African Tick Bite Fever in a Taiwanese Traveler Returning from South Africa: Molecular and Serologic Studies

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Abstract. We report the first imported case of African tick bite fever (ATBF) in a patient from Taiwan who returned from a 10-day trip to South Africa. Diagnosis was confirmed by polymerase chain reaction (PCR) from eschar biopsies. Portions of rickettsial ompA (491 bp) and ompB (273 bp) genes were amplified and subsequent sequencing of PCR product showed its 100% identity with R. africae. Microimmunofluorescence (MIF) assay of patient’s serum on Days 14 and 46 after the onset of illness revealed IgG seroconversion when tested with spotted fever group (SFG) rickettsiae antigens, including R. africae. The patient clinically improved on the third day of 14-day treatment with a combination of ciprofloxacin and minocycline. Based on the patient’s travel history and chronology of clinical symptoms, we strongly suspect that the tick-biting event occurred in Kruger National Park.

INTRODUCTION

African tick bite fever (ATBF), caused by Rickettsia africae, is mainly diagnosed in travelers returning from sub-Saharan Africa and the French West Indies. ATBF typically occurs in clusters and has recently emerged as a common cause of acute febrile illness in international travelers to rural sub-Saharan Africa. A recent worldwide report showed the incidence of rickettsial infection of 5.6% in the group of travelers who developed acute febrile infection after coming back from sub-Saharan Africa. It is the second most frequent identified etiology (the first is malaria) for systemic febrile illness. R. africae is transmitted by hard ticks (Amblyomma hebraeum and A. variegatum), which are not host specific. Amblyomma ticks have been noted to attack different vertebrate hosts, including humans. They are abundant and aggressive, therefore, often occurs in clusters of humans and often results in multiple inoculation eschars. The disease is seasonal, so months associated with a high risk of ATBF include those between November and April in rural sub-Saharan Africa. Clinically, ATBF manifests as an acute febrile and influenza-like illness frequently accompanied with maculopapular rash (46%), regional lymphadenopathy (51%), and inoculation eschars (95%). Multiple eschars occur in 54% of cases. Symptoms of ATBF infection are usually mild, but the disease may be more severe in the elderly.

Although R. africae has been detected in Amblyomma ticks and patients from > 14 African countries, the reported case incidence rate of ATBF was only 60–80 per 100,000 patients each year in Zimbabwe. Although multiple cases of ATBF in autochthonous Africans and travelers from Europe, America, and Australia have been reported, no cases have been described for international travelers from Asia. It is possible that many Asian travelers returning from sub-Saharan Africa were infected with R. africae without recognition because of difficulty in serologic diagnosis to differentiate ATBF from other spotted fever group rickettsial diseases.

The diagnosis of ATBF is usually based on serology (MIF, Western blotting, and cross-adsorption assays), polymerase chain reaction (PCR), and isolation of etiologic agent. PCR is a specific and frequently applied tool widely used in clinical molecular laboratories. The successful isolation of pathogen’s DNA is often unpredictable or even impossible if acute phase blood is not available. Several studies reported lower sensitivity of whole blood samples compared with eschar specimens by PCR as shown in Table 1. Although culturing of rickettsiae from an eschar biopsy specimen has been shown to be effective, this technique is labor intensive, time-consuming (taking ~7–14 days for shell-vial centrifugation technique), and requires special equipment. Recently, the eschar biopsy had been successfully used for the immunohistochemical (IHC) staining as an early confirmation test in ATBF diagnosis. Similarly, eschar PCR has been suggested as a useful test for the diagnosis of rickettsioses even in patients with scrub typhus receiving the appropriate antibiotic therapy. Studies comparing immunofluorescence antibody test (IFA), the gold standard, and nested PCR among scrub typhus patients have shown a sensitivity and specificity of 86% and 100%, respectively, for eschar PCR.

Commercial antigen slides for the diagnosis of rickettsial infection are usually not species specific. Several studies have shown that cross-reactivity of rickettsiae of the spotted fever group (SFG) is very common. Therefore, serologic diagnosis for rickettsial diseases, although very helpful, cannot establish the exact Rickettsia species involved. However, it is very useful for epidemiologic studies. The strategy combining MIF assays and Western blot with a cross-adsorption assay was most efficient for the diagnosis of rickettsioses; however, these techniques are only available in reference laboratories and together show a sensitivity of only 56% compared with PCR. Serologic testing for ATBF using acute-phase serum samples is usually negative, and the diagnosis must be confirmed with a second convalescent-phase sample. The clinical usefulness of performing eschar PCR or culturing rickettsiae from eschars in ATBF patients, in which 95% of human cases appeared as inoculation eschars, remains to be evaluated.

