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Primary Isolation of *Ehrlichia chaffeensis* from Patients with Febrile Illnesses: Clinical and Molecular Characteristics

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*Ehrlichia chaffeensis* was sought among patients with a history of tick exposure and fever, and the accuracy of other diagnostic tests was compared with that of primary isolation. Among the 38 patients enrolled, *E. chaffeensis* was isolated from the blood of 7 (18%) and from cerebrospinal fluid specimens of 2 of these 7. All 7 patients also were positive by polymerase chain reaction (PCR) of blood, and 6 patients developed diagnostic titers of antibody to *E. chaffeensis*. The isolates were characterized by molecular analysis of the 16S rRNA gene, the 120-kDa protein gene, and the variable-length PCR target (VLPT) of *E. chaffeensis*. On the basis of the 120-kDa and VLPT genotypes, the cerebrospinal fluid and blood isolates from the same patients were identical. This study demonstrates that both PCR and culture of blood for *E. chaffeensis* have high diagnostic yields. More frequent isolation of *E. chaffeensis* from patients with infection should further our understanding of the pathogenesis of this infection.

Infection by *Ehrlichia chaffeensis* may be mild or may cause severe or fatal disease. Although human monocytotropic ehrlichiosis (HME) was first recognized in 1986 [1], it was not until 1990 that the causative organism, *E. chaffeensis*, was isolated from a military recruit with a febrile illness after a tick bite while at Fort Chaffee, Arkansas [2]. Only 8 other primary isolates have been reported subsequently [3-6], presumably because of the complex growth requirements of this obligate intracellular bacterium. In contrast, successful isolation of the agent of human granulocytotropic ehrlichiosis (HGE), the other common form of human ehrlichiosis in the United States, has been reported in as many as one-third of patients with acute infection from whom isolation has been attempted [7].

Because isolation of *E. chaffeensis* from clinical specimens is rare, the accuracy of indirect diagnostic tests is unknown. More frequent isolation of this organism also is needed to further define the range of clinical characteristics and the pathophysiology of infection. Therefore, we sought to determine whether a systematic effort to culture clinical specimens from patients with suspected HME would result in more frequent isolations of *E. chaffeensis*. We attempted to define the accuracy of the currently available diagnostic techniques by use of culture of the organism as the reference standard for comparison. We further sought to characterize each isolate by molecular analysis of genes with known diversity [4, 5, 8, 9].

**Methods**

**Prospective enrollment.** Between 15 April and 15 October 1998, we enrolled patients with possible tickborne infection who presented to the 3 Vanderbilt University-affiliated hospitals. Enrollment criteria included fever (>37.5°C), a history of tick bite or exposure, and a clinical suspicion of tickborne rickettsial infection sufficient to warrant doxycycline therapy. All enrolled patients were interviewed with a standard questionnaire, and their medical records were reviewed. Peripheral blood smears from all patients were evaluated for the presence of intraleukocytic ehrlichial inclusions (morulae) at the time of enrollment.

For each patient, an acute-phase serum specimen and an EDTA-anticoagulated whole blood specimen were collected at the time of presentation, and a convalescent-phase serum specimen was obtained 4-6 weeks later. Lumbar punctures were done for patients whose clinical condition suggested meningitis, and a 1- to 3-mL cerebrospinal fluid (CSF) specimen was collected for study purposes.

**Primary isolation of E. chaffeensis.** Blood and CSF specimens were transported and processed for culture immediately after collection. The EDTA-anticoagulated whole blood specimen (2-3 mL) was diluted with 2 v of sterile Hanks’ balanced salt solution (HBSS;...
Life Technologies Gibco BRL, Gaithersburg, MD), layered onto Histopaque 1083 (Sigma, St. Louis), and centrifuged at 800 g for 20 min. The leukocyte-enriched fraction was collected and diluted in 10 mL of HBSS and centrifuged at 400 g for 10 min. The CSF specimens were diluted in 10 mL of HBSS and centrifuged at 400 g for 10 min. The cell pellet was resuspended in 4 mL of cell culture medium, which was prepared as described elsewhere [5] except that the fetal bovine serum concentration was 6.5%. The cell suspension was inoculated onto a confluent layer of DH82 cells (canine peritoneal macrophage; ATCC 10389) in a 25-cm² cell culture flask and incubated at 37°C in a 5% CO₂ atmosphere. The culture medium was changed at 48 h and then twice weekly thereafter for 60 days. Cultures were monitored for infection by use of Leukostat-stained (Fisher Scientific, Philadelphia) cyto centrifuged preparations at least weekly for the first 30 days and serially thereafter.

