

## RICKETTSIA AFRICAE, A TICK-BORNE PATHOGEN IN TRAVELERS TO SUB-SAHARAN AFRICA

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### ABSTRACT

**Background** African tick-bite fever occurs after contact with ticks that carry *Rickettsia africae* and that parasitize cattle and game. Sporadic reports suggest that this infection has specific clinical and epidemiologic features.

**Methods** We studied patients who were tested for a rickettsial disease after returning from a visit to Africa or Guadeloupe. To assess the value of the microimmunofluorescence assay, Western blotting, and cross-adsorption assays, we compared the results of these tests in 39 patients in whom African tick-bite fever had been confirmed by the polymerase-chain-reaction assay, cell culture, or both; 50 patients with documented *R. conorii* infection; and 50 blood donors. These diagnostic criteria were then applied to 376 additional patients who had returned from southern Africa and 2 who had returned from Guadeloupe and whose serum was being tested for rickettsial disease.

**Results** In the 39 patients with direct evidence of *R. africae* infection, the combination of microimmunofluorescence assay, Western blotting, and cross-adsorption assays showing antibodies specific for *R. africae* had a sensitivity of 0.56; however, each test had a positive predictive value and a specificity of 1.0. An additional 80 patients were found to have an *R. africae* infection on the basis of these serologic criteria. Infections with *R. africae* were acquired by visitors to 11 African countries and Guadeloupe. The illness was generally mild and was characterized by a rash in 46 percent of the patients; the rash was usually maculopapular or vesicular and rarely purpuric. Ninety-five percent of patients had an inoculation eschar or eschars, and 54 percent of these patients had multiple eschars, a finding that is unusual in patients with rickettsial infection.

**Conclusions** In this series, *R. africae* was the cause of nearly all cases of tick-bite rickettsiosis in patients who became ill after a trip to sub-Saharan Africa. (N Engl J Med 2001;344:1504-10.)

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**R**ICKETTSIA *conorii* infection was once considered the only tick-transmitted rickettsial infection in Europe and Africa, causing both Mediterranean spotted fever and African tick-bite fever. At that time, African tick-bite fever, which occurred after contact with ticks (amblyomma species) found on cattle and game and was characterized by signs of mild infection, usually with no rash,<sup>1</sup> was considered a rural form of Mediterra-

nean spotted fever. Mediterranean spotted fever was transmitted by dog ticks (rhhipicephalus species) and was characterized by fever, headache, myalgias, a maculopapular rash, and a single inoculation eschar at the site of the tick bite.<sup>2</sup> In 1936, Pijper suggested that these two diseases were caused by different agents,<sup>3</sup> but it was not until 1992 that Kelly et al. demonstrated the causative role of a new rickettsia isolated from a patient who presented with fever, an inoculation eschar, and regional lymphadenopathy but no rash.<sup>4</sup> This isolate was named *R. africae*; it was distinct from all other previously described rickettsiae but similar to a bacterium previously isolated from *Amblyomma hebraeum* ticks.<sup>4,5</sup> Subsequently, *R. africae* has been implicated as the etiologic agent in several cases of African tick-bite fever in travelers returning from southern Africa<sup>6,7</sup> and in one woman who was bitten by a tick in Guadeloupe.<sup>4,8,9</sup> We evaluated a large series of patients with *R. africae* infections in order to describe the epidemiologic, clinical, and diagnostic features of African tick-bite fever and to validate serologic methods for the diagnosis of this disease.

### METHODS

#### Patients

We tested serum samples, blood samples, and skin-biopsy specimens that were obtained between January 1996 and March 2000 from patients who had had an influenza-like syndrome that was not due to malaria (as indicated by a negative blood smear) after returning from a trip to Africa or Guadeloupe. For each patient, data on epidemiologic and clinical features were obtained by the attending physician through the use of a standardized questionnaire. Information was obtained about whether the patient had been bitten by a tick (or had handled a tick) during the trip and, if so, the geographic area in which the bite had occurred; whether the patient had had any contact with animals during the trip; whether the patient had any underlying diseases; and whether the patient had had the following clinical symptoms: fever, headache, an in-

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oculation eschar or eschars (and their locations if present), regional adenopathy, a rash, and if present, the type of rash (maculopapular, vesicular, or purpuric). Information about laboratory results and the outcome of the infection was also obtained. For each patient, a serum sample was obtained for serologic testing during the acute phase of the illness and, when possible, during convalescence. Some of the patients have been described previously.<sup>7,10</sup> Each patient provided written informed consent.