Patients with ATBF, similar to those with other spotted fever rickettsioses, usually respond well to a short course of doxycycline (100 mg orally twice daily) treatment to prevent
Sensitivities between whole blood sample and eschar biopsy samples by different diagnostic methods within confirmed ATBF human cases

<table>
<thead>
<tr>
<th>Case no.</th>
<th>PCR (no. positive/no. test)</th>
<th>Culture (no. positive/no. test)</th>
<th>IHC (no. positive/no. test)</th>
<th>Antibiotics</th>
<th>Country of origin*</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1†</td>
<td>16/109</td>
<td>2/18</td>
<td>11+ (15)‡ /23+ (15)</td>
<td>8/18</td>
<td>–</td>
<td>Dc, Mc, Em, Cf</td>
</tr>
<tr>
<td>2</td>
<td>0/1§</td>
<td>–</td>
<td>–</td>
<td>1/1</td>
<td>Cf</td>
<td>United States (ZB)</td>
</tr>
<tr>
<td>3</td>
<td>0/5+1</td>
<td>0/5</td>
<td>3/8</td>
<td>4/8</td>
<td>Dc</td>
<td>France (SA)</td>
</tr>
<tr>
<td>4</td>
<td>0/6+1</td>
<td>0/6</td>
<td>–</td>
<td>6/6</td>
<td>NA</td>
<td>NA (SA)</td>
</tr>
<tr>
<td>5</td>
<td>1/9+1</td>
<td>0/9+1</td>
<td>1/1</td>
<td>1/1</td>
<td>–</td>
<td>Dc, Bl</td>
</tr>
<tr>
<td>6</td>
<td>0/1+1</td>
<td>1/1</td>
<td>–</td>
<td>1/1</td>
<td>–</td>
<td>Dc</td>
</tr>
<tr>
<td>7</td>
<td>0/1+1</td>
<td>1/1</td>
<td>–</td>
<td>1/1</td>
<td>–</td>
<td>Dc</td>
</tr>
<tr>
<td>8</td>
<td>0/1+1</td>
<td>1/1</td>
<td>–</td>
<td>1/1</td>
<td>–</td>
<td>Mc + Cp</td>
</tr>
<tr>
<td>Total</td>
<td>13.39 (17/127)</td>
<td>5.88 (2/34)</td>
<td>71.19 (42/59)</td>
<td>50.00 (18/36)</td>
<td>77.78 (7/9)</td>
<td>§</td>
</tr>
</tbody>
</table>

*Infection occurred in South Africa (SA), Zimbabwe (ZB), Swaziland (SZ), Botswana (BW), Namibia (NA), Lesotho (LS), Gambia (GM), Tanzania (TZ), Kenya (KE), Gabon (GA), Central African Republic (CF), Côte d’Ivoire (CI), and Guadeloupe (GP).
†The detailed course of antibiotics therapy was unknown.
§Bold characters mean the numbers of blood samples or eschar specimens were obtained after antibiotics administration.
¶This case.
– = not done; NA = not available. Bl = beta-lactam antibiotics; Cf = ciprofloxacin; Dc = doxycycline; Em = erythromycin; Mc = minocycline.

Clinical deterioration and potentially death. Effective antibiotic therapy, including doxycycline, erythromycin, ciprofloxacin, and minocycline, has been used in 88 cases of ATBF, which were diagnosed based on clinical information, and all patients recovered without any sequela. In vitro susceptibility tests using 13 antimicrobials showed that R. africae is susceptible to both tetracyclines and fluoroquinolones. The new fluoroquinolone compounds, including ciprofloxacin, pefloxacin, ofloxacin, and sparfloxacin, are also effective against spotted fever group rickettsiae. However, a prospective study of a cohort of 940 travelers to rural sub-equatorial Africa showed one case of treatment failure after 3 days of doxycycline and was successfully treated only after initiation of ciprofloxacin (500 mg twice daily) treatment.

Here we report a case of a patient with R. africae infection, as shown by both eschar PCR and MIF, after returning from South Africa. This case highlights the global health issue for international travelers and the need for specific diagnostics and pre-travel information on ATBF and other tick-borne diseases when traveling to rural sub-Saharan Africa.

**MATERIALS AND METHODS**

**Case history.** A 62-year-old Taiwanese woman had a 10-day trip to South Africa, including Johannesburg, Cape Town, Mossel Bay, Oudsthoorn, George Town, Knysna, Wilderness, Port Elizabeth, Sun City, Gold Reef City, Kruger National Park, and Pretoria in April 2006. An organized traveling history in South Africa is shown in Figure 1. She denied having been bitten by any arthropods or animals during her visit to South Africa. However, she did ride an ostrich to take pictures while visiting Oudsthoorn and walked on grassland during a 3-day stay in Kruger National Park.

One day before the onset of the disease she noted two pustular nodules surrounded by erythema on her left inner thigh and left shoulder. In the course of the infection they finally developed into typical eschars. The onset was with a low-grade fever but no myalgias or lymphadenopathy. Six days later, asymptomatic erythematous papules and vesicles were observed near the eschars, as described previously. The chronology of clinical symptoms and antibiotic treatment is shown in Figure 2.

The patient received oral cephalaxin for presumed furunculosis, but with no clinical response. Malarial infection was excluded by blood film analysis by the Centers for Disease Control (CDC), Taiwan. Cutaneous anthrax and ecthyma gangrenosum (Pseudomonas infection) were excluded by the laboratory tests of eschar biopsy specimens.

The patient clinically recovered within 2 days after receiving a combination of ciprofloxacin (500 mg: twice daily) and minocycline (100 mg; twice daily), and therapy was completed in a total of 14 days.

**Serologic and molecular diagnosis.** The patient’s serum samples on days 14 (3 days after minocycline and ciprofloxacin administration), 21, and 46 after the onset of illness were
obtained for serological testing. Immunofluorescence assays were performed using commercial antigens to detect antibodies against *R. conorii*, *R. rickettsii*, and *R. typhi* (Panbio, Columbia, MD) at the Taiwan CDC, according to the manufacturer’s recommendations. A 4-fold rise in titer to ≥1:64 in paired sera is considered to be diagnostic using PanBio commercial kit. The paired serum samples (Days 14 and 46) were also sent to Unité des Rickettsies, a WHO Collaborating Center for Rickettsial Reference and Research in Marseille, France. A multiplex-antigen MIF assay was used as previously described. In brief, a panel of antigens including *R. conorii*, *R. slovaica*, *R. helvetica*, *R. massiliae*, *R. aferca*, *R. aeschlimannii*, *R. mongolotimonae*, *R. israelii*, *R. typhi*, *R. felis*, *Coxiella burnetii*, *Francisella tularensis*, *Bartonella henselae*, *Anaplasma phagocytophilum*, and *Borrelia burgdorferi* were deposited on slides with a pen nib and air dried. After fixing the slides in methanol for 10 minutes, 2-fold dilutions of serum samples were tested. Binding of the sera was detected using fluorescein isothiocyanate (FITC)-conjugated goat anti-human immunoglobulin G+I+M (IgGAM; Jackson Immuno Research Laboratories, West Grove, PA) diluted 1:100 in phosphate-buffered saline containing 0.2% Evans blue (BioMerieux, Marcy-l’Etoile, France). Antibody titers against rickettsiae antigens ≥1:128 of IgG antibody or ≥1:32 of IgM antibody are usually considered significant.

