

NOTES

Comparison of Immunofluorescence, Western Blotting, and Cross-Adsorption Assays for Diagnosis of African Tick Bite Fever

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In testing paired serum samples from 40 consecutive cases of African tick bite fever, we detected diagnostic antibodies against spotted fever group rickettsiae in 45% of the patients by immunofluorescence assay (IFA) and in 100% of the patients by Western blotting (WB) ($P < 0.01$). A specific diagnosis of *Rickettsia africae* infection could be established in 15% of the patients by IFA and in 73% of the patients by a combination of WB and cross-adsorption assays ($P < 0.01$).

African tick bite fever (ATBF) is a flu-like illness frequently accompanied by inoculation eschars, headache, and neck myalgia (7). The disease is caused by *Rickettsia africae*, a recently identified spotted fever group (SFG) rickettsia, and is transmitted by cattle ticks in large parts of rural sub-Saharan Africa (9). ATBF typically occurs in clusters and has recently emerged as a common cause of acute febrile illness in international visitors to the region (2, 4, 8, 13, 14). Serology is the most widely used microbiological method for diagnosing rickettsial infections. Several tests are available, but with the exception of immunofluorescence assay (IFA), Western blotting (WB), and cross-adsorption assay, most are not recommended (10, 11). IFA, which can detect immunoglobulin G (IgG) and IgM antibodies, has a sensitivity rate ranging from 84 to 100% (1, 6, 13, 15). Poor species specificity is a major drawback, but this may be circumvented by using a multiple-antigen IFA where reactions to several species can be compared directly (4, 13). WB detects both early occurring antibodies against nonspecific lipopolysaccharide (LPS) antigens and late occurring antibodies against specific protein antigens (SPAs) located in the rickettsial outer membrane (15). In cases where WB is unable to identify the causative species, a cross-adsorption assay followed by a second WB on the resulting supernatant may be conclusive (11).

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Eighty serum samples collected between days 1 and 45 after the onset of symptoms from 40 consecutive patients with ATBF were obtained from a prospective cohort study of tropical fevers in Norwegian safari travelers to rural sub-Saharan Africa in 1999 to 2001 (8). All cases met the epidemiological and clinical criteria for ATBF, including myalgia and fever commencing no later than 10 days after a safari, and 26 (65%) cases occurred in clusters. Twenty-nine (73%) patients were males, and the mean age was 38.3 years (range, 14 to 57 years). Nineteen (48%) patients were treated with doxycycline, one (3%) had a complicated course (reactive arthritis), and one (3%) was hospitalized.

A multiple-antigen IFA was performed as previously reported (4), using seven SFG rickettsial antigens: *R. africae* strain ESF-5, *R. conorii* strain seven (Malish) ATCC VR-613T, *Rickettsia sibirica mongolotimonae* strain HA-91T, *Rickettsia aeschlimