

Rickettsia africae sp. nov., the Etiological Agent of African Tick Bite Fever

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We propose the name *Rickettsia africae* sp. nov. (with type strain Z9-Hu) for a distinct species of spotted fever group (SFG) rickettsiae that is the etiological agent of African tick bite fever in humans. This rickettsia has a distinct natural cycle and can be phenotypically distinguished from the other SFG rickettsiae by microimmunofluorescence serotyping, by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and by Western blotting (immunoblotting). Genotypic differences between *R. africae* and the other SFG rickettsiae can be demonstrated by PCR restriction fragment length polymorphism analysis, pulsed-field gel electrophoresis, and sequencing of the 16S rRNA gene.

Until recently, *Rickettsia conorii* was the only spotted fever group (SFG) rickettsia that had been reliably reported from Africa. Studies in our laboratories, however, have shown that there is another SFG rickettsia which is pathogenic in humans and ecologically, phenotypically, and genotypically distinct from *R. conorii* and the other SFG rickettsiae. In this paper we describe the characteristics of this organism and formally propose that it should be named *Rickettsia africae*.

Background. In the 1930s South African workers studied the relationship between tick bite fever, a disease described in southern Africa in 1911 (25, 27, 36), and fièvre boutonneuse, which had been described in North Africa a year earlier (5). On the basis of clinical and epidemiological data, Adrianus Pijper considered the two diseases distinct and probably caused by different etiologic agents (30–32, 39). He noted that while fièvre boutonneuse was a more severe disease characterized by a skin rash and contact with dog ticks, in particular *Rhipicephalus sanguineus* (6), tick bite fever was a mild disease that was not associated with complications, mortality, or skin rash and occurred following travel into the veld and contact with ticks of cattle and wild animals, in particular *Amblyomma* spp. Also, experiments performed with guinea pigs failed to demonstrate cross-immunity between the agents of tick bite fever and fièvre boutonneuse (31). However, the findings of Pijper were not confirmed by subsequent workers (9, 13, 14), and the epidemiological differences between tick bite fever and fièvre boutonneuse were explained as the result of age-related differences in behavior and susceptibility (8–11). Finally, the causative agent of fièvre boutonneuse, *R. conorii* Malish 7^T (= ATCC VR-613^T) (T = type strain), (4), was isolated in South Africa, and subsequently, all cases of tick bite fever have been considered cases caused by infection with *R. conorii* (12).

Recent reports from southern Africa have provided strong epidemiological evidence that *Amblyomma hebraeum*, a tick known to readily feed on humans (16, 26), is the major vector

of tick bite fever in the region. This tick has been implicated in outbreaks of the disease in Rhodesian troops during the Zimbabwe War of Independence (26) and in United States airborne troops deployed in Botswana (17). In Zimbabwe, *A. hebraeum* is most prevalent in rural areas in the south of the country, where it mainly parasitizes domestic and wild ungulates (26). The southern part of Zimbabwe is also the area that has the highest incidence of clinical cases of tick bite fever (22) and the area shown by serosurveys to have the highest incidence of SFG rickettsial infections in people (19, 22) and domestic ruminants (21, 23). It has been shown that a high percentage (72%) of *A. hebraeum* ticks are infected with rickettsia-like organisms (1), and feeding experiments performed with susceptible hosts have demonstrated that *A. hebraeum* can maintain SFG rickettsiae transtadially and transovarially and that each feeding stage can transmit the organisms (20, 23).

When six rickettsial isolates obtained from *A. hebraeum* ticks collected in widely separated areas of Zimbabwe and an isolate obtained from *Amblyomma variegatum* in Ethiopia (28) were compared by using microimmunofluorescence serotyping, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and Western blotting (immunoblotting), they were phenotypically indistinguishable from one another but distinct from the other SFG rickettsiae (18). As determined by microimmunofluorescence serotyping, the specificity differences (SPDs) between these seven isolates and the other SFG rickettsiae varied from 4 to 14. These SPDs were calculated by the usual method, as follows: $SPD = (Aa + Bb) - (Ab + Ba)$, where Aa or Bb is $-\log_2$ of the end point titer between serum A or B and homologous antigen a or b and Ab or Ba is $-\log_2$ of the end point titer of serum A or B against heterologous antigen b or a. If the SPD is <3 , two strains are assumed to be identical, and if the SPD is ≥ 3 , the strains are different (29). In addition, an isolate obtained from a patient in Zimbabwe who had a history of tick bites and signs of tick bite fever but no rash (24) was indistinguishable from isolates obtained from *Amblyomma* ticks as determined by the techniques mentioned above but was distinct from the other SFG rickettsiae. Therefore, the isolates obtained from *Amblyomma* ticks and the human isolate have been called the agent of African tick bite fever (AATBF) (18), and workers have obtained evidence that the

