2001

Infections with Ehrlichia chaffeensis and Ehrlichia ewingii in Persons Coinfected with Human Immunodeficiency Virus

ChristopheR D. Paddock
Centers for Disease Control and Prevention

Scott M. Folk
Adult Infectious Diseases,Tallahassee Community Hospit

G. Merrill Shore
St. Vincent's Medical Center, Jacksonvill

Linda J. Machado
University of Oklahoma Health Sciences Center

Mark M. Huycke
University of Oklahoma Health Sciences Center

See next page for additional authors

Authors
ChristopheR D. Paddock, Scott M. Folk, G. Merrill Shore, Linda J. Machado, Mark M. Huycke, Leonard N. Slater, Allison M. Liddell, Richard S. Buller, Gregory A. Storch, Thomas P. Monson, David Rimland, John W. Sumner, Joseph Singleton, Karen C. Bloch, Yi-Wei Tang, Steven M. Standaert, and James E. Childs

Infections with *Ehrlichia chaffeensis* and *Ehrlichia ewingii* in Persons Coinfected with Human Immunodeficiency Virus

Christopher D. Paddock,[1] Scott M. Folk,[3,a] G. Merrill Shore,[4] Linda J. Machado,[5] Mark M. Huycke,[5] Leonard N. Slater,[5] Allison M. Liddell,[6] Richard S. Buller,[6] Gregory A. Storch,[6] Thomas P. Monson,[7] David Rimland,[2] John W. Sumner,[1] Joseph Singleton,[1] Karen C. Bloch,[8] Yi-Wei Tang,[8] Steven M. Standaert,[8,a] and James E. Childs[1]

[1]Viral and Rickettsial Zoonoses Branch, Division of Viral and Rickettsial Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, and [2]Division of Infectious Diseases, Department of Medicine, Veterans' Affairs Medical Center, Atlanta; [3]Adult Infectious Diseases, Tallahassee Community Hospital, Tallahassee, and [4]Department of Pathology, St. Vincent's Medical Center, Jacksonville, Florida; [5]Division of Infectious Diseases, Department of Medicine, University of Oklahoma Health Sciences Center and Veterans' Affairs Medical Center, Oklahoma City; [6]Departments of Pediatrics and Medicine, Washington University School of Medicine, St. Louis; [7]Infectious Diseases Section, Department of Medicine, University of Arkansas for Medical Sciences, Little Rock, Arkansas; [8]Division of Infectious Diseases, Departments of Medicine (Infectious Diseases) and Preventive Medicine and of Pathology, Vanderbilt University Medical Center, Nashville, Tennessee

The clinical course and laboratory evaluation of 21 patients coinfected with human immunodeficiency virus (HIV) and *Ehrlichia chaffeensis* or *Ehrlichia ewingii* are reviewed and summarized, including 13 cases of ehrlichiosis caused by *E. chaffeensis*, 4 caused by *E. ewingii*, and 4 caused by either *E. chaffeensis* or *E. ewingii*. Twenty patients were male, and the median $CD4^+$ T lymphocyte count was 137 cells/μL. Exposures to infecting ticks were linked to recreational pursuits, occupations, and peridomestic activities. For 8 patients, a diagnosis of ehrlichiosis was not considered until $\geq$4 days after presentation. Severe manifestations occurred more frequently among patients infected with *E. chaffeensis* than they did among patients infected with *E. ewingii*, and all 6 deaths were caused by *E. chaffeensis*. Ehrlichiosis may be a life-threatening illness in HIV-infected persons, and the influence of multiple factors, including recent changes in the epidemiology and medical management of HIV infection, may increase the frequency with which ehrlichioses occur in this patient cohort.

During the first 2 decades of the AIDS epidemic, a diverse collection of pathogens emerged as causes of opportunistic diseases in persons infected with HIV. During this same period, 3 species of tickborne bacteria in the genus *Ehrlichia*, namely *Ehrlichia chaffeensis*, the as-yet-unnamed agent of human granulocytic ehrlichiosis, and *Ehrlichia ewingii*, were identified as agents of newly recognized diseases in the United States, which were collectively termed the ehrlichioses [1–3]. Concurrent infection with *E. chaffeensis* in an HIV-infected patient was first described in 1993, and subsequent reports have been sporadic [4–9]. Infections with the agent of human granulocytic ehrlichiosis or *E. ewingii* have not been described in this patient cohort.

In persons infected with HIV, ehrlichiosis caused by *E. chaffeensis* is often life threatening [4, 6, 7, 9]; however, the disease responds well to specific therapy with tetracyclines, particularly when these antibiotics are given early in the course of the infection. In this report, we review and summarize the clinical courses and laboratory evaluations of 6 previously described patients and 15 newly reported patients who were coinfected with HIV and either *E. chaffeensis* or *E. ewingii*.

Received 1 March 2001; revised 12 June 2001; electronically published 24 September 2001.

[a] Current affiliations: Division of Infectious Diseases, Heartland Health System, St. Joseph, Missouri (S.M.F.); Infectious Disease Consultants, Olympia, Washington (S.M.S.).

Reprints or correspondence: Dr. Christopher D. Paddock, Viral and Rickettsial Zoonoses Branch, Mailstop G-13, Centers for Disease Control and Prevention, 1600 Clifton Rd., Atlanta, GA 30333 (cdp9@cdc.gov).

Clinical Infectious Diseases 2001;33:1586–94

1058-4838/2001/3309-0022$03.00

METHODS

Identification of patients. Published cases of ehrlichiosis in persons infected with HIV were reviewed and summarized [4–9]. When available, supplementary clinical data were collected from the authors of these reports. Additional cases were identified passively in the course of routine patient care by infectious disease physicians practicing in clinics, hospitals, or medical centers in Arkansas, Florida, Georgia, Missouri, Oklahoma, and Tennessee from May 1997 through July 2000. Confirmed and suspected cases were reported to Centers for Disease Control and Prevention (CDC; Atlanta) during consultations or requests for confirmatory laboratory testing. Epidemiological and clinical data for each patient were abstracted from clinic and hospital records.

Laboratory confirmation. Clinical samples were obtained from patients during or shortly after their illnesses and tested using confirmatory assay(s). Peripheral blood, buffy coat, or bone marrow aspirate smears were stained with eosin-azure (Romanovsky)–type stains and examined for the presence of characteristic intracellular bacterial aggregates (morulae) in patient leukocytes. Patient serum or plasma samples were evaluated for antibodies reactive with *E. chaffeensis* by using indirect immunofluorescence assays (IFAs), as described elsewhere [10].

Nucleic acid amplification was done with DNA extracted from samples of whole blood, peripheral blood leukocyte preparations, serum, plasma, or bronchoalveolar lavage fluid, as described elsewhere [3, 11]. One or more genomic regions were amplified from extracted DNA using PCR assays. Regions of the 16S rRNA gene of *E. chaffeensis* were amplified with primer pairs HE1 and HE3 [12] or EhrlU (5′-AGAACGAACGCTGGCGGCAAG) and EhrlL (5′-TAGGTACCGTCATTATCTTCCCTA) in a direct assay, or by a nested assay with primers 8F and 1448R in the primary reaction and primers HE1 and GA1UR in the nested reaction [13]. Primers FB3 and FB5 were used to amplify the VLPT gene [11], and primers F1 and R2 were used to amplify the 120-kDa protein gene [14] of *E. chaffeensis.* Regions of the 16S rRNA gene of *E. ewingii* were amplified directly by using primers EWF1 (5′-TCGAACGAACAATTCCTAAA) and HE3 or EWI and HE3 [3]. A region of the *groESL* heat-shock operon of *E. ewingii* was amplified in a nested PCR with primers HS1 and HS6 in the primary reaction and primers EWNF1 and EWNR2 in the nested reaction [15]. PCR products were evaluated by complete or by partial sequencing to verify identity, as described elsewhere [3, 11, 13, 15].

Formalin-fixed, paraffin-embedded bone marrow biopsy samples or autopsy tissues were stained with immunohistochemical stains, as described elsewhere [4, 16, 17]. For isolation of *E. chaffeensis,* peripheral blood leukocytes were separated from 3–5 mL of acute-phase whole blood and inoculated into DH82 cell cultures, as described elsewhere [7, 13]. The identity of each isolate was confirmed by means of PCR and sequencing.

Statistical analyses. Significance tests were done with Fisher's exact test, for dichotomous variables, and the Mann-Whitney *U* test, for continuous variables; a *P* value of $<.05$ was considered significant. Analyses were done using SPSS software for Windows (SPSS) [18].

RESULTS

Epidemiology and patient demographics. Ehrlichiosis was diagnosed in 21 HIV-infected patients during the period of 1992–2000, including 17 patients in 1997–2000. Thirteen cases of disease were caused by *E. chaffeensis,* 4 by *E. ewingii,* and 4 by either *E. chaffeensis, E. ewingii,* or an antigenically related *Ehrlichia* species. Patients infected with *E. chaffeensis* were identified from northern Arkansas, southern Illinois, central Georgia, northern Florida, central and southern Missouri, and central Tennessee. Infections with *E. ewingii* were confirmed in patients from central and southern Oklahoma, southern Missouri, and central Tennessee.

Twenty patients (95%) were male, and the median age was 43 years (range, 31–56 years). Absolute $CD4^+$ T lymphocyte counts were available for 20 patients; 14 patients (70%) had counts of <200 cells/μL at or near the time that they presented for care. All patients but 1 had HIV infection diagnosed before they presented with ehrlichiosis. In general, patients infected with *E. chaffeensis* had lower $CD4^+$ T lymphocyte counts and had been aware of their HIV-seropositive status for fewer years than had patients infected with *E. ewingii* at the time they presented for care (table 1). Eleven patients (52%) had no prior opportunistic infection. For 5 patients (24%), oropharyngeal candidiasis had been the only prior infection associated with their immunodeficiency. Severe or life-threatening opportunistic infections (e.g., *Pneumocystis carinii* pneumonia, *Candida* esophagitis, tuberculosis, or disseminated infection with *Mycobacterium avium*) had occurred in 5 patients (24%) before the onset of their infection with an *Ehrlichia* species. According to the CDC surveillance classification for HIV infection, 14 patients presented with AIDS. Clinical categories were available for 20 patients in this series and included A2 (4 patients), A3 (7 patients), B2 (2 patients), B3 (3 patients), and C3 (4 patients) [19].

Fourteen patients (67%) were receiving antibiotics that contained sulfa as chemoprophylaxis for *P. carinii.* Approximately half of the patients were receiving combination antiretroviral regimens that included either a viral protease inhibitor or a nonnucleoside reverse transcriptase inhibitor (i.e., highly active antiretroviral therapy [HAART]). Four patients (19%) were not taking antiretroviral therapy at presentation (table 1).

Onset of ehrlichial infections occurred from April through September of each year; 16 patients (76%) became ill during May or June. Eleven patients (52%) reported a recent tick bite and 8 other patients (38%) had witnessed ticks on their clothing or on

Table 1. Demographic and clinical characteristics of HIV infection in 21 patients with ehrlichiosis caused by *Ehrlichia chaffeensis* or *Ehrlichia ewingii*.

Demographic or clinical parameter	Patients infected with *E. chaffeensis* (*n* = 13)	Patients infected with *E. ewingii* (*n* = 4)	All patients[a] (*n* = 21)
Age, median years (range)	41 (31–56)	46 (40–49)	43 (31–56)
No. male	12	4	20
Duration of known HIV seropositivity, median years (range)	4 (<1–13)	7 (1–11)	4 (<1–13)
Most recent $CD4^+$ cell count, median cells/μL (range)[b]	98 (13–462)	176 (106–226)	137 (13–462)
HIV plasma RNA, median copies/mL (range)[c]	1950 (<50 to >750,000)	6736 (<50 to 231,380)	1900 (<50 to >750,000)
No. with prior opportunistic infection	6	2	10
No. receiving HAART	6	3	11

NOTE. HAART, highly active antiretroviral therapy.

[a] Includes 4 patients for whom specific ehrlichial agent could not be ascribed.

[b] Data available for 20 patients.

[c] Data available for 15 patients.

pets or other persons in their household or neighborhood within days to weeks preceding the onset of illness. Activities associated with tick bites or presumed exposure included recreational pursuits (hiking, camping, collecting arrowheads, fishing, picnicking, or canoeing) for 9 patients (47%); occupation (landscaping, field biology, gathering earthworms, or farming), for 6 patients (32%); and peridomestic activities (working or recreating in yard), for 4 patients (21%). For 2 patients who had no identifiable recent tick exposure, presumptive exposures were associated with residence in or travel to rural or semirural areas.

Clinical and laboratory characteristics. Patients presented for medical care a median of 4 days after the onset of illness (range, 1–21 days). All patients described fever as an initial component of their illnesses, and 13 patients presented with temperatures of ≥38.5°C (median, 38.8°C; range, 36.3°C–41.0°C). Patients infected with *E. chaffeensis* were more likely to present with ≥1 gastrointestinal symptom, cough, or rash than were persons infected with *E. ewingii* (table 2). Rash patterns were described as diffusely erythematous or morbilliform, or scattered petechiae or macules. Rashes showed varied distributions, from focal involvement of extremities to extensive involvement of chest and abdomen. All patients in this series presented with ≥1 cytopenia, and thrombocytopenia was observed in all but 1 patient. Patients infected with *E. chaffeensis* were more likely to present with pancytopenia than were those infected with *E. ewingii*. Elevated hepatic aminotransferase levels were observed at presentation in all patients infected with *E. chaffeensis* but in only 2 (50%) of 4 patients infected with *E. ewingii*. Approximately 80% of all patients presented with mild to moderate hyponatremia (table 2). Less frequently noted electrolyte abnormalities included hypocalcemia, hypomagnesemia, and hypophosphatemia.

Eighteen patients (86%) were hospitalized for their illnesses (table 3), with a median duration of stay of 6 days (range, 2–17 days). Ehrlichiosis was considered among the differential diagnoses within 24 h of evaluation for 13 patients (62%). Initial diagnoses other than ehrlichiosis included gastroenteritis (in 3 patients), fever of undetermined etiology (in 3), pneumonia (in 2), unspecified viral syndrome (in 2), volume depletion (in 2), urinary tract infection (in 1), pharyngitis (in 1), subdural

Table 2. Selected initial signs, symptoms, and laboratory abnormalities of ehrlichioses in patients coinfected with HIV.

Clinical characteristic	Patients infected with *Ehrlichia chaffeensis* (*n* = 13)	Patients infected with *Ehrlichia ewingii* (*n* = 4)	All patients[a] (*n* = 21)
Fever	13 (100)	4 (100)	21 (100)
Malaise	10 (83)	2 (50)	15 (75)
Myalgia	7 (58)	2 (50)	13 (65)
Headache	5 (42)	1 (25)	10 (50)
Cough	7 (58)	0 (0)	8 (40)
Nausea	5 (42)	1 (25)	8 (40)
Vomiting	5 (42)	1 (25)	8 (40)
Diarrhea	5 (42)	0 (0)	6 (30)
Rash	6 (46)	0 (0)	8 (38)
Leukopenia	11 (88)	3 (75)	18 (86)
Thrombocytopenia	13 (100)	3 (75)	20 (95)
Anemia	8 (62)	3 (75)	12 (57)
Pancytopenia	7 (54)	1 (25)	9 (43)
Elevated aspartate aminotransferase level	13 (100)	2 (50)	18 (86)
Elevated alanine aminotransferase level	10 (83)	1 (33)	11 (73)
Hyponatremia	11 (85)	3 (75)	17 (81)

NOTE. Data are no. (%). Percentages were calculated from patients for whom data were available.

[a] Includes 4 patients for whom specific ehrlichial agent could not be ascribed.

Table 3. Comparison of clinical characteristics and laboratory results for HIV-infected patients coinfected with *Ehrlichia chaffeensis* or *Ehrlichia ewingii*.

Clinical characteristic or laboratory value	Patients infected with *E. chaffeensis* (n = 13)	Patients infected with *E. ewingii* (n = 4)	P[a]
No. hospitalized	12	2	.121
No. of deaths	6	0	.237
No. of patients with			
Pulmonary manifestations[b]	8	0	.082
Acute renal failure	6	0	.237
Cardiac manifestations[c]	5	0	.261
Neurological manifestations[d]	4	0	.519
Disseminated intravascular coagulopathy	3	0	.541
Spontaneous hemorrhage	3	0	.541
Secondary fungal infection	3	0	.541
Nadir WBC count, median $\times 10^9$ cells/L (range)	1.8 (0.4–3.7)	3.4 (2.6–4.0)	.003
Nadir platelet count, median $\times$ 10^9 cells/L (range)	15 (2–42)	80 (34–255)	.003
Nadir hemoglobin level, median g/L (range)	9.8 (8.2–13.8)	11.9 (7.9–12.2)	.785
Peak aspartate aminotransferase level, median U/L (range)	293 (89–1107)	60 (23–131)	.003
Peak serum creatinine level, median mg/dL (range)	2.4 (1.0–9.0)	1.1 (0.7–1.4)	.045
Nadir serum sodium level, median mmol/L (range)	127 (121–134)	134 (132–135)	.015

[a] P values were determined using Fisher's exact test, for dichotomous variables, and Mann-Whitney U test, for continuous variables.
[b] Includes acute respiratory distress syndrome, pneumonia, and acute hypoxia.
[c] Includes atrial fibrillation, dilated cardiomyopathy, congestive heart failure, myocarditis, and acute myocardial infarct.
[d] Includes confusion, delirium, generalized seizures, and slurred speech.

hemorrhage (in 1), and sepsis (in 1). For 8 patients (38%), ehrlichiosis was not considered until ≥4 days after initial evaluation. All persons for whom a diagnosis of ehrlichiosis was considered received doxycycline within the first 24 h after presenting for care, and those who survived their illnesses generally became afebrile within 2–3 days after receiving this antibiotic.