### Laboratory Diagnosis

A microimmunofluorescence assay was used as the reference method and was carried out as described previously with the use of both *R. conorii* strain Seven (Malish, American Type Culture Collection VR-613T) and *R. africae* strain ESF-5 (provided by Dr. G. Dasch) as antigens.<sup>9</sup> Titers of 1:64 in the case of IgG antibody or of 1:32 in the case of IgM antibody (or both) were considered evidence of recent infection by a rickettsia species.<sup>11,12</sup> Western blotting, cross-adsorption assays, cell or tissue culture, polymerase-chain-reaction (PCR) assays, and "suicide" PCR assays (this is a nested PCR whose name comes from the fact that the primers are used only once)<sup>13</sup> were performed if adequate blood samples or tissue specimens were available. Western blotting procedures were performed as previously described<sup>14</sup>; 20  $\mu$ g of *R. africae* antigen or *R. conorii* antigen was used per lane. Cross-adsorption assays<sup>15</sup> for serologic testing were performed as previously described.<sup>16</sup> We estimated the positive predictive value, specificity, and sensitivity of the microimmunofluorescence assay, Western blotting, and the cross-adsorption assay in 39 patients who had confirmed *R. africae* infection on the basis of cell-culture results, PCR assay, or both, by comparing their results with those of 50 patients with direct evidence of *R. conorii* infection contracted in France and 50 randomly chosen blood donors from Marseille. We considered any of the following to be definite serologic evidence of an *R. africae* infection: titers of IgG antibody and IgM antibody against *R. africae* antigen that were at least two serial dilutions higher than titers of IgG and IgM antibody against *R. conorii* (e.g., 1:64 vs. 1:16), a Western blot profile that showed only *R. africae*-specific antibodies, or cross-adsorption studies demonstrating that the homologous antibodies were directed against *R. africae*.<sup>17</sup> We then applied these serologic diagnostic criteria to serum samples from 376 additional patients who had returned from southern Africa and 2 who had returned from Guadeloupe and who had provided serum samples for the diagnosis of rickettsiosis.

The microorganism was isolated from skin-biopsy specimens and heparin-treated blood samples that were inoculated into shell vials, and the cultures were then cultivated as previously reported.<sup>9</sup> For the molecular detection and identification of *R. africae*, DNA was extracted from ground eschar-biopsy specimens, from the leukocyte layer in the sediment of EDTA-treated blood samples, or from 200  $\mu$ l of shell-vial supernatant with the QIAmp Tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations. The standard PCR assay of the DNA extracts was conducted as described previously.<sup>18</sup> In an effort to increase the sensitivity of DNA detection and to avoid false positive amplifications, we used a suicide PCR assay to test, when enough of the specimen or sample remained, all skin-biopsy specimens that were negative on the standard PCR assay and the serum sample obtained from each patient during the acute phase of the illness.<sup>13</sup> Three series of samples were tested successively with the use of three sets of two pairs of single-use primers that amplified nonoverlapping fragments of the gene encoding outer-membrane protein A (*ompA*), a gene present in all spotted-fever-group rickettsia. The first suicide PCR assay used the primers AF1F (5'CACTCGGTGTGCTGCA3') and AF1R (ATTAGTGCAGCATTCGTC3') and AF2F (GCTGCAGGAGCATTTAGTG3') and AF2R (5'TATCGGCAGGAGCATCAA3') for the nested amplification. The second used the primers AF3F (5'GGTGGTGGTAACGTAATC3') and AF3R (5'CGTCAGTTATTGTAACGGC3') and AF4F (5'GGAACAGT-TGCAGAAATCAA3') and AF4R (5'CTGCTACATTAATCCTAATA3') for the nested amplification. The third used the primers