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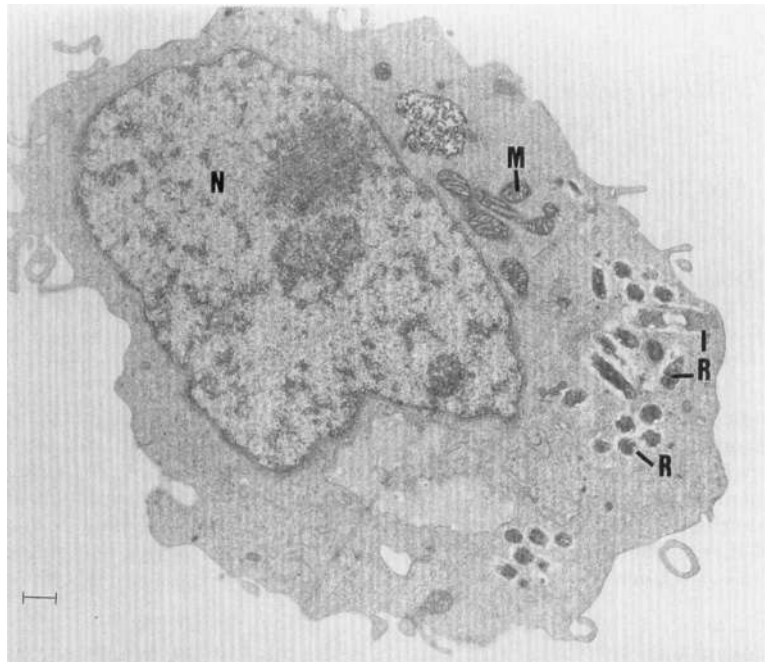


FIG. 1. Electron micrograph of *R. africae* infecting an L929 cell. R, rickettsia; M, mitochondrion; N, nucleus. Bar = 0.5 μ m.

organisms are infective (38) and pathogenic in humans (unpublished data).

There are also genotypic differences between the AATBF and the other SFG rickettsiae. When we performed a PCR and restriction fragment length polymorphism analysis in which primers for the 120- and 190-kDa surface protein genes of *Rickettsia rickettsii* and digestion with *RsaI* and *PstI* (33) were used, AATBF could be differentiated from all other SFG rickettsiae except *Rickettsia parkeri* (7). The average genome size of the AATBF (1,248 kb) was estimated by digesting the DNA with *EagI*, *SmaI*, and *BssHIII* and separating the resulting preparation by pulsed-field gel electrophoresis (PFGE), and the value obtained was consistent with the genome sizes of other SFG rickettsiae. However, the PFGE restriction profiles were different from those of the other SFG species (34). Moreover, the sizes of the digestion fragments on which the citrate synthase, the 120-kDa surface protein, and the 190-kDa surface protein genes were located were different from the sizes of the fragments obtained with the other SFG rickettsiae. A genetic relationship dendrogram established from the PFGE patterns obtained after *SmaI*, *EagI*, and *BssHIII* digestion by using the Dice coefficient, the unweighted pair group with mathematical average method, and the Philip package program shows that the estimated levels of genetic divergence between the AATBF and other organisms are as follows: 35% with the closest relative, *Rickettsia sibirica*; 46% with a cluster of species containing *R. parkeri*, *R. conorii*, and the Israeli spotted fever rickettsia; and 47 to 74% with all other SFG rickettsiae (34). Sequencing studies have shown that the AATBF has a 16S rRNA gene sequence that differs from the 16S rRNA gene sequences of all other SFG rickettsiae; the levels of homology range from 97.9% with *Rickettsia akari*, a relatively distantly related SFG species, to 99.6% with *R. parkeri* and 99.7% with *R. sibirica*, the closest relatives (35).

Justification for a new species. Currently, serological typing by microimmunofluorescence (29) is the standard method for determining taxonomic relationships between SFG rickettsiae

(2, 3, 40). Genomic species definitions of prokaryotic taxa are supposed to rely on DNA-DNA relatedness analysis data (43) rather than 16S rRNA sequence data, particularly when the levels of 16S ribosomal DNA similarity are 97% or higher (37). The levels of homology among SFG rickettsiae are more than 97.8% (35). However, criteria which have been established primarily by analyzing enterobacteria may not be suitable for the study of rickettsiae, which are strictly intracellular organisms characterized by small genomes (34) whose evolution is probably subjected to the different selective mechanisms inherent in their complex ecological and epidemiological cycles. Although the results of a DNA-DNA homology analysis indicated that the SFG rickettsiae belong to a single species (42), until the validity of such criteria for all eubacterial taxa is confirmed (41), a global approach based on phenotypic, immunological, genotypic, ecological, and clinical data seems to be more suitable for the definition of rickettsial species.

On the basis of the distinctive clinical, epidemiological, phenotypic, and genotypic features described above, and to differentiate between the two pathogenic SFG rickettsiae that have been found in Africa, we propose that the isolates that were obtained from *Amblyomma* spp. and a patient with a history of tick bites and clinical signs of tick bite fever should be considered a separate taxonomic species, for which we propose the name *Rickettsia africae*.