Moderate to severe disease manifestations were reported for 15 patients (71%), predominantly among those infected with *E. chaffeensis* (table 3). Three (23%) of 13 patients infected with *E. chaffeensis* had acute respiratory distress syndrome diagnosed, and 2 others developed pulmonary failure that required mechanical ventilatory support. Pulmonary, intracranial, or gastrointestinal hemorrhage occurred in 3 patients (23%) who were infected with *E. chaffeensis*. Secondary fungal infections, including pulmonary and tracheobronchial aspergillosis and tracheobronchial candidiasis, occurred in 3 *E. chaffeensis*–infected patients (23%) during the course of hospital stays that lasted ≥10 days. Hematologic and biochemical abnormalities were significantly more pronounced in persons infected with *E. chaffeensis* than they were in *E. ewingii*–infected patients (table 3). Platelet count nadirs of $<50 \times 10^9$ cells/L were observed in 17 patients (81%) in this series; however, all 8 patients who had counts of $<20 \times 10^9$ cells/L were infected with *E. chaffeensis*. Similarly, all 7 patients who had serum sodium levels of <130 mmol/L and all 8 patients who had serum creatinine levels of >1.5 mg/dL were infected with this agent.

Lumbar punctures were performed for 6 patients (29%). Three patients (50%) had mildly elevated WBC counts (range, 6–8×10^6 cells/L), with 85%–100% lymphocytes. Protein concentration was elevated in 4 patients (67%; range, 0.48–0.96 g/L). Cerebrospinal glucose levels were abnormally low (2.4 mmol/L) in 1 patient (17%) and elevated (4.5 mmol/L) in 1 patient.

Six patients died either directly of infection with *E. chaffeensis* or of complications associated with the illness. All patients with

Table 4. Comparison of selected clinical events and characteristics associated with fatal and nonfatal infections with *Ehrlichia chaffeensis* in persons coinfected with HIV.

Clinical event or characteristic	Patients with Fatal *E. chaffeensis* infection (n = 6)	Patients with Nonfatal *E. chaffeensis* infection (n = 7)	P[a]
Time from illness onset to presentation for medical care, median days (range)	3.5 (1–7)	4.0 (1– 6)	.836
Time from presentation to consideration of ehrlichiosis, median days (range)	5.5 (1–18)	1.0 (<1–5)	.073
$CD4^+$ T lymphocyte count, median cells/μL (range)[b]	64 (13–164)	232 (30–462)	.088
Receipt of ≤1 antiretroviral drug	4	1	.102
Receipt of HAART	1	5	.102
Nadir platelet count of <20 × 10^9 cells/L	5	3	.266
Peak serum creatinine level of >3.0 mg/dL	5	1	.029
Peak aspartate aminotransferase level of >300 U/L	5	1	.029
Nadir serum sodium level of <130 mmol/L	3	4	1.000

NOTE. Data are no. of patients, unless otherwise indicated. HAART, highly active antiretroviral therapy.

[a] P values were determined using Fisher's exact test, for dichotomous variables, and Mann-Whitney U test, for continuous variables.

[b] Data available for 5 patients.

fatal disease for whom $CD4^+$ T lymphocyte counts were available (5 patients) had counts of <200 cells/μL; 4 patients (80%) had counts of <100 cells/μL, and 2 patients (40%) had counts of <50 cells/μL. Case-fatality ratios for patients infected with *E. chaffeensis* at each of these $CD4^+$ cell count break points were 62%, 67%, and 50%, respectively. Patients died a median of 13 days (range, 8–18 days) after the onset of symptoms. Four patients (67%) had been evaluated for their symptoms and released from at least 1 emergency room or medical clinic with a diagnosis other than ehrlichiosis within 1–2 weeks before admission. Most patients with fatal disease exhibited multisystem organ failure, including ≥2 of the following manifestations: acute renal failure (in 5 patients); pneumonia, severe hypoxia, or acute respiratory distress syndrome (in 4), atrial fibrillation or myocarditis (in 3); hepatic failure (in 3); metabolic acidosis (in 3); disseminated intravascular coagulopathy or spontaneous hemorrhage (in 3); and seizures or delirium (in 2). Specific causes of death included pulmonary hemorrhage, pneumonia, respiratory failure, and cerebral edema with brainstem herniation. In general, patients with fatal *E. chaffeensis* infections were more likely to have a later diagnosis of ehrlichiosis and develop acute renal failure, severe thrombocytopenia, and profound hepatic aminotransferase elevations and were less likely to be taking HAART than were patients who recovered from infection with this agent; however, only peak creatinine levels of >3.0 mg/dL and aspartate aminotransferase levels of >300 U/L were statistically significant predictors of fatal outcome (table 4).

Laboratory confirmation. Infection with an *Ehrlichia* species was confirmed for each patient by using ≥1 of the following laboratory results: amplification of gene sequences specific for an *Ehrlichia* species from a clinical sample (for 16 patients); ≥4-fold change in titer of antibodies reactive with *E. chaffeensis* antigens (for 12); immunohistochemical staining of *E. chaffeensis* in biopsy or autopsy tissue specimens (for 3); morulae identified in peripheral blood or bone marrow leukocytes (for 7); or isolation of *E. chaffeensis* in cell culture (for 6; table 5).

For 16 patients (76%), ≥1 serum or plasma sample was obtained and tested for antibodies reactive with *E. chaffeensis.* Initial specimens (n = 4) obtained from patients with fatal disease were collected a median of 5 days after onset (range, 4–15 days), and none had diagnostic IgG titers (i.e., ≥64). Initial samples (n = 12) obtained from patients who survived their infections were collected a median of 6.5 days after onset (range, 1–61 days), and 7 samples (58%) had titers of ≥64. Twelve (86%) of 14 patients from whom paired serum samples were obtained demonstrated a ≥4-fold change in titer; 11 patients (79%) had titers of ≥512 in the second sample, and these specimens were obtained a median of 41 days after onset (range, 9–74 days). The geometric mean of the highest IgG antibody titer reactive with *E. chaffeensis* from the 9 patients for whom end points were available was 2048 (range, 512–16,384). Both patients with fatal disease for whom a second serum sample was available (obtained 8 days after onset of illness for one patient and 16 days after onset for the other) failed to develop anti–*E. chaffeensis* antibody titers of ≥64 before their deaths.

