGG 3'f</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EC19</td>
<td>5' AAGGATCCACTCATGTTTACAGCGTG 3'</td>
<td>757</td>
<td>57-826</td>
</tr>
<tr>
<td>EC20</td>
<td>5' AATCTAGACATGCGTACACGAC 3'</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The restrictions sites (either XbaI or BamHI) in each primer used for cloning are underlined. EC9, EC10, EC11, and EC12 are modified versions of universal primers previously described (19).
* The approximate size obtained upon amplification of *Ehrlichia* sp. 16S rDNA.
* The approximate range of the 16S rDNA amplified relative to the *E. coli* sequence (20).
* Where D = A, T, or G.
* Where H = C, T, or A.
* Where M = A or C.
additional fragment of approximately 325 bp that upon sequencing was shown to be unrelated to bacterial 16S rDNA. Each strain of ehrlichiae tested yielded only one strong PCR product upon amplification with EC9 and EC10 primers (lanes C and E to I); two strains of *E. phagocytophila* are not shown. A no-template control was included in every run to ensure that amplification reagents were not contaminated with bacterial DNA (lane J). Likewise, primers EC11 and EC12, which are modified P0mod and PC3mod (19), produced a 767-bp fragment upon amplification of ehrlichial templates, and primers EC19 and EC20 produced a 757-bp fragment subsequent to amplification (data not shown). Upon cloning and sequencing of these three different PCR products (two to three times each), approximately 1,430 nucleotides of the 16S rRNA gene could be unequivocally determined. This sequence roughly corresponds to nucleotides 33 to 1,487 of the *E. coli* rRNA sequence (20). When the same samples were amplified, cloned, and sequenced in duplicate, approximately 50% of the duplicate sequences disagreed by one to three nucleotides. In all cases, a third independent determination of the sequence agreed with either the first or second sequence determination, and the two agreeing sequences were assumed to be correct.

The results of pairwise alignments for each of the ehrlichial 16S rDNA sequences are shown in Table 3; included in this comparison is *E. risticii* Illinois, which has previously been sequenced (18). The HE agent was most closely related to *E. canis* (98.1% to the Florida strain and 98.2% to the Oklahoma strain) and less related to *E. equi* (92.7%) and *E. phagocytophila* (92.8%). *E. risticii* was found to be the member of the genus most phylogenetically distant from the HE agent (84.2%), with both strains of *E. sennetsu* 84.4% related to the HE agent. To determine whether the 1.8 and 1.9% rDNA sequence divergences between the two strains of *E. canis* and the HE agent were significant enough to warrant separate species designation within the genus *Ehrlichia* (as would be the case for most genera), two different strains each of *E. canis*, *E. phagocytophila*, and *E. sennetsu* were included in the sequencing. *E. canis* Oklahoma and *E. canis* Florida had a single nucleotide difference (0.07% divergence), whereas *E. sennetsu* Miyayama and *E. sennetsu* 11908 had identical sequences. The 16S rDNA sequence obtained from the amplified products of a sheep infected with the OS strain of *E. phagocytophila* and a strain originally obtained from a feral goat (FG strain) infected with *E. phagocytophila* were identical. These results indicate that sequence divergence within the 16S rRNA gene at the strain level, within the genus *Ehrlichia*, is virtually nonexistent.

The dendrogram shown in Fig. 2 illustrates the relationships among the organisms studied. It also generally accurately reflects phylogenetic relationships that have been previously reported (18). The dendrogram (Fig. 2) and 16S rDNA gene sequence comparisons (Table 3) indicate that members of the genus *Ehrlichia* seem to fall into three different phylogenetic groups, with *E. sennetsu* and *E. risticii* forming a closely related group (1.0% sequence divergence). These same two species (*E. sennetsu* and *E. risticii*) are quite distantly related to the second group represented by both *E. canis* and the HE agent (between 15.6 and 16.0% divergence). A third group is represented by *E. equi*, which has a sequence divergence of 7.3% with the HE agent, 7.6 or 7.7% with the two strains of *E. canis*, 15.0% with *E. sennetsu*, and 15.1% with *E. risticii*. This third group is also represented by *E. phagocytophila*, which has 16S rDNA sequences (for both strains tested) essentially identical to that of *E. equi* (99.9% related). This observation also suggests that these two currently recognized species of ehrlichiae, *E. equi* and *E. phagocytophila*, need to be reclassified as a single species. The comparative sequence analysis of the 16S rRNA gene indicates that the genus