Description of *Rickettsia africae* sp. nov. *Rickettsia africae* (a'fri. cae. L. gen. n. *africae*, from Africa, the continent where the organism was isolated) is an obligately intracellular gram-negative bacterium. This organism can be grown in the yolk sacs of developing chicken embryos, which die 5 to 7 days following inoculation, and in L929, Vero, and human embryonic lung fibroblast cells. Plaque formation does not occur in infected Vero cells. Gimenez-stained (15) infected cells contain intracellular, rod-shaped organisms that are 0.3 to 0.5 by 0.9 to 1.6 μ m. Electron microscopy shows that the organisms occur free in the cytoplasm and have an outer slime layer and a trilaminar cell wall (Fig. 1).

R. africae can be isolated from humans with a history of tick bites and clinical signs consistent with African tick bite fever and from *A. hebraeum* and *A. variegatum*. Stage-to-stage and transovarial transmission of the organism has been demonstrated in *A. hebraeum*, and all feeding stages of this tick can transmit the organism to rabbits. Adults can transmit the organism to goats. In these species and in Swiss Webster and BALB/c mice, infections are subclinical, although antibodies to the organism are produced. At 4 to 6 days after intraperitoneal inoculation, guinea pigs may develop a mild fever (>39.5°C for 1 to 2 days), but they do not develop scrotal edema. In micro-immunofluorescence tests the SPDs between *R. africae* and the other SFG rickettsiae are >3 (the SPD between *R. africae* and *R. parkeri* is 7, and the SPD between *R. africae* and *R. sibirica* is 5.5). As determined by SDS-PAGE, *R. africae* produces three distinct high-molecular-weight protein bands, at 117, 135, and 138 kDa; the most prominent band is the 117-kDa band. Sera from mice inoculated with *R. africae* have antibodies that react with these bands. *R. africae* can be differentiated from all other SFG rickettsiae, except *R. parkeri* by the results of PCR restriction fragment length polymorphism analysis. When amplified rickettsial DNA derived from PCRs with primers Rr190.70p and Rr190.602n is digested with restriction endonuclease *RsaI*, 343- and 220-bp fragments are obtained (a 107-bp fragment and a double 223-bp band are obtained with *R. sibirica* [7]); restriction endonuclease *PstI* digests the DNA into of 267-, 124-, and 81-bp fragments. *RsaI* digestion of the products obtained from PCRs performed with primers BG 1-21 and BG 2-20 yields 163-, 133-, 102-, 88-, and 60-bp fragments.

As determined by PFGE, the estimated genome size of *R. africae* is 1,248 kDa. The PFGE profiles of the DNA of the organism obtained following digestion with *EagI*, *SmaI*, and *BssHII* are distinct from those of the other SFG rickettsiae. After digestion with *BssHII*, the profile of *R. africae* is characterized by five fragments, the *R. parkeri* profile contains four bands (one in common with *R. africae*), and the *R. sibirica* profile contains four bands (two in common with *R. africae*); after restriction with *EagI* the profiles of these three organisms contain 16 bands, 7 of which are shared by *R. africae* and *R. sibirica* and 8 of which are shared by *R. africae* and *R. parkeri*; after digestion with *SmaI* 8 of 25 fragments are shared by *R. africae* and *R. parkeri* and 18 fragments of 26 are shared by *R. africae* and *R. sibirica* (34). The bands on which the citrate synthase and 190- and 120-kDa surface protein genes are located are distinct from the bands on which these genes are located in the other SFG rickettsiae following PFGE of the digestion products of endonucleases *BssHII* and *SmaI*. *R. africae* has a 16S rRNA gene sequence which differs from the 16S rRNA gene sequences of the other SFG rickettsiae. Compared with *R. rickettsii*, *R. africae* has a TTT insertion at position 85/6 and an A mutation at position 78.

The illness. Previously, tick bite fever and fièvre boutonneuse have been considered different names for a single disease resulting from *R. conorii* infection. Therefore, the clinical features of these diseases have been considered to be the same (12). There is now strong evidence, however, that tick bite fever has a different etiological agent, and additional studies will be needed to determine more precisely the clinical features, prognosis, and therapy for the disease. The data available and our unpublished findings suggest that tick bite fever results from bites of infected *Amblyomma* spp. encountered during travel into rural areas. An eschar develops at the bite site, and regional lymphadenopathy, mild fever, and severe headache follow. The absence of a skin rash appears to be a characteristic feature of the disease.

Type strain. The type strain of *R. africae* is strain Z9-Hu, an isolate obtained from a person with tick bite fever; this strain has been deposited in the Collection of the World Health Organization Collaborative Center for Rickettsial Reference, Marseille, France.

Nucleotide sequence accession number. The 16S rRNA gene sequence of *R. africae* Z9-Hu^T has been deposited in the GenBank data library under accession number L36098.

We thank Guy Vestris for expert technical assistance.

Funding was provided by the Research Board of the University of Zimbabwe and the European Community-funded link between the Veterinary Faculties of the Universities of Zimbabwe and Utrecht.

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