Polymerase chain reaction (PCR). DNA extraction and nested PCR of the 16S rRNA gene of E. chaffeensis, the HGE agent, and Rickettsia rickettsii were done on all blood specimens (and CSF specimens when available) from enrolled patients and all primary isolates, as described elsewhere [10], except for the addition of an R. rickettsii species-specific primer (RR1F, CTAATTGGGGCCTGCTGTCATTAG) of our own design. Briefly, the product from the first-round reaction with wide-range 16S rDNA primers, 8F and 1448R [10], was used as the DNA template for the second (nested) reaction. For each specimen, 3 simultaneous second-round reactions were done with species-specific primers for E. chaffeensis, the HGE agent, and R. rickettsii in pairwise combination with a primer with enhanced specificity for Ehrlichia species, GA1UR [10]. Both positive- and negative-control specimens were tested simultaneously with each reaction. The positive-control DNA specimens were obtained from culture-derived E. chaffeensis, the HGE agent (provided by J. S. Dumler, Johns Hopkins University Hospital, Baltimore), and R. rickettsii (provided by D. H. Walker, University of Texas Medical Branch, Galveston). The negative-control specimens were sterile water, DNA obtained from the blood of an uninfected person (S.M.S.), and uninfected DH82 cells. For nucleotide sequencing of the 16S rRNA gene from the patients with PCR evidence of E. chaffeensis infection and the respective primary isolates, the second-round reaction was done with primers 15F (ATCATGGCTCAAGACGAACG) and 208R (CGTAGGCTCATCTAATAGCG) to amplify a variable region of the gene useful for species identification. The sequences of these products were compared with the published sequences of the previous known isolates of E. chaffeensis (GenBank: Arkansas, M73222; Sapulpa, U60476; 91HE17, U23503; Jax, U86664; St. Vincent, U86665).

To further characterize each E. chaffeensis isolate, PCR amplification of the 120-kDa protein gene and the variable-length PCR target (VLPT) gene were done on all isolates, as well as the original blood or CSF clinical specimen from which the isolate was obtained. Both genes contain several tandem repeat units (240 bp in the 120-kDa protein gene and 90 bp in the VLPT gene), which have been reported to vary between strains [5, 6, 8, 9]. All reactions were done as described elsewhere [5]; primers F1 and R2 were used for the 120-kDa protein gene and FB3 and FB5 for the VLPT gene. All PCR reagents were supplied by Qiagen (Chatsworth, CA), and reactions were done in a DNA thermal cycler (Perkin-Elmer, Norwalk, CT).

Serologic studies. All serum specimens from enrolled patients were examined at the Centers for Disease Control and Prevention in a blinded fashion. An indirect immunofluorescence assay was used to identify antibodies reactive with E. chaffeensis, the HGE agent, and R. rickettsii, as described elsewhere [2].

Case definitions. A confirmed case of HME, HGE, or Rocky Mountain spotted fever (RMSF) was defined as occurring in any patient whose blood specimen was positive by PCR testing with species-specific primers for 16S rDNA or whose serum specimens revealed a ≥4-fold change in antibody titer for E. chaffeensis, the HGE agent, or R. rickettsii, respectively [11]. A culture-confirmed case of HME was defined as occurring in any patient whose blood specimen yielded a primary isolate of E. chaffeensis.

Statistical analysis. Significance tests were done with the Mann-Whitney U test for continuous variables and Fisher’s exact test for dichotomous variables performed by use of Epi Info [12].

Results

Confirmed cases of HME. A total of 38 patients were enrolled during the 6-month study period. Of these, E. chaffeensis was isolated from blood cultures from 7 patients (18%), who thus were defined as having culture-confirmed cases of HME (table 1). We have designated these 7 E. chaffeensis isolates as Vanderbilt 1–7 (V1–7), on the basis of the order in which the patients presented during the study. E. chaffeensis also was isolated from the CSF specimens of 2 of these patients (V4C and V6C).

Assessment of E. chaffeensis diagnostics. Each of the 7 patients with culture-confirmed HME also was positive by E. chaffeensis-specific PCR testing of whole blood (table 2). Although the true clinical specificity of the PCR is not known, these data suggest that it is very high, because it was not positive for any of the 31 culture-negative patients. Of the 7 patients with confirmed HME, examination of the peripheral blood smears revealed characteristic ehrlichial morulae in 2 (29%).