<table>
<thead>
<tr>
<th>Strain</th>
<th>HE agent</th>
<th>E. canis Oklahoma</th>
<th>E. canis Florida</th>
<th>E. equi</th>
<th>E. phagocytophila OS</th>
<th>E. phagocytophila FG</th>
<th>E. phagocytophila OS</th>
<th>E. risticii</th>
<th>E. sennetsu Miyayama</th>
<th>E. sennetsu 11908</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. sennetsu</em> 11908</td>
<td>84.4</td>
<td>84.2</td>
<td>84.2</td>
<td>85.0</td>
<td>85.1</td>
<td>99.0</td>
<td>100.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. sennetsu</em> Miyayama</td>
<td>84.4</td>
<td>84.2</td>
<td>84.2</td>
<td>85.0</td>
<td>85.1</td>
<td>99.0</td>
<td>0.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. risticii</em> (12)</td>
<td>84.2</td>
<td>84.0</td>
<td>84.0</td>
<td>84.9</td>
<td>84.9</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. phagocytophila</em> FG</td>
<td>92.8</td>
<td>92.5</td>
<td>92.4</td>
<td>99.9</td>
<td>100.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. phagocytophila</em> OS</td>
<td>92.8</td>
<td>92.5</td>
<td>92.4</td>
<td>99.9</td>
<td>100.0</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><em>E. equi</em></td>
<td>92.7</td>
<td>92.4</td>
<td>92.3</td>
<td>0.1</td>
<td>0.1</td>
<td>15.1</td>
<td>14.9</td>
<td>14.9</td>
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</tr>
<tr>
<td><em>E. canis</em> Florida</td>
<td>98.1</td>
<td>99.9</td>
<td>7.7</td>
<td>7.6</td>
<td>7.6</td>
<td>16.0</td>
<td>15.0</td>
<td>15.0</td>
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</tr>
<tr>
<td><em>E. canis</em> Oklahoma</td>
<td>98.2</td>
<td>0.1</td>
<td>7.6</td>
<td>7.5</td>
<td>7.5</td>
<td>15.9</td>
<td>15.8</td>
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<tr>
<td>HE agent</td>
<td>1.8</td>
<td>1.9</td>
<td>7.3</td>
<td>7.2</td>
<td>7.2</td>
<td>15.8</td>
<td>15.6</td>
<td>15.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*16S rDNA sequences were determined as described in the text, and the entire sequence was aligned by using a method previously described (3).*

### TABLE 3. Relatedness and divergence among *Ehrlichia* 16S rDNA sequences

 FIG. 2. Dendrogram showing the phylogenetic relationships among the ehrlichiae studied and *R. rickettsii*. The dendrogram was prepared by plotting the percent rRNA sequence difference (obtained from the rDNA) for each *Ehrlichia* sp., using *R. rickettsii* as a point of reference. The sequence of *E. phagocytophila* is virtually identical to that of *E. equi* (see Table 3).
Ehrlichia is a phylogenetically deeply branching taxon. Although some members of the genus Mycoplasma have been reported to exhibit >20% rRNA sequence divergence (20), the rRNA sequence divergence reported here for the genus Ehrlichia is far greater than for most other eu-bacteria. These results indicate that the HE agent is related to E. canis but is sufficiently different to rule out strain variation within the species, as demonstrated by little or no strain variation within E. canis, E. phagocytophila, and E. sen-
nettus. This conclusion is supported by the fact that rRNA gene sequences of the HE agent and of E. canis differ to a greater extent (1.8 or 1.9%) than do those of E. sennettus and E. risticii (1.0%), two currently recognized distinct species of ehrlichiae. We therefore propose that a new species designation be given the HE agent, and a description fol-

**Description of Ehrlichia chaffeensis sp. nov. E. chaffeensis**

(Chaf.fe.en.sis, N. L. fem. adj. chaffeensis, coming from Fort Chaffee, Ark., where the isolate originated) exhibits all the characteristics of the genus Ehrlichia; i.e., it exhibits cytoplasmic growth, appears as compact clusters (“morula”), is found in circulating leukocytes, and has strong serologic cross-reactivity with the type species (E. canis). The type strain is the Arkansas strain (ATCC accession no. CRL 10679), which was isolated from a U.S. Army recruit at Fort Chaffee, Ark., who was diagnosed as having human ehrlichiosis.

**Discussion**

We have proposed a new species designation within the genus Ehrlichia for the HE agent on the basis of comparative 16S rDNA sequence analysis. Using universal primers that are conserved among eubacterial 16S rRNA genes, we were able to amplify, clone, and sequence 16S rDNA from all five currently recognized species of ehrlichiae, including two different strains each from three of the five species. This technique allows comparisons by the same means both for ehrlichiae that are cultivatable and for those that are not. This type of methodology has recently been used to identify the etiologic agent of basilar angiomasis in humans (13) and to identify *Anaplasma marginale*, which causes disease in ruminants (17); both of these agents belong to the order Rickettsiales.

