M. Gagua,‡ Alexandre B. Predtechenski,§ Irina V. Tarasevich,¶ and Didier Raoult*†‡
*Université de la Méditerranée, Marseille, France; †Russian Academy of Medical Sciences, Moscow, Russia; ‡Moscow Municipal Disinfection Center, Moscow, Russia; and §Research Center of Virology, Russia

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Letters

Tick-Transmitted Infections in Transvaal: Consider *Rickettsia africae*

To the Editor: We report a case of African tick-bite fever (ATBF) in a 54-year-old French hunter returning to France on 21 April 1997, after a 15-day visit to Transvaal, South Africa. While
traveling in the veld, the hunter removed (but did not keep) two ticks from his left leg. Two days later, he observed eschars at the bite sites. Within 5 days, he had high fever (39.5°C) and headache and decided to fly back to France, where he was admitted to the Infectious Diseases Department in the Hotel Dieu Hospital in Clermont-Ferrand. The patient’s clinical symptoms were persistent fever, severe headache, and two inflammatory eschars on the left leg. Laboratory results were normal. On 22 April, an acute-phase serum sample and eschar biopsy were sent to our laboratory. The patient was treated with 200 mg per day doxycycline for 10 days. His symptoms resolved. A second serum sample was collected on 13 May.

Microimmunofluorescence was performed as previously described (1). Although the acute-phase serologic results were negative, the convalescent-phase serum exhibited anti–R. africæ and anti–R. conorii titers of 16 for immunoglobulin (Ig) G and 8 for IgM. Sera were adsorbed with R. conorii and R. africæ antigens (2), and serologic testing and Western blot analysis (1) were performed on the resultant supernatants. Cross-adsorption of the convalescent-phase serum caused the homologous and heterologous antibodies to disappear when adsorption was performed with R. africæ antigens; only homologous antibodies disappeared when adsorption was performed with R. conorii. Western immunoblot, performed with the same adsorbed serum, indicated R. africæ infection by demonstrating a specific reactivity pattern with R. africæ–specific antigens in the 110-kDa to 145-kDa region (2). An inoculation eschar biopsy specimen was injected into human embryonic lung fibroblasts, according to the centrifugation shell-vial technique (3). After 6 days’ incubation at 32°C, a Gimenez staining of methanol-fixed human embryonic lung fibroblasts showed rickettsialike bacilli. The strain was identified by direct immunofluorescence performed on the cells with an anti–R. africæ monoclonal antibody (4). Moreover, DNA was extracted from the ground eschar biopsy specimen and from 200 µL of shell-vial supernatant, by using a QIAMP Tissue kit (QIAGEN GmbH, Hilden, Germany), according to the manufacturer’s instructions. These extracts were used as templates with primers complementary to a portion of the coding sequence of the rOmpA encoding gene in a polymerase chain reaction (PCR) assay (5), and the base sequences of the resulting PCR products were determined (5). The sequence obtained by both methods was the same as the R. africæ sequence in Genbank (100% similarity).

Since first described in Africa in 1910, tick-transmitted rickettsioses have been imputed to a single rickettsial species, Rickettsia conorii, although two distinct clinical illnesses have been observed (6): an urban form in patients in contact with dogs and their ticks (Rhipicephalus spp.) characterized by fever, headache, myalgia, cutaneous rash, and a lesion at the site of the tick bite (7), and a rural form in patients in contact with cattle or game and their ticks (Amblyomma spp.) characterized by mild signs and frequent lack of rash (8).

Although R. conorii was initially isolated from Amblyomma cattle ticks in 1973, the first evidence of its pathogenic role in humans was seen in 1992 in a patient who, after a tick bite, had fever, an inoculation eschar, regional lymphadenopathy, but no cutaneous rash (9). Since then, an additional 20 cases of R. africæ–related infections have been reported in travelers returning from Zimbabwe and South Africa (2,10).

R. conorii has long been considered the only African spotted fever group rickettsia, responsible for both Mediterranean spotted fever and ATBF. Since the first case was described (9), most of the 20 reported cases of ATBF occurred as outbreaks (2,10) in Europeans returning from Zimbabwe and South Africa. The occurrence of concomitant ATBF cases is unusual since Mediterranean spotted fever is generally sporadic and is likely related to the biologic characteristics of the recognized vector of R. africæ, Amblyomma spp. ticks. While both are nonnidioculous ticks, Amblyomma spp. and Rhipicephalus spp. exhibit very different host-seeking behavior (11). Amblyomma spp. are ticks of cattle and wild ungulates, are not host-specific, and can readily feed on humans; they are “hunter ticks” and exhibit an “attack strategy” (in response to stimuli they specifically converge on nearby hosts). Rhipicephalus spp. are dog ticks and vectors of R. conorii; very host-specific, they exhibit an “ambush strategy” (they are passive and remain quiescent in their habitat until a vertebrate host passes). Up to 72% of A. hebraeum are infected with Rickettsia-like organisms, in particular R. africæ (12); Amblyomma spp. are widely distributed in rural
areas in sub-Saharan Africa (13) and prevalence of *A. hebraeum* ticks, incidence of ATBF cases, and prevalence of *R. africae* antibodies have been strongly linked (14). Rural Africans are also commonly infected with *R. africae*, usually at a young age (14). In Zimbabwe, Kelly et al. (15) demonstrated that 55% of the tested human sera had antibodies against *R. africae*.