A ≥4-fold change in antibody titer reactive with *E. chaffeensis* was the only positive confirmatory test for 4 patients; however,

Table 5. Results of confirmatory tests for ehrlichioses in 21 patients coinfected with HIV.

Results of laboratory tests	Patients infected with *Ehrlichia chaffeensis* (*n* = 13)	Patients infected with *Ehrlichia ewingii* (*n* = 4)	All patients[a] (*n* = 21)
Ehrlichial DNA detected by use of PCR	12/12	4/4	16/18
≥4-fold change in titer of antibody reactive with *E. chaffeensis*	4/6	4/4	12/14
Morulae identified in peripheral blood or bone marrow leukocytes	6/9	1/2	7/11
Ehrlichiae identified in tissue by immunohistochemical stain	3/3	NT	3/3
Ehrlichiae isolated in DH82 cells	6/6	0/1	6/7

NOTE. Data are no. of patients with positive test result/no. tested. NT, none tested.

[a] Includes 4 patients for whom specific ehrlichial agent could not be ascribed.

because persons infected with either *E. chaffeensis* or *E. ewingii* may generate antibodies that react with *E. chaffeensis* antigens (in this series, 4 patients each), infection with these agents cannot be differentiated by using IFA as the sole confirmatory test [3, 15].

Morulae were visualized in peripheral blood or bone marrow leukocytes of ~60% of patients in this series for whom whole blood, buffy coat, or bone marrow aspirate smears were evaluated. When quantified, morulae of *E. chaffeensis* were identified in ~1%–25% of leukocytes, predominantly monocytes, and occasionally in metamyelocytes and band neutrophils. Morulae of *E. ewingii* were seen in ~5% of mature and immature neutrophils and in rare eosinophils of 1 patient.

Ehrlichial DNA was amplified from all but 2 patients tested; both negative specimens were acute-phase serum samples obtained from patients with ≥8-fold increases in antibody titer reactive with *E. chaffeensis.* Twelve samples yielded the characteristic 389-bp product from the 16S rRNA gene of *E. chaffeensis* when the DNAs were amplified by means of PCR. Appropriately sized fragments of the VLPT gene (369 bp, 459 bp, or 639 bp) and 120-kDa protein gene (~1250 bp or 1500 bp) were amplified from DNA samples from 6 patients from whom isolates of *E. chaffeensis* were obtained; fragments of identical sizes were obtained from the corresponding cell culture isolates. Sequenced PCR products from patients and isolates in all cases matched reported sequences for the corresponding genes of *E. chaffeensis.* A 354-bp segment of the 16S rRNA gene and a 1416-bp segment of the *groESL* heat-shock operon of *E. ewingii* were obtained from 2 patients by using nested PCR, and identities of these gene sequences were confirmed by sequencing. Expected 354-bp and 389-bp segments of the 16S rRNA gene of *E. ewingii* were amplified from the blood samples obtained from 2 additional patients by use of PCR assays specific for *E. ewingii.*

Antigens and distinct morulae of *E. chaffeensis* were identified by use of immunohistochemical staining in mononuclear cells of tissue specimens obtained from all 3 patients tested by means of this method. Ehrlichiae were visualized in various tissues and were especially abundant in bone marrow and spleen.

E. chaffeensis was cultured from whole blood samples obtained from each of 6 patients for whom isolation was attempted. Blood samples ranged in age from 1 to 5 days and had been obtained from patients within 3–5 days after the onset of symptoms. Distinct morulae were visible in DH82 cells within 2–8 days after inoculation of peripheral blood leukocytes. An attempt to isolate *E. ewingii* in DH82 cells was unsuccessful.

DISCUSSION

This report documents clinical, laboratory, and epidemiological features of ehrlichioses in 21 patients coinfected with HIV, occurring over broad geographic regions of the southeastern and midwestern United States. This is the first description of infections with *E. ewingii* in persons with HIV disease and the first report of laboratory-confirmed infections with *E. ewingii* in patients from Oklahoma and Tennessee.

In the United States, ehrlichiae were first identified as human pathogens in 1986. Expanding public and physician awareness of and improved epidemiological surveillance for infections caused by *Ehrlichia* species have contributed to increasing recognition of ehrlichioses in the general population. This is exemplified by recent increases in reported cases: ~1200 cases of ehrlichioses were reported to state health departments from 1986 through 1997 [20], but nearly 1300 cases were reported in 1998 and 1999 alone (CDC, unpublished data). Similarly, 81% of the patients described in this series of HIV-infected patients were identified during the period of 1997–2000. However, enhanced surveillance and awareness are perhaps only 2 of several elements involved in the emergence of *E. chaffeensis*– and *E. ewingii*–associated illnesses in patients coinfected with HIV. Recent changes in the geographic distribution of persons with HIV disease and progress in the medical management of HIV-infected patients may also be contributing to the emergence of ehrlichioses in this patient population.

Most patients in this series resided or worked in nonmetropolitan areas, which is true of the majority of persons who have ehrlichioses diagnosed [20, 21]. Although the absolute preva-

lence of HIV among persons in nonmetropolitan areas remains significantly lower than it is in urban centers, the number of HIV-infected persons residing in nonmetropolitan areas has increased steadily [22, 23]. From 1992 through 1995, the average annual increase in AIDS cases in nonmetropolitan areas was 30.0%, which exceeded the 25.8% increase observed in the largest metropolitan areas [24]. The increase in HIV infection in rural populations has been attributed to increased transmission in these areas and to migration of persons to their native communities for treatment and supportive care after acquiring HIV infection in urban centers [25–27]. The diffusion of HIV into rural populations is particularly evident in the southeastern United States [25–29], which was the region with the greatest number of HIV infections during the mid-1990s [30]. This region also represents the origin of the majority of ehrlichiosis cases caused by *E. chaffeensis* [20].