AF5F (5'GTATAACATTACACGCTGG3') and AF5R (5'GCAA-GTGTTCCTATAGTTG3') and AF6F (5'TATAGATTTGGAG-CCAAGGA3') and AF6R (5'CCGTAAGTAACCTTTGTATAA3') for the nested amplification. DNA extracted from skin-biopsy specimens from a patient who had died of cancer and from serum specimens from blood donors were used as negative controls for PCR amplification from skin-biopsy specimens and serum samples from the study patients, respectively. One negative control was used for every seven samples. Testing was done in a blinded fashion. All positive PCR products were sequenced as previously described for the identification of the pathogenic rickettsial species.<sup>17,19</sup>

### Statistical Analysis

We used Fisher's exact test with Epi Info version 6.0<sup>20</sup> to compare the sensitivity of the suicide PCR assay and blood or eschar culture. Observed differences were considered to be significant when the resulting two-tailed P value was less than 0.05.

## RESULTS

### Laboratory Results

A total of 417 patients were tested for *R. africae* infection, and the diagnosis was established in 133, including 39 with direct evidence of *R. africae* on PCR assay, cell culture, or both and 94 with only a specific serologic response to *R. africae* on microimmunofluorescence assay. However, among the 417, 14 patients for whom epidemiologic and clinical information was missing were excluded from the study. On cell or tissue culture, *R. africae* was isolated from the blood of 2 of 18 patients who were tested and from the skin-biopsy specimens of 8 of 18 patients who were tested. A PCR assay carried out according to standard methods was positive in 1 of 8 blood samples that were tested and in 11 of 23 skin-biopsy specimens. All 15 skin-biopsy specimens that were evaluated with the suicide PCR assay were positive (including 7 that were negative on the standard PCR assay). Fragments of 330, 240, and 170 bp were obtained with use of the primer pairs AF2F and AF2R, AF4F and AF4R, and AF6F and AF6R, respectively. Of the 109 serum specimens tested with the use of this technique, 16 were positive. None of the negative controls were positive.

Amplification of DNA from skin-biopsy specimens with the use of the suicide PCR assay was significantly more sensitive than culture of skin-biopsy specimens (15 of 15 positive samples identified correctly, as compared with 7 of 17;  $P < 0.01$ ). However, suicide PCR assay of serum was not more sensitive than blood culture (16 of 109 positive samples identified correctly, as compared with 2 of 18;  $P = 0.50$ ). In every patient with positive results on either or both of the PCR assays, the sequence of the amplicon was 100 percent homologous to and specific for that of *R. africae*.

When we compared the 39 patients with confirmed *R. africae* infection with 50 patients with confirmed *R. conorii* infection and 50 blood donors, 10 of our patients with *R. africae* infection but none of the controls had levels of IgG antibody and IgM anti-

body against *R. africae* antigen that were higher than the IgG and IgM antibody levels against *R. conorii* by at least two dilutions (positive predictive value, 1.0; specificity, 1.0; and sensitivity, 0.26). Five of six patients with *R. africae* infection but none of the controls had an antibody response specific for *R. africae* infection on cross-adsorption testing (positive predictive value, 1.0; specificity, 1.0; and sensitivity, 0.83). Eighteen of 34 patients but none of the controls had antibodies specific for *R. africae* by Western blotting (positive predictive value, 1.0; specificity, 1.0; and sensitivity, 0.53) (Fig. 1). The remaining 16 patients had antibodies against both *R. africae* and *R. conorii*.