The patient provided written informed consent for blood samples and skin biopsy. After the eschar biopsy specimen (~1 cm in diameter) was taken on Day 15 (4 days after minocycline and ciprofloxacin administration), and a sterile swab was used to immediately dry the wound and placed into a sterile capped tube. The tubes containing the eschar and swab were shipped on dry ice to the Taiwan CDC. For molecular detection and differentiation of rickettsial species, DNA was extracted from whole blood samples (Day 14), eschar biopsy specimens (Day 15), and the samples obtained from swabs (Day 15) using the QIAamp Tissue kit (Qiagen, Hilden, Germany) according to the manufacturer’s recommendations. Rickettsial PCR was performed with the outer membrane protein A (*ompA*) (Rr190.70p: 5'-ATG GCC AAT ATT TCT CCA AAA-3'; Rr190.60R: 5'-ATG GCA GCA TTC GCT CCC CCT-3') and outer membrane B (*ompB*) (F22: 5'-ATG GTR TAT GGG CWA AAC CTT TCT ATA-3'; R25: 5'-TAG MMT CGA AGA ATG AAC GCT GAC TTT-3') genes. PCR amplification was performed in 50-μL volumes according to the manufacturer’s suggested protocol (Qiagen). The reaction mixture contained 0.2 μmol/L of each of the *Rickettsia*-specific primer sets, 0.5 mmol/L dNTPs, 10× Qiagen PCR buffer, 5× Q-solution, and 2.5 U of *Taq* DNA polymerase (Qiagen). Specific fragments were amplified using the following conditions: 3 minutes at 94°C, 35 cycles of 1 minute at 94°C, 45 seconds at annealing temperature, and 1 minute at 72°C, and one cycle of 10 minutes at 72°C. The annealing temperatures for *ompA* and *ompB* primer pairs were 46°C and 55°C, respectively. Suicide nested PCR was used to evaluate the sensitivity of the eschar specimens using *ompA* primer pairs (outer primer pair: AF1F: 5'-CAC TCG GTG TTG CTG CA-3'; AF1R: 5'-ATT AGT GCA GCA TTC GCT C-3'), inner primer pair: AF2F: 5'-GCT GCA GGA GCA TTT AGT G-3'; AF2R: 5'-TAT CGG CAG GAG CAT CAA-3'). DNA extracted from cell culture of *R. japonica* and distilled water were used as the positive and negative controls, respectively. After electrophoresis, the gels were stained with ethidium bromide (EtBr), and amplicons were visualized with a UV transilluminator. PCR products were purified using the QIAquick Gel Extraction Kit (Qiagen). All PCR-positive amplicons were purified from agarose gel, cloned into pCR4-TOPO vector using the TOPO TA Cloning kit (Invitrogen, Carlsbad, CA), and sequenced twice in each direction to identify pathogenic rickettsial species.

**RESULTS**

PCR in whole blood samples obtained at Day 14 after the onset was negative. However, DNA extracted from the eschar biopsy and the samples obtained using cotton swabs were positive. DNA sequencing of PCR products showed that sequences of both *ompA* and *ompB* genes were completely identical to *R. aferca* (491/491 bp; accession no. EU622980) and (273/273 bp; accession no. AF123706), respectively. Suicide nested PCR also showed the expected fragment (330 bp) in both eschar and cotton swab samples.

Serologic tests showed increased antibody responses in IgG titers among Day 14 (IgM/IgG = 0/256), Day 21 (IgM/IgG = 32/2,048), and Day 46 (IgM/IgG = 16/256) serum samples using commercial slides (*R. conorii* or *R. rickettsii* as antigen). Further analyses performed in Unité des Rickettsies showed 2-fold higher antibody titers against *R. aferca* compared with *R. conorii* using serum samples at day 46 after onset. Antibody titers against *R. aferca* on Days 14 and 46 were ≤8/8 (IgM/IgG) and ≤16/≥256 (IgM/IgG), respectively. Serum from Day 46 showed cross-reactivity at an IgG titer ≥256 with *R. slovaica*, *R. helvetica*, *R. aeschlimannii*, and *R. israelii* and at an IgG titer of 128 with *R. massiliae*, *R. mongolotimonae*, and *R. felis*. We did not show antibodies against typhus group rickettsiae or other tick-borne disease antigens (*C. burnetii*, *F. tularensis*, *B. henselae*, *A. phagocytophilum*, and *B. burgdorferi*).

Both molecular and serologic assays provided strong proof of *R. aferca* as an etiologic agent of acute febrile illness in the patient. Epidemiologic, clinical, and laboratory data are also consistent with diagnosis.

**DISCUSSION**

To our knowledge, this is the first report of an imported ATBF case from South Africa to Asia (Taiwan). Previous
In conclusion, we emphasize that, with the increasing popularity of backpacking, safaris, game hunting, and adventure travel in South Africa, international travelers to endemic areas should be informed of the risk of ATBF and be prepared with personal protection. Although chemoprophylaxis for rickettsioses has been studied in guinea pigs infected with *Rickettsia rickettsii*, prescription of chemoprophylaxis before visiting high-risk areas is still being evaluated in clinical trials.46,20,41 Insect repellents serve as protection against ticks transmitting ATBF, and products containing > 20% diethyltoluamide have been proposed to provide substantial, but temporary, protection.42

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