Acute and convalescent sera were available for 34 (89%) of the enrolled patients. Six of the 7 patients with confirmed HME were positive by serologic testing (table 2). The patient with one of the confirmed cases of RMSF was positive by serologic testing and also was positive by PCR (V1). Of the 10 patients whose sera were positive by serologic testing and culture and who were negative by PCR, 7 (70%) were positive by serologic testing alone, and 3 (30%) were positive by both PCR and serologic testing. Of the 3 patients whose sera were positive by serologic testing and culture and who were negative by PCR, 2 (67%) were positive by serologic testing alone, and 1 (33%) was positive by both PCR and serologic testing.

Table 1. Diagnostic findings for 38 enrolled study patients with febrile illness and history of tick exposure.

| Organism         | Culture (n = 38) | PCR (n = 38) | Serology (n = 34) | Total no. of subjects in whom pathogen identified |
|------------------|------------------|--------------|-------------------|-----------------------------------------------|
| Ehrlichia chaffeensis | 7    | 7           | 6     | 7   |
| HGE agent        | ND   | 0           | 0     | 0   |
| Rickettsia rickettsii | ND   | 3           | 3     | 6   |
| Negative         | 31   | 28          | 22   | —   |

NOTE. Data (except for negative results) are no. of patients positive by diagnostic test. HGE, human granulocytic ehrlichiosis; ND, not done; PCR, polymerase chain reaction.

a. E. chaffeensis was isolated from both blood and cerebrospinal fluid of 2 patients.
b. PCR with E. chaffeensis-specific primers was positive in both blood and CSF of 1 patient.
c. One patient (V1) with E. chaffeensis infection did not have detectable antibody in either acute or convalescent serum specimen and is included with other 21 serologically negative patients.
d. Three of these patients also were PCR-positive for R. rickettsii.
developed diagnostic antibody titers within 14–43 days of the onset of illness (range of reciprocal titers, 512–4096; table 2). One patient (V1) with confirmed HME who had received doxycycline therapy during the first 12 h after the onset of fever never developed diagnostic serum titers of antibody to *E. chaffeensis*.

In addition to the cases of HME, there were 6 (16%) confirmed cases of RMSF and no cases of HGE (table 1). Among the remaining 25 patients, there was no evidence of infection with *E. chaffeensis*, the HGE agent, or *R. rickettsii* by any modality; only the 21 patients for whom both acute and convalescent specimens were available were used for subsequent comparisons.

**Clinical and epidemiologic features of HME cases.** All cases of HME occurred between late April and the first week of August, whereas the cases of RMSF extended to late September (figure 1). The patients with HME originated from four counties in Tennessee and a single county in Kentucky, and there was no geographic clustering of cases. There were no significant differences in demographic characteristics between patients with HME, patients with RMSF, and those without either infection, although 12 (92%) of the 13 patients with either HME or RMSF were male and 10 (77%) required hospitalization (table 3). A nonspecific, fleeting maculopapular rash on the face and neck occurred in 2 patients with HME (23%), whereas a typical petechial rash occurred in 4 patients with RMSF (67%). A nonspecific maculopapular rash occurred in 6 patients for whom no infecting agent was identified. The only laboratory findings that were significantly different between patients with HME and those without either HME or RMSF were the degree of thrombocytopenia and the proportion with elevated serum aspartate aminotransferase. However, 71% of patients with HME had leukopenia and none had an abnormally elevated white blood cell count.

Of the 21 patients without either HME or RMSF, a definitive diagnosis was determined for only 2; 1 patient had *Staphylococcus aureus* bacteremia and the other patient had AIDS and acute hepatitis A virus infection. There were no deaths among the patients enrolled in the study.

**Characteristics of the primary isolation of E. chaffeensis.** For the 9 samples that yielded *E. chaffeensis*, the median time between collecting the blood and CSF specimens from patients and inoculating the leukocyte-enriched fraction into cell culture was 3 h (range, 1–18). This interval did not differ from that for the 31 culture-negative patients (median, 3 h; range, 1–10). Primary isolation of ehrlichiae from the blood specimens, as evidenced by visualization of morulae in the cytoplasm of DH82 cells, occurred between 2 and 12 days (median, 11) after inoculation. There was no significant relationship between the rapidity of inoculation and either the success or rapidity of primary isolation in cell culture (data not shown). Of the 2 isolates from CSF, 1, from a patient (V4) with obvious meningitis (310 white blood cells/mm³), was seen 16 days after cell culture inoculation. In contrast, in patient V6, in whom only a single CSF white blood cell and no red blood cells were present, *E. chaffeensis* was isolated 50 days after cell culture inoculation.