ATBF usually has specific clinical features: shorter incubation period than for Mediterranean spotted fever, multiple inoculation eschars (related to the host-seeking behavior and host-specificity of *Amblyomma* spp. ticks, which are “attack ticks” [15]), regional lymphadenopathies, frequent lack of cutaneous rash or a pale vesicular eruption, and absence of complications (2). Although only 22 proven cases have been described so far (including the present case), ATBF has been recognized as a commonly encountered disease in southern Africa since 1900 (8,16). Epidemiologic and clinical features indicate that several cases previously diagnosed on the basis of serology results only as *R. conorii*-caused may have been caused by *R. africae*.

Given the serologic cross-reactivity among spotted fever group rickettsiae, microimmunofluorescence, the easiest serologic method, may not be sufficient for the etiologic diagnosis of a rickettsial spotted fever. A definitive diagnosis of ATBF requires either additional serologic procedures, such as cross-adsorption or Western blot, or the use of PCR or culture. As for PCR, rOmpA-amplification possesses sufficient sequence heterogeneity among the spotted fever group rickettsiae to be used as an identification tool (5). The centrifugation-shell vial-cell culture (3), used routinely in our laboratory, reliably isolates strictly intracellular bacteria, including rickettsia, from blood and tissue specimens, especially eschar biopsies (the specimen of choice for isolation procedures or genomic detection). We noted cross-reactions between *R. africae* and *R. conorii*. Cross-adsorption between anti-*R. africae* and anti-*R. conorii* antibodies and Western blots confirmed that the antibodies we detected were directed specifically at *R. africae*. Furthermore, both PCR and cell culture confirmed the diagnosis of *R. africae* infection.

ATBF appears to be an important emerging disease in visitors to rural areas of southern Africa. *R. africae* should be considered a potential pathogen in patients returning from such areas who have fever, headache, multiple inoculation eschars, or regional lymphadenopathy after a tick bite.

Pierre-Edouard Fournier,* Jean Beytout,† and Didier Raoult*

*Université de la Méditerranée, Marseille, France; and †Centre Hospitalier Régional Hotel Dieu, Clermont-Ferrand, France

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Extended-Spectrum Beta-Lactamase-Producing Salmonella Enteritidis in Trinidad and Tobago

To the Editor: Salmonella Enteritidis, a predominantly localized pathogen of the human gastrointestinal tract, can become invasive in very young, very old, malnourished, and immunocompromised patients. In recent years, S. Enteritidis has emerged as a major intestinal pathogen in Trinidad and Tobago (population 1.2 million); in 1997, S. Enteritidis caused 79 (66%) of 119 culture-confirmed salmonella infections, in contrast to 18 (18%) of 99, 48 (47%) of 102, and 107 (61%) of 178 in 1994, 1995, and 1996, respectively. Increased incidence of S. Enteritidis infections has been reported worldwide (1,2). Of 216 human S. Enteritidis isolates tested for antimicrobial susceptibility between 1994 and 1996 in Trinidad, none were resistant to cephalosporins, aminoglycosides, ampicillin, trimethoprim-sulphamethoxazole, chloramphenicol, and norfloxacin/ciprofloxacin by the Kirby-Bauer disk diffusion method, which uses the National Committee for Clinical Laboratory Standards (NCCLS) breakpoints (3).

Here we report an unusual isolate of S. Enteritidis resistant to all penicillins and cephalosporins—including third-generation cephalosporins, gentamicin, tobramycin, and trimethoprim-sulphamethoxazole—by the Kirby-Bauer disk diffusion method. Amoxicillin-clavulanate and piperacillin-tazobactam disks gave zone sizes of 15 mm and 19 mm, respectively, which are classified as intermediate in the NCCLS guidelines. This isolate was recovered from the blood culture of a febrile, nonneutropenic patient with multiple myeloma on two occasions 24 hours apart in March 1998. The isolate was sensitive only to ofloxacin and imipenem. Admitted to the hospital with compressed fracture of the spine for physiotherapy in December 1997, the patient had several febrile episodes and received several courses of multiple empirically prescribed antibiotics (cefotaxime, gentamicin, and piperacillin). The patient had not traveled abroad during the previous 6 months.

Because cephalosporin resistance in salmonellae has not been reported before in the Caribbean, we investigated the mechanism behind this third-generation cephalosporin resistance further. Using amoxicillin-clavulanate in combination with ceftazidime, ceftriaxone, and aztreonam, we performed the double disk synergy test to determine whether this strain was an extended-spectrum beta-lactamase producer as described elsewhere (3); augmentation of the zone at the junction of amoxicillin-clavulanate and aztreonam/ceftriaxone/ceftazidime zones confirmed that indeed it was.

In the past few years, third-generation cephalosporin resistance in S. Enteritidis has been described in Europe (4), the United States (5), Turkey (6), India (7,8), and Argentina (9). Few reports exist of extended-spectrum beta-lactamase–mediated third-generation cephalosporin resistance in Salmonella spp. To our knowledge, this is the first report of this type of resistance among S. Enteritidis in the Caribbean. This patient was treated with ciprofloxacin for 1 week; subsequent blood cultures were negative.

This unusual isolate highlights the need to establish an antimicrobial resistance surveillance network for Salmonella isolates, including S. Enteritidis, to monitor the trends and new types of resistance mechanisms in the Caribbean. An epidemiologic study of S. Enteritidis infections is being planned to describe the extent of the problem and to define risk factors and vehicles of human infections in three Caribbean countries, including Trinidad and Tobago.

B.P. Cherian,* Nicole Singh,* W. Charles,* and P. Prabhakar†
*Port of Spain General Hospital, Port of Spain, Trinidad; and †Caribbean Epidemiology Center (CAREC), Port of Spain, Trinidad

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