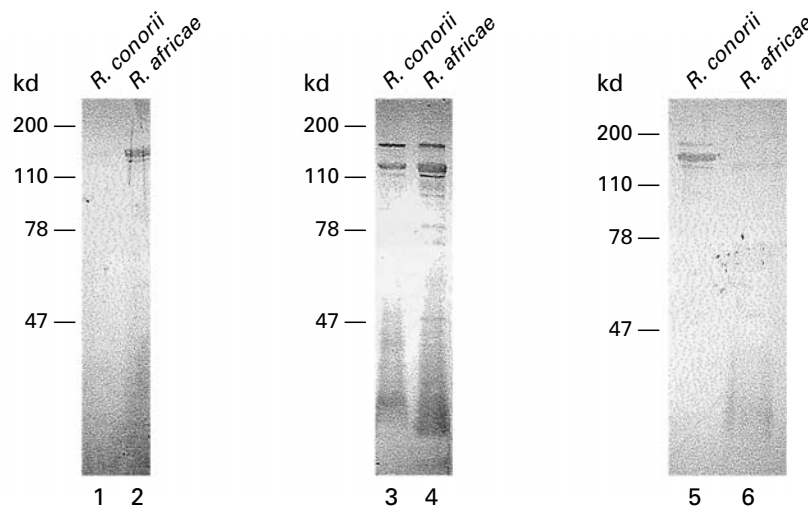
When we combined the results of the microimmunofluorescence assay, Western blotting, and cross-adsorption tests, the results of at least one of these three tests were positive in 22 of the 39 patients with confirmed infection (56 percent). When we applied these serologic tests to 378 other patients with potential *R. africae* infection, 81 patients for whom clinical information was available had positive results (Table 1). Serum samples obtained during the acute phase of the illness were available for 77 patients, whereas samples obtained during convalescence were available for 47. The microimmunofluorescence assay indicated that 68 patients were seropositive; titers of IgG antibody against *R. africae* antigen ranged from 0 to 1:32 and 0 to 1:2048 in samples obtained during the acute phase and convalescence, respec-

tively, whereas titers of IgM antibody ranged from 0 to 1:256 in samples obtained during the acute phase and from 0 to 1:1024 in samples obtained during convalescence. In 33 patients the levels of IgG and IgM antibody against *R. africae* antigen were higher than the levels of these antibodies against *R. conorii* antigen, and 46 had a specific antibody response against *R. africae* antigen on Western blotting. In an additional 33 patients, the presence of antibodies against both *R. africae* and *R. conorii* antigen prevented us from determining the causative pathogen.

By combining the results of the microimmunofluorescence assay and Western blotting, we obtained a specific diagnosis in 66 patients. In 14 patients for whom the levels of IgG and IgM could not be used to identify the infecting organism and for whom the results of Western blotting were negative but who had positive results on the microimmunofluorescence assay, the cross-adsorption assay confirmed the presence of an *R. africae* infection. Of the 46 patients with positive results on Western blotting, 13 had no detectable antibodies on the microimmunofluorescence assay. One additional patient who had returned from South Africa had *R. conorii* infection on the basis of serologic analysis after cross-adsorption assay (Table 1).

#### Epidemiologic and Clinical Findings

Of the 119 patients with *R. africae* infection, 106 (89 percent) were members of 21 tourist groups. In 88 cases (74 percent) the infections occurred in clus-



**Figure 1.** Results of Western Blot Assay of Serum from Three Patients.

A Western blot assay of the serum of a patient with epidemiologic and clinical features consistent with African tick-bite fever showed specific reactivity with the outer membrane proteins of *Rickettsia africae* (lane 2) but not with *R. conorii* antigen (lane 1). A microimmunofluorescence assay for antibodies against *R. africae* antigen was negative in this serum sample. A Western blot assay of the serum of a patient with culture-proved *R. africae* infection showed nonspecific reactivity with the outer membrane proteins and lipopolysaccharide of *R. conorii* (lane 3) and *R. africae* (lane 4). A Western blot assay of the serum of a patient with culture-proved *R. conorii* infection showed specific reactivity with the outer membrane proteins of *R. conorii* (lane 5) but not with *R. africae* antigen (lane 6).

**TABLE 1.** RESULTS FROM 80 PATIENTS WITH A SEROLOGIC DIAGNOSIS OF AFRICAN TICK-BITE FEVER AND 1 PATIENT WITH A SEROLOGIC DIAGNOSIS OF MEDITERRANEAN SPOTTED FEVER.\*