**Molecular characteristics of E. chaffeensis isolates.** Nested PCR with the *E. chaffeensis*–specific primers HE1 and GA1UR and DNA extracts of the 9 isolates from all 7 patients yielded the characteristic 389-bp 16S rRNA gene product (data not shown). The nucleotide sequence of the variable 190-bp region of the 16S rRNA gene (as determined by use of primers 15F and 208R) from each patient’s blood specimen and the 7 isolates was identical to the GenBank sequence for the previous *E. chaffeensis* isolate.

PCR amplification of the 120-kDa protein gene and the VLPT gene produced amplicons varying from ~1000 to 1500 bp and 369 to 539 bp, respectively, from the 9 isolates (table 4). The number of repeat units, as inferred from the size of these products, ranged from 2 to 4 repeats for the 120-kDa protein gene and 3 to 5 repeats for the VLPT gene (table 4). Nucleotide sequencing of the VLPT gene PCR products confirmed these findings (data not shown). Because of the large size of the 120-kDa gene, confirmatory nucleotide sequencing was not done. On the basis of the number of repeats of these 2 genes, 5 distinct molecular patterns were observed among the 9 isolates. For each patient, the PCR product obtained directly from the blood had the same apparent migration as the product obtained from the corresponding *E. chaffeensis* isolate (figure 2); the product from each of the CSF isolates also was identical to that of the corresponding blood isolate.

**Discussion**

Primary isolation of *E. chaffeensis* from infected patients has been difficult. Attempts to isolate the organism have been limited by the requirement for antibiotic-free cell culture techniques and a concern for the potential risk of laboratory-acquired
infections, as has been reported with *R. rickettsii* [13]. Despite numerous attempts, only 9 primary isolates have been described in the previous 8 years [2–6]. In this study, *E. chaffeensis* was isolated from blood specimens from 7 patients in one locale over a 6-month period; isolation was 100% sensitive, on the basis of both PCR and serologic criteria. The time to culture positivity was substantially shorter in this study (median, 11 days) than reported for the other *E. chaffeensis* isolates (median, 20 days) [2–5]. This rapidity likely was due to the proximity of our laboratory to the patients, resulting in brief (median, 3-h) intervals until specimen inoculation in cell culture. Although isolation of *E. chaffeensis* was not more likely when culture inoculation occurred early within the 18-h range, the preincubation time likely was much longer for previous *E. chaffeensis* isolation attempts, given the inherent delays in transporting clinical specimens to research centers. The proximity of infected patients to a specialized research laboratory probably also contributed to the first successful isolation of the HGE agent [14]. The lower concentration (6.5%) of fetal bovine serum used in our cell culture media compared with previous efforts (8.8%–12%) [2–6] may have contributed to *E. chaffeensis* isolation, as suggested elsewhere [3].

Despite the high sensitivity of primary isolation of *E. chaffeensis* from clinical specimens, the required technical expertise and prolonged incubation time may curtail its widespread diagnostic use. However, we and others [15, 16] have found PCR to be both rapid and accurate for diagnosing *E. chaffeensis* infections and, thus, useful in initial patient management. PCR-based techniques are increasingly becoming standardized and clinically available. In contrast, the sensitivity of early serologic testing for *E. chaffeensis* infection is poor, because only 2 (29%) of the 7 patients with confirmed HME in our series had detectable antibody levels on initial presentation, and both patients already had been ill for 14 days. Despite the rapidity and increased sensitivity of PCR testing, empirical therapy with doxycycline should be administered immediately without waiting for the results of diagnostic testing, because any delay in therapy can lead to increased morbidity and mortality [17].

The improved sensitivity of the nested PCR protocol we used may be offset by an increased risk of contamination, because specimens must be handled more frequently. Nonetheless, we chose to use this technique during our study for 2 reasons. First, the same 16S rDNA amplicon from the first reaction can be used as the template for subsequent reactions to test for several different organisms simultaneously. In addition, we have found that a nested reaction is required to detect *R. rickettsii* from blood specimens, because the number of organisms in peripheral blood is so few. As with any laboratory test, simultaneous reactions with adequate positive and negative control specimens are essential. Laboratories that handle a large number of clinical specimens may find that a nested protocol is too problematic to be used for routine diagnostic purposes. However, our data indicate that this approach is feasible, especially when the PCR amplicon identity is confirmed by nucleotide sequencing.