We believe that the technique of using broad-range universal PCR primers, coupled with sequencing, will allow the identification of a number of new, previously uncharacterized pathogens and is especially useful for characterizing fastidious (or uncultivable) organisms such as the rickettsiae. However, because of the broad range of amplification of these primers, care must be taken to ensure that PCR reagents do not become contaminated with unwanted bacterial DNA. Another potential drawback to this method is Taq polymerase incorporation errors. Presumably, this is why approximately 50% of all our samples disagreed at one to three nucleotides between duplicate determinations. However, in all cases a third determination agreed with the first or second. Thus, the cause of this disagreement is probably Taq polymerase incorporation errors and not multiple versions of the 16S rRNA gene, as has been reported for *E. coli* (1a, 8). Regardless, we feel that at least duplicate and, when required, triplicate independent sequence determinations are essential for the accurate sequencing of cloned, PCR-amplified 16S rDNA.

In the past, the designation of species-level taxa for bacteria has been accomplished by a variety of phenotypic and genetic means, with DNA-DNA hybridization the most commonly applied genetic technique. However, small subunit rRNA sequencing has been used rather extensively to determine phylogenetic relationships (6, 20) among bacteria as well as to distinguish bacterial taxa at the genus level. If full-length rRNA sequencing is performed carefully (and at least in duplicate), small differences (>0.5% for the genus Ehrlichia) are significant at the species level since the 16S rRNA gene is so highly conserved, with mutations fixed at very low frequencies (20).

Although species recognition is usually supported by phenotypic as well as genotypic criteria, we do not have this luxury for the genus Ehrlichia. Some species (e.g., *E. phagocytophila* and *E. equi*) have not yet been cultivated in vitro, while others are difficult to grow and purify in amounts large enough to facilitate exhaustive comparative biochemical or genetic studies. Consequently, current differential characteristics of the species within the genus Ehrlichia include type of leukocytes infected, natural host, geographic distribution, and vector (14). These characteristics, while valuable, provide no direct information comparing genotypic or phenotypic traits of the ehrlichiae themselves. For this reason, we propose that 16S rRNA sequencing be recognized as the standard for future species-level identification of ehrlichiae until further technological developments allow additional taxonomic criteria to be utilized.

The comparative 16S rDNA sequence analysis presented here describes a scheme for classifying members of the genus Ehrlichia, including one new species (*E. chaffeensis*). This scheme agrees extremely well with the existing classification of the genus, with one exception. *E. phagocytophila* and *E. equi*, two currently recognized species of ehrlichiae, were found to be essentially identical. From the comparison of 16S rDNA sequences, these two ehrlichiae should be recognized as one species. Stannard et al. (15) have noted that a number of similarities exist between equine ehrlichiosis (presumably caused by *E. equi*) and tick-borne fever in goats and sheep (presumably caused by *E. phagocytophila*). Thus, *E. equi* and *E. phagocytophila* may be strain variants of the same species.

The data presented here provide clear evidence that an agent associated with HE is a new distinct species of ehrlichiae. A previous report suggested, primarily on the basis of the morphology of infected leukocytes and serological cross-reactivity, that HE was caused by *E. canis* (10). We believe HE is caused by a new *Ehrlichia* species that is related to, but distinct from, *E. canis*. Bacterial 16S rRNA gene sequences found in blood specimens from two different patients, infected in two different states, who were diagnosed as having HE as well as that of an isolate recovered from one of the patients were identical. Thus, it is likely that *E. chaffeensis* sp. nov. is the sole etiologic agent of ehrlichiosis in humans; this hypothesis is supported by results obtained in our laboratory by using *E. chaffeensis*-specific PCR primers that do not amplify *E. canis* DNA. When these specific primers were used to amplify DNA from blood specimens obtained from five persons, diagnosed as having ehrlichiosis, from different geographic regions within the United States, all five were positive, indicating that *E. chaffeensis* was the etiologic agent for all five cases (1). Further studies are needed to determine whether strain variants of *E. chaffeensis* exist and are capable of causing human ehrlichiosis.
ACKNOWLEDGMENTS

We are grateful to the U.S. Army, Fort Chaffee, Ark., for providing blood samples from suspected human ehrlichiosis patients as well as to Richard Corstvet, Gregory Dasch, Gordon Scott, and Cynthia Holland for providing ehrlichiae in various forms as indicated in Table 1. We thank Theodore Tzianabos for extracting DNA from the human blood samples and Brian Holloway, Edwin George, and Misao Olsen-Rasmussen for synthesizing oligonucleotide primers used for sequencing and PCR amplification. We also thank Don J. Brenner and James Olson for constructive comments regarding the manuscript. We thank Thomas MacAdoo, Associate Professor Emeritus of Greek and Latin, Virginia Polytechnic Institute and State University, Blacksburg, for advice on naming the human ehrlichiosis agent.

REFERENCES