NO. OF PATIENTS	MICROIMMUNOFLUORESCENCE ASSAY FOR <i>R. AFRICAE</i>		TITERS OF IgG AND IgM ANTIBODY AGAINST <i>R. AFRICAE</i> ANTIGEN HIGHER THAN IgG AND THAN IgM ANTIBODY TITERS AGAINST <i>R. CONORII</i> ANTIGEN BY AT LEAST 2 DILUTIONS	WESTERN BLOTTING		CROSS-ADSORPTION ASSAY FOR SPECIFIC <i>R. AFRICAE</i> INFECTION
	ACUTE-PHASE SERUM SAMPLE	CONVALESCENT-PHASE SERUM SAMPLE		SPECIFIC FOR <i>R. AFRICAE</i>	SPECIFIC FOR <i>R. CONORII</i>	
2	+	+	+	-	-	ND
5	+	NA	+	-	-	ND
4	NA	+	+	-	-	ND
7	-	+	+	-	-	ND
1	+	+	+	+	-	ND
7	+	NA	+	+	-	ND
5	-	+	+	+	-	ND
3	+	+	-	+	-	ND
9	+	NA	-	+	-	ND
8	-	+	-	+	-	ND
13	-	NA	-	+	-	ND
14	-	+	-	-	-	+
2	-	+	+	ND	ND	ND
1	+	+	-	-	+	ND
<b>TOTAL</b>			<b>No./TOTAL No. (%)</b>			
81	68/81 (84)		33/81 (41)	46/79 (58)	1/79 (1)	14/14 (100)

\*The microimmunofluorescence assay was considered to be positive if the IgG antibody titer was at least 1:64 or the IgM antibody titer was at least 1:32 or if both criteria were met. The acute-phase serum samples were obtained within 15 days after the onset of symptoms, and the convalescent-phase samples were obtained more than 15 days after onset. Plus signs indicate positive results, and minus signs negative results. NA denotes not available, and ND not done.

ters. One hundred sixteen patients (97 percent) had traveled to 1 of 11 African countries (Table 2). Three patients acquired the disease outside Africa: two were infected in Guadeloupe, and the other was a young postdoctoral student in Great Britain who was bitten by an imported South African amblyomma tick that he was studying (Table 2). Seventy-four patients were male (62 percent), and 45 were female (38 percent). Their mean ( $\pm$ SD) age was  $44.6 \pm 15.7$  years (range, 15 to 77). Tick bites or handling of ticks was reported by 52 patients (44 percent) (Table 3), and we used this information to determine the mean incubation time of  $6.6 \pm 3.0$  days.

Symptoms at onset included fever in 105 patients (88 percent) and an influenza-like syndrome in 75 (63 percent). One hundred thirteen patients (95 percent) presented with inoculation eschars; 52 patients had a single eschar (46 percent), and 61 had multiple eschars (54 percent) (Fig. 2). Eschars were located on the arms in 12 patients (11 percent); the legs in 70 (62 percent); the chest, abdomen, or groin in 20 (18 percent); the back or the buttocks in 5 (4 percent), and the face, scalp, or neck in 6 (5 percent). Fifty-one of the 119 patients (43 percent) presented with regional lymphadenopathy. Fifty-five patients

**TABLE 2.** COUNTRY OF ORIGIN AND COUNTRY IN WHICH THE DISEASE WAS ACQUIRED IN THE CASE OF 119 PATIENTS WITH *RICKETTSIA AFRICAE* INFECTION.

COUNTRY OF ORIGIN	NO. OF PATIENTS	COUNTRY IN WHICH INFECTION OCCURRED	NO. OF PATIENTS
France	62	South Africa	71
Denmark	14	Swaziland	10
Norway	13	Lesotho	14
Italy	5	Zimbabwe	14
Great Britain	6	Botswana	1
Austria	12	Gambia	1
United States	3	Tanzania	1
Canada	2	Kenya	1
Switzerland	1	Gabon	1
Luxembourg	1	Central African Republic	1
		Côte d'Ivoire	1
		Great Britain*	1
		Guadeloupe	2

\*The tick was from South Africa.