All patients with *E. chaffeensis* infection became ill between late April and the first week of August (figure 1). A similar seasonal distribution of HME in Tennessee has been noted earlier [15] and may reflect the seasonal activities of the tick vector, *Amblyomma americanum* [18, 19]. *E. chaffeensis* is not believed to be passed transovarially within the tick, and therefore only adults and nymphs are potentially infectious. Questing *A. americanum* adults and nymphs are present from early spring to midsummer in Tennessee, but beyond August
Table 3. Demographic characteristics and laboratory results for 34 study patients with history of tick exposure by tickborne illness.

| Demographic or laboratory characteristic | Human monocytic ehrlichiosis (n = 7) | Rocky Mountain spotted fever (n = 6) | No agent identified (n = 21) |
|-----------------------------------------|-------------------------------------|----------------------------------|-----------------------------|
| Age, years                              | 36 (10–57)                          | 33 (0.6–69)                      | 37 (7–72)                   |
| No. male                                | 6                                   | 6                                | 11                          |
| No. hospitalized                        | 5                                   | 5                                | 12                          |
| No. with reported tick bite             | 4                                   | 2                                | 7                           |
| No. with rash                           | 2                                   | 4                                | 6                           |
| White blood cell count, cells × 10^9/mm³| 2.1 (1.4–7.5)                       | 10.2 (1.4–16.8)                 | 5.1 (1.9–11.5)              |
| No. with leukopenia (<4.5 × 10^9 white blood cells/mm³) | 5                                   | 1                                | 9                           |
| Hematocrit, %                           | 37 (31–45)                          | 40 (32–43)                       | 41 (29–48)                  |
| No. with anemia (for age and sex)       | 4                                   | 2                                | 8                           |
| Platelet count, cells × 10^9/mm³        | 75 (37–206)                         | 159 (21–367)                     | 167 (62–327)                |
| No. with thrombocytopenia (<150 × 10⁹ platelet/mm³) | 6                                   | 3                                | 10                          |
| Serum aspartate aminotransferase level, IU | 155 (52–281)                       | 54 (17–167)                      | 36 (17–2173)                |
| Bilirubin level, mg/dL                  | 2.3 (0.7–5.3)                       | 1.0 (0.4–3.1)                    | 0.7 (0.2–6.5)               |
| No. with abnormal serum aspartate aminotransferase value (>40 IU) | 3                                   | 3                                | 5                           |
| No. with abnormal bilirubin value (>1.0 mg/dL) | 4                                   | 2                                | 4                           |

NOTE. Data with parentheses are median (range). Only acute serum specimens were obtained from 4 patients, and they were excluded from original 38 patients. All 4 were negative by culture and polymerase chain reaction for organisms of interest.

* P < 0.05 vs. patients with no agent identified.

* P < 0.05 vs. patients with Rocky Mountain spotted fever.

* Determined for only 11 patients.

only larval ticks can be found. In contrast, the adult stage of Dermacentor variabilis, the predominant vector of R. rickettsii in Tennessee, is present late into the fall [20], which is consistent with our identification of patients with RMSF in September.

Meningitis has been increasingly recognized as a feature of E. chaffeensis infection [21, 22]. Although E. chaffeensis has been detected in CSF by PCR [23], we now report its isolation. Lumbar puncture was done for only 2 of the 7 patients with E. chaffeensis infection, and a primary isolate identical to the blood isolate was recovered from both specimens. Since 1 of these patients (V6) had no laboratory evidence of meningitis, the frequency of E. chaffeensis invasion of the CSF during acute infection and then its elimination, either by antibiotic therapy or the host immune response, is unknown. The findings in patient V6, that PCR yielded negative results and culture required 50 days, suggest that the actual number of Ehrlichiae in the CSF was small. Although contamination of the CSF with the blood isolate during the lumbar puncture may have occurred, we believe this to be unlikely, because no red blood cells were detected in the CSF specimen from this patient.