Approximately half of the patients in this series were receiving HAART, and most were in otherwise good health before the onset of their infection with an *Ehrlichia* species. Combination antiretroviral regimens have significantly slowed the progression of HIV disease in many persons, with concomitant decreases in the rates of hospitalization, morbidity, and mortality in patients for whom these drugs are available [30–33]. In this context, new therapies for HIV offer a level of health that facilitates occupational and recreational pursuits that perhaps were not previously possible. Some of these activities (e.g., hunting, hiking, camping, or working outdoors) involve incursions into tick-infested habitats and are associated with risks of acquiring tickborne diseases. Severe disease caused by *Rickettsia rickettsii,* the agent of Rocky Mountain spotted fever, and *Babesia microti,* an agent of human babesiosis, have been reported recently for persons with advanced HIV disease [34, 35]. In this context, the emergence of a healthier HIV-infected patient population exposed to increasingly diverse environments may paradoxically accentuate increases in the incidence of some infectious diseases, such as ehrlichioses.

The full clinical spectrum of illnesses that result from infections with *E. chaffeensis* or *E. ewingii* in HIV-infected patients may eventually reveal milder forms of disease; however, it is apparent that ehrlichioses, and illness caused by *E. chaffeensis* in particular, can be severe or life threatening in this patient cohort. All but 3 patients in this series were eventually hospitalized, creating a possible selection bias for those patients with more-severe disease; however, comparisons with other series that include data for hospitalized patients suggest that ehrlichiosis caused by *E. chaffeensis* is more severe in HIV-infected persons than it is in the general population. In a series of 237 patients from the general population that included 146 hospitalized persons (62%), renal failure, disseminated intravascular coagulopathy, spontaneous hemorrhages, or cardiac manifestations attributable to ehrlichiosis were identified in only 3%–11% of persons infected with *E. chaffeensis* [21]. These same manifestations occurred in ~15%–30% of the HIV-infected persons in this series. Contrasting mortality as a measure of disease severity, no deaths were reported among 41 patients hospitalized with *E. chaffeensis* infection described in separate prospective studies in Georgia, Missouri, North Carolina, and Tennessee from 1987 through 1998 [13, 36–38]; however, one-third of the hospitalized HIV-infected patients in this series died of infection with this agent.

Severe disease attributable to *E. chaffeensis* has been described in patients with compromised immunity from other causes, including immunosuppressive therapies [39–42], monoclonal gammopathy [17], or asplenia [5, 43]. A resurgent T cell lymphocytosis is frequently described in persons recovering from infection with *E. chaffeensis* [37, 44], suggesting that intact T cell host responses are important for clearance of ehrlichiae. In this series, all patients with fatal disease had $CD4^+$ T lymphocyte counts of <200 cells/μL. Case-fatality ratios for persons with <100 cells/μL were $\geq$50%, in contrast with a case-fatality ratio in the general population of ~3% [20]. In this context, ehrlichiae may represent opportunistic pathogens in HIV-infected persons.

Only 4 patients infected with *E. ewingii* have been reported previously in the literature, and 3 of these had underlying immunodeficiencies [3]. It is unclear whether *E. ewingii* causes disease primarily in immunosuppressed persons or if this bacterium is responsible for illnesses in a broader patient population, in which only the most clinically advanced cases have been recognized in a sentinel cohort. Only 2 (50%) of 4 patients with confirmed *E. ewingii* infection in this series were hospitalized, and all patients recovered completely from infection with this pathogen. The *E. ewingii*–infected patients reported in this series developed fewer disease manifestations and complications than did patients infected with *E. chaffeensis.* Although these findings suggest that *E. ewingii* may be less pathogenic than *E. chaffeensis,* the small number of patients evaluated to date precludes broad comparisons of severity between these 2 forms of ehrlichiosis in this patient population.

Host factors responsible for disease severity remain relatively undefined in persons infected with *E. chaffeensis* [7, 13]. A wide range of clinical outcomes was recognized soon after the initial identification of human ehrlichiosis in the United States [45]; a spectrum of disease severity continues to be observed among persons infected with ehrlichiae. Severe and sometimes fatal illnesses have occurred in otherwise healthy young adults and children [5, 9, 46]. In contrast, relatively moderate disease caused by *E. chaffeensis* or *E. ewingii* occurred in several HIV-infected patients in this series, including some patients with $CD4^+$ T lymphocyte counts of <200 cells/μL. Genetic heterogeneity exists among different isolates of *E. chaffeensis* [7, 11, 13, 14], which suggests that different strains of this bacterium

may vary in phenotypic or biological characteristics that influence pathogenicity [7, 13]. Other studies have demonstrated that factors extrinsic to the host or pathogen, particularly delay in treatment with an appropriate antibiotic (e.g., a tetracycline), can be associated with an increased risk for disease complications and death in persons infected with ehrlichiae [21]. A combination of factors, including the immune status of the patient, the interval from symptom onset to correct diagnosis and initiation of effective therapy, and, perhaps, the virulence of the bacterial isolate infecting the patient, may ultimately represent important and interacting determinants of the clinical outcome of ehrlichioses.

Most presenting signs and symptoms (e.g., fever, headache, malaise, myalgia, and nausea) were nonspecific and similar in frequency to those described in other case series of patients infected with *E. chaffeensis* or *E. ewingii* [3, 21, 37, 43, 47]. Diagnosis of ehrlichioses in persons coinfected with HIV may be confounded by clinical similarity with various community-acquired or conventionally recognized opportunistic infections in this patient population. As with many other systemic diseases in persons infected with HIV, ehrlichioses may present with multiple and varied signs and symptoms, including vomiting, diarrhea, rash, or cough. Leukopenia, thrombocytopenia, and anemia occurred with frequencies similar to those described in prospective series of *E. chaffeensis*–infected patients in the general population; however, median nadir values for each value were markedly lower in HIV-infected patients [13, 36, 37, 47]. Cytopenia, although a characteristic finding of ehrlichioses, is perhaps less indicative of these illnesses in HIV-infected patients than it is in the general population, and it can be caused by HIV disease, other opportunistic infections, or various antimicrobial therapies used by this patient cohort [48, 49].

Several of the patients in this series initially had other conditions diagnosed, and one-third of the patients received empirical therapy with $\geq$1 antibiotic that was ineffective against ehrlichiae (e.g., cephalosporins, macrolides, and sulfa antimicrobials). Doxycycline, the drug of choice for ehrlichioses, is not routinely administered as empirical therapy for febrile patients infected with HIV. Two-thirds of the patients were receiving trimethoprim-sulfamethoxazole or dapsone as chemoprophylaxis for *P. carinii* pneumonia at the time of their illness. It has been suggested that drugs that contain sulfa may exacerbate the severity of infection with *E. chaffeensis* [50]; however, further studies are necessary to confirm this observation.

Several patients who died of infection with *E. chaffeensis* failed to mount IgG antibody responses by week 2 or 3 of illness; however, most patients in this series showed antibody responses that are similar both temporally and in magnitude to those observed in the general population [13, 36, 51]. All patients who survived their illness developed robust IgG antibody levels, including some patients with $CD4^+$ T lymphocyte counts of <200 cells/μL. As noted in earlier studies, diagnostic levels of IgG antibodies were not present in the first serum sample obtained from the majority of acutely ill patients in this series. Restricting serological evaluation to a single sample obtained during the acute phase of the illness may preclude laboratory confirmation of ehrlichioses [51]. For 4 patients, laboratory confirmation was obtained by using IFA as the only test. Patients infected with either *E. chaffeensis* or *E. ewingii* may demonstrate $\geq$4-fold changes in titers of antibodies reactive with *E. chaffeensis;* this finding may prevent assignment of a specific agent if IFA is used as the sole confirmatory test [3, 15]. In this context, testing multiple samples by use of a combination of serological and molecular assays may be necessary to confirm the disease and to ascribe a species-specific etiology.

Successful outcomes after *Ehrlichia*-associated illnesses in HIV-infected patients can be achieved by using fundamental principles that apply to successful management of ehrlichioses in the general population. In this context, improving awareness among physicians and patients of the seasonal and geographic distribution of diseases caused by *Ehrlichia* species, eliciting pertinent epidemiological data in the patient history (e.g., a recent tick bite or exposure to ticks), and using an early presumptive diagnosis to guide empirical treatment with doxycycline remain the best approaches to combat these potentially fatal diseases.

Acknowledgments

We thank Michael Crutcher (Oklahoma State Department of Health, Oklahoma City), Donald Bartley (St. Vincent's Medical Center, Jacksonville, Florida), and Jerry Harris (Tallahassee Memorial Hospital, Tallahassee, Florida), for their assistance in identifying patients in this series; Joan Barenfanger (Memorial Medical Center, Springfield, Illinois) and Stevan Whitt (Columbia University Medical Center, Columbia, Missouri), for providing additional data regarding their previously published reports; Sherif Zaki (CDC, Atlanta), for the immunohistochemical testing of patient tissues; John O'Connor, for editorial comments; and Jonathan Kaplan, William Nicholson, Andy Comer (CDC), and several anonymous reviewers, for their critical manuscript review.

References

1. Maeda K, Markowitz N, Hawley RC, et al. Human infection with *Ehrlichia canis,* a leukocytic rickettsia. N Engl J Med **1987**; 316:853–6.
2. Bakken JS, Dumler JS, Chen SM, et al. Human granulocytic ehrlichiosis in the upper midwest United States: a new species emerging? JAMA **1994**; 272:212–8.
3. Buller RS, Arens M, Hmiel SP, et al. *Ehrlichia ewingii,* a newly recognized agent of human ehrlichiosis. N Engl J Med **1999**; 341:148–55.
4. Paddock CD, Suchard DM, Grumbach KL, et al. Brief report: fatal

seronegative ehrlichiosis in a patient with HIV infection. N Engl J Med **1993**; 329:1164–7.
5. Fichtenbaum CJ, Peterson LR, Weil GJ. Ehrlichiosis presenting as a life-threatening illness with features of the toxic shock syndrome. Am J Med **1993**; 95:351–7.
6. Barenfanger J, Patel PG, Dumler JS, Walker DH. Identifying human ehrlichiosis. Lab Med **1996**; 27:372–4.
7. Paddock CD, Sumner JW, Shore GM, et al. Isolation and characterization of *Ehrlichia chaffeensis* strains from patients with fatal ehrlichiosis. J Clin Microbiol **1997**; 35:2496–502.
8. Whitt SP, Everett ED, Roland W, Dolan S. *Ehrlichia chaffeensis*–associated cardiomyopathy in a patient with AIDS. Clin Infect Dis **1999**; 28:140.
9. Martin GS, Christman BW, Standaert SM. Rapidly fatal infection with *Ehrlichia chaffeensis*. N Engl J Med **1999**; 341:763–4.
10. Dawson JE, Anderson BE, Fishbein DB, et al. Isolation and characterization of an *Ehrlichia* sp. from a patient diagnosed with human ehrlichiosis. J Clin Microbiol **1991**; 29:2741–5.
11. Sumner JW, Childs JE, Paddock CD. Molecular cloning and characterization of the *Ehrlichia chaffeensis* variable length PCR target: an antigen-expressing gene that exhibits interstrain variation. J Clin Microbiol **1999**; 37:1447–53.
12. Anderson BE, Sumner JW, Dawson JE, et al. Detection of the etiologic agent of human ehrlichiosis by polymerase chain reaction. J Clin Microbiol **1992**; 30:775–80.
→ Standaert SM, Yu T, Scott MA, et al. Primary isolation of *Ehrlichia chaffeensis* from patients with febrile illnesses: clinical and molecular characteristics. J Infect Dis **2000**; 181:1082–8.
14. Yu XJ, Crocquet-Valdes P, Walker DH. Cloning and sequencing of the gene for a 120-kDa immunodominant protein of *Ehrlichia chaffeensis*. Gene **1997**; 184:149–54.
15. Sumner JW, Storch GA, Buller RS, et al. PCR amplification and phylogenetic analysis of *groESL* operon sequences from *Ehrlichia ewingii* and *Ehrlichia muris*. J Clin Microbiol **2000**; 38:2746–9.
16. Dumler JS, Dawson JE, Walker DH. Human ehrlichiosis: hematopathology and immunohistologic detection of *Ehrlichia chaffeensis*. Hum Pathol **1993**; 24:391–6.
17. Dawson JE, Paddock CD, Warner CK, et al. Tissue diagnosis of *Ehrlichia chaffeensis* in patients with fatal ehrlichiosis by using in situ hybridization, immunohistochemistry, and polymerase chain reaction. Am J Trop Med Hyg (in press).
18. SPSS for Windows, release 10.0.7, standard version. Chicago: SPSS, **2000**.
19. Centers for Disease Control and Prevention. 1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. MMWR Morb Mortal Wkly Rep **1992**; 41(RR-17):1–19.
20. McQuiston JH, Paddock CD, Holman RC, Childs JE. Human ehrlichioses in the United States. Emerg Infect Dis **1999**; 5:635–42.
21. Fishbein DB, Dawson JE, Robinson LE. Human ehrlichiosis in the United States, 1985 to 1990. Ann Intern Med **1994**; 120:736–43.
22. Gardner LI, Brundage JF, Burke DS, et al. Evidence for spread of the human immunodeficiency virus epidemic into low prevalence areas of the United States. J Acquir Immune Defic Syndr **1989**; 2:521–32.
23. Lam NS, Liu K. Spread of AIDS in rural America, 1982–1990. J Acquir Immune Defic Syndr **1994**; 7:485–90.
24. Fordyce EF, Thomas P, Shum R. Evidence of an increasing AIDS burden in rural America. Stat Bull Metrop Insur Co **1997**; 78:2–9.
25. Verghese A, Berk SL, Sarubbi F. *Urbs in rure:* human immunodeficiency virus infection in rural Tennessee. J Infect Dis **1989**; 160:1051–5.
26. Berry DE. The emerging epidemiology of rural AIDS. J Rural Health **1993**; 9:293–304.
27. McCoy CB, Metsch LR, McCoy HV, Weatherby NL. HIV seroprevalence across the rural/urban continuum. Subst Use Misuse **1999**; 34: 595–615.
28. Rumley RL, Shappely NC, Waivers LE, Esinhart JD. AIDS in rural eastern North Carolina–patient migration: a rural AIDS burden. AIDS **1991**; 5:1373–8.
29. Cohn SE, Klein JD, Mohr JE, et al. The geography of AIDS: patterns of urban and rural migration. South Med J **1994**; 87:599–606.
30. Centers for Disease Control and Prevention. HIV AIDS Surveillance Report **1999**; 11:1–43.
31. Palella FJ, Delaney KM, Moorman AC, et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. N Engl J Med **1998**; 338:853–60.
32. Kovacs JA, Masur H. Prophylaxis against opportunistic infections in patients with human immunodeficiency virus infection. N Engl J Med **2000**; 342:1416–29.
33. Masur H. Human immunodeficiency virus–related opportunistic infections in the era of highly active antiretroviral therapy. In: Scheld WM, Craig WA, Hughes JM, eds. Emerging infections 4. Washington, DC: ASM Press, **2000**:165–86.
34. DiNubile MJ. Reliving a nightmare: a hard and tragic lesson in humility. Clin Infect Dis **1996**; 23:160–4.
35. Falagas ME, Klempner MS. Babesiosis in patients with AIDS: a chronic infection presenting as a fever of unknown origin. Clin Infect Dis **1996**; 22:809–12.
36. Fishbein DB, Kemp A, Dawson JE, et al. Human ehrlichiosis: prospective active surveillance in febrile hospitalized patients. J Infect Dis **1989**; 160:803–9.
37. Everett ED, Evans KA, Henry B, McDonald G. Human ehrlichiosis in adults after tick exposure. Ann Intern Med **1994**; 120:730–5.
→ Carpenter CF, Gandhi TK, Kong LK, et al. The incidence of ehrlichial and rickettsial infection in patients with unexplained fever and recent history of tick bite in central North Carolina. J Infect Dis **1999**; 180: 900–3.
39. Manian FA, Weidner J, Costello J, et al. Human ehrlichiosis. Mo Med **1989**; 86:691–5.
40. Marty AM, Dumler JS, Imes G, et al. Ehrlichiosis mimicking thrombotic thrombocytopenic purpura: case report and pathological correlation. Hum Pathol **1995**; 26:920–5.
41. Anthony SJ, Dummer JS, Hunter E. Human ehrlichiosis in a liver transplant recipient. Transplantation **1995**; 60:879–81.
42. Sadikot R, Shaver MJ, Reeves WB. *Ehrlichia chaffeensis* in a renal transplant recipient. Am J Nephrol **1999**; 19:674–6.
43. Eng TR, Harkess JR, Fishbein DB, et al. Epidemiologic, clinical, and laboratory findings of human ehrlichiosis in the United States, 1988. JAMA **1990**; 264:2251–8.
44. Caldwell CW, Everett ED, McDonald G, et al. Lymphocytosis of γ/δ T cells in human ehrlichiosis. Am J Clin Pathol **1995**; 103:761–6.
45. Taylor JP, Betz TG, Fishbein DB, et al. Serological evidence of possible human infection with *Ehrlichia* in Texas. J Infect Dis **1988**; 158:217–20.
46. Berry DS, Miller RS, Hooke JA, et al. Ehrlichial meningitis with cerebrospinal fluid morulae. Pediatr Infect Dis J **1999**; 18:552–5.
47. Standaert SM, Dawson JE, Schaffner W, et al. Ehrlichiosis in a golf-oriented retirement community. N Engl J Med **1995**; 333:420–5.
48. Perkocha LA, Rodgers GM. Hematologic aspects of human immunodeficiency virus infection: laboratory and clinical considerations. Am J Hematol **1988**; 29:94–105.
49. Sloand EM, Klein HG, Banks SM, et al. Epidemiology of thrombocytopenia in HIV infection. Eur J Haematol **1992**; 48:168–72.
50. Peters TR, Edwards KM, Standaert SM. Severe ehrlichiosis in an adolescent taking trimethoprim-sulfamethoxazole. Pediatr Infect Dis J **2000**; 19:170–1.
51. Childs JE, Sumner JW, Nicholson WL, et al. Outcome of diagnostic tests using samples from patients with culture-proven human monocytic ehrlichiosis: implications for surveillance. J Clin Microbiol **1999**; 37:2997–3000.