**TABLE 3.** SIGNS AND SYMPTOMS OF PATIENTS WITH AFRICAN TICK-BITE FEVER, PATIENTS WITH MEDITERRANEAN SPOTTED FEVER, AND PATIENTS WITH ROCKY MOUNTAIN SPOTTED FEVER.\*

VARIABLE	AFRICAN TICK-BITE FEVER (N=119)	MEDITERRANEAN SPOTTED FEVER (N=199)	ROCKY MOUNTAIN SPOTTED FEVER (N=262)
Agent	<i>R. africae</i>	<i>R. conorii</i>	<i>R. rickettsii</i>
Main tick vector	Amblyomma species	Rhipicephalus species	Dermacentor species
Ratio of male patients to female patients	1.64	1.49	1.22
Mean age — yr	45	51	15
Reported tick bite or handling of tick — % (no.)	44 (52)	37	84
Mean incubation time — days	6.6	6	7
Temperature $\geq 38.5^{\circ}\text{C}$ — % (no.)	88 (105)	100	99
Inoculation eschar — % (no.)	95 (113)	72	Rare
Multiple eschars — % (no./total no.)	54 (61/113)	0	0
Location of eschar — % (no./total no.)			
Legs	62 (70/113)	36†	
Arms	11 (12/113)		
Chest, abdomen, or groin	18 (20/113)	36‡	
Back or buttocks	4 (5/113)		
Face, scalp, or neck	5 (6/113)	8	
Regional lymphadenopathy — % (no.)	43 (51)	NA	NA
Myalgia — % (no.)	63 (75)	36	83
Rash — % (no.)	46 (55)	97	88
Maculopapular — % (no./total no.)	51 (28/55)	90	39
Purpuric — % (no./total no.)	4 (2/55)	10	49
Vesicular — % (no./total no.)	45 (25/55)	0	0
Cases clustered — % (no.)	74 (88)	Rare	4–5
Deaths — % (no.)	0	2.5	4

\*Data on African tick-bite fever are from the current study, data on Mediterranean spotted fever are from Raoult et al.,<sup>2</sup> and data on Rocky Mountain spotted fever are from Helmick et al.<sup>21</sup> and Rotz et al.<sup>22</sup> NA denotes not available.

†The location of the eschar was listed as the limbs.

‡The location of the eschar was listed as the trunk.

(46 percent) had a rash; among these 55 patients the rash was maculopapular in 28 (51 percent), vesicular in 25 (45 percent), and purpuric in 2 (4 percent).

Information about treatment was available for 88 patients; 74 percent received doxycycline for a mean of  $6.3 \pm 3.1$  days (range, 7 to 15), 1 percent received minocycline, 6 percent received erythromycin, 3 percent received ciprofloxacin, and 16 percent received no antibiotic therapy. All patients recovered without any sequelae.

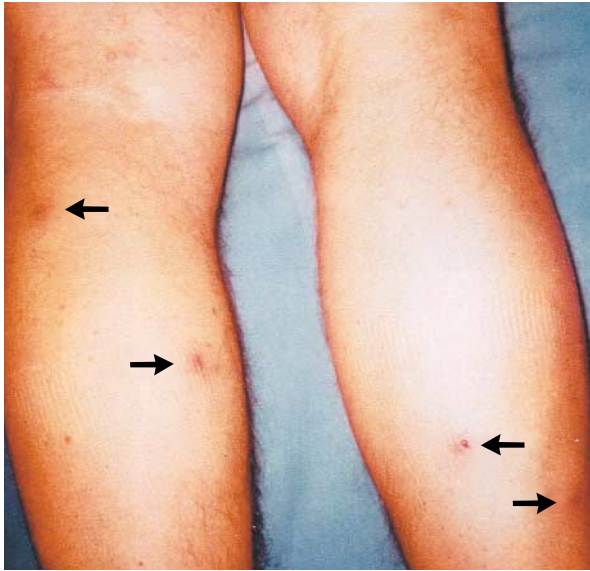
## DISCUSSION

African tick-bite fever is highly prevalent in Africa (seroprevalence, 30 to 56 percent),<sup>12</sup> and it is also an important disease among visitors to this region.<sup>23</sup> Most of the reported cases of African tick-bite fever have been part of outbreaks.<sup>4,7,24</sup> The rate of suspected *R. africae* infections among U.S. Army troops deployed to Botswana was 14 percent.<sup>25</sup> During an outbreak of *R. africae* infections among participants in an adventure race in South Africa, the estimated rate of infection was 3.9 to 7.6 percent.<sup>26</sup> In our series of 120 patients, 117 of whom had returned from a trip to Africa, who had been given a diagnosis of tick-bite–

related disease and for whom the infecting rickettsia could be identified, 119 were infected with *R. africae* and 1 with *R. conorii*.