E. chaffeensis isolates are polymorphic in the number of repetitive sequences within the genes encoding the 120-kDa surface protein and VLPT [4–6, 8, 9], and the isolates we obtained illustrate this phenomenon. Isolate V5 had only 2 repeat units in the 120-kDa gene, a novel finding (table 4). On a practical level, these studies are useful by indicating that neither the initial diagnostic PCR results nor the isolated organisms were falsely positive as a result of contamination with laboratory strains of E. chaffeensis. From a biologic and epidemiologic perspective, further study of the genetic polymorphisms of the VLPT and the 120-kDa protein genes would be interesting to determine if they can be correlated with pathogenicity or geographic distribution of different strains. Several pathogenic organisms, including Rickettsia species, have genes with repeat

Table 4. Size variation in 120-kDa protein gene and variable-length polymerase chain reaction (PCR) target (VLPT) gene in 18 known Ehrlichia chaffeensis isolates.

| Previous isolate [ref.] | New isolate | No. of repeats
|-------------------------|-------------|----------------|
| Wokulla [6]             | V7          | 4              |
| 91HE17 [3]              |             | 4              |
| Arkansas, Jax, Liberty  | V1, V4b     | 4              |
| [2, 5, 6]               |             | 4              |
| St. Vincent, Sapulpa,   | V3          | 3              |
| West Paces [4, 5]      | V2, V6b     | 3              |
| Osceola [6]             | V5          | 2              |

a As determined by nucleotide sequencing for VLPT and from size of PCR product for 120-kDa gene (figure 2).

b For individual patient, cerebrospinal fluid and blood isolates showed identical patterns by PCR.
domains that encode for surface proteins involved in antigenic variation and possibly in adherence to host cells [24–27]. We and others have found no geographic clustering of *E. chaffeensis* strains on the basis of the genotypes examined, although the number of samples examined is low [5, 6, 9]. In addition, none of the patterns observed among the 7 new isolates was predictive of a particular clinical outcome. Further analysis of these potential relationships may be more revealing as we increase the number of isolates from infected patients.

One-third of the patients enrolled in this study were found to have a tickborne rickettsial infection, either HME or RMSF. Equally provocative, however, is the cause of illness in the 19 patients without an identifiable rickettsial or other known infection. All recovered from their illness without consequence, and several appeared to respond to doxycycline therapy. Yet-to-be-identified organisms that infect humans could be contributing to this group of undefined, apparently doxycycline-responsive febrile illnesses [28]. The development of molecular tools, such as broad-range PCR primers, may permit identification of novel rickettsial (including *Ehrlichia*) species in humans.

This study represents the first systematic effort to isolate *E. chaffeensis* from patients with febrile illness following tick exposure. In contrast to previous investigations, we were able to isolate the organism from each patient in our study who had any evidence of *E. chaffeensis* infection. Because the diagnostic accuracy of the other available modalities (detection of morulae, PCR, and serology) has not been defined, the ability to readily culture the organism now can serve as a reference standard for comparison. Although the prolonged time for culture (median, 11 days) precludes its application for rapid diagnosis, examination of peripheral blood by means of PCR and staining may be clinically useful. Finally, our demonstration of the feasibility of culturing *E. chaffeensis* directly from patient blood and CSF specimens should improve understanding of the phylogeny of this organism and the epidemiology and pathogenesis of infection.

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**Figure 2.** Variation of no. of tandem repetitive sequences in 120-kDa protein gene and variable length polymerase chain reaction (PCR) target (VLPT) identified by agarose gel electrophoresis of PCR products of clinical specimens (lanes B, whole blood; lanes C, cerebrospinal fluid [CSF]) from patients V7, V4, and V3 and corresponding *Ehrlichia chaffeensis* isolates (lanes I) from culture in DH82 cells. Patient V4 had meningitis, and isolate (I') was obtained from CSF. Both genes encode variable number of tandem repeats that vary between strains. Arrows represent no. of repeat units observed for 120-kDa protein gene (4 repeats = 1500 bp, 3 repeats = 1250 bp) and VLPT gene (5 repeats = 549 bp, 4 repeats = 459 bp, 3 repeats = 369 bp). Amplicon sizes of clinical blood and CSF specimens are all identical to that of corresponding *E. chaffeensis* culture isolate from that specimen, indicating that isolated strain was detected by direct PCR of clinical specimens. Nos. of repeat units noted for genes are independent of each other: for example, amplicon sizes for 120-kDa protein gene are identical in all specimens from patient V7 and V4, but sizes of VLPT amplicons are different. Lane L, molecular size standard ladder; lane N, negative control (uninfected DH82 cells).
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