Mediterranean spotted fever and Rocky mountain spotted fever are generally sporadic, and the vectors of these diseases are very host-specific.<sup>27</sup> In contrast, amblyomma ticks, which are parasites of cattle and wild ungulates and have been found to carry *R. africae* in all tested areas,<sup>12,28</sup> are not host-specific. They readily feed on humans, which may explain why cases of *R. africae* infection often occur as clusters and why patients often present with multiple inoculation eschars. On the basis of our data, African tick-bite fever appears to be acquired after travel in the countryside and through contact with ticks that parasitize cattle or wild animals, especially amblyomma ticks.

African tick-bite fever is characterized by an incubation period of six to seven days. Specific features include its tendency to occur in clusters, multiple inoculation eschars, regional lymphadenopathy, the frequent absence of a rash or the presence of a pale vesicular eruption, and the absence of complications. Such features are uncommon in patients with Mediterranean spotted fever and those with Rocky moun-



**Figure 2.** Four Inoculation Eschars (Arrows) on the Legs of a Patient Who Presented with African Tick-Bite Fever after Returning from a Safari in South Africa.

tain spotted fever (Table 3).<sup>21</sup> These potentially severe diseases most often occur as single cases after a bite by a dog tick, in particular rhipicephalus species or dermacentor species, or a wood tick.<sup>29</sup> Although clusters of cases of other tick-borne rickettsioses have been described,<sup>22</sup> clustering appears to be characteristic of cases of African tick-bite fever.

The usual method of diagnosing rickettsiosis is a microimmunofluorescence assay of a serum sample. However, serologic cross-reactions are common among rickettsiae in the spotted-fever group. The three serologic tests that we used were highly specific for and predictive of *R. africae* infection in patients with direct evidence of *R. africae* infection and allowed us to identify 80 patients as being infected with *R. africae* and 1 as being infected with *R. conorii*. A difference in specific IgG or IgM antibody titers has been reported to be useful for distinguishing *R. prowazekii* from *R. typhi* infections.<sup>30</sup> Such a difference was evident in 33 patients with African tick-bite fever, including 18 for whom the Western blot assay was not specific. We have previously demonstrated that the Western blot assay is positive earlier in the course of illness than is the microimmunofluorescence assay in patients with Mediterranean spotted fever.<sup>31</sup> Antibodies against high-molecular-weight proteins were detected by Western blotting in 13 patients with a negative microimmunofluorescence assay and were the only proof of infection in these patients. The cross-adsorption assay was informative in 47 per-

cent of the serum samples that were tested. Overall, the combination of these three serologic criteria confirmed the diagnosis in only 56 percent of the 39 with confirmed infection on the basis of a PCR assay, cell culture, or both. Therefore, some of the 284 patients for whom all serologic tests were negative may have been infected by *R. africae*, and the frequency of the disease may have been underestimated.

Since rickettsiae multiply at the site of inoculation, the eschar should be the preferred source of a biopsy specimen for isolation procedures or genomic detection. In our study, PCR-based methods, using *ompA*-derived primers,<sup>19</sup> were very sensitive and specific for both the detection and the identification of *R. africae* from skin-biopsy specimens. The suicide PCR assay appeared to be very efficient, since it allowed us to confirm the diagnosis in all 15 skin-biopsy specimens and in 16 of 109 serum samples that were tested, some of which were negative on the standard PCR assay. Most patients in our series were treated with 200 mg of doxycycline per day for 7 to 15 days. Given the benign nature of African tick-bite fever, this regimen could probably be shortened to a single day, as has been proposed for the treatment of Mediterranean spotted fever.<sup>32</sup> We found that the majority of cases of rickettsiosis among patients returning from sub-Saharan Africa are caused by *R. africae* and not by *R. conorii*.<sup>33</sup> Therefore, physicians need to recognize that African tick-bite fever is a disease of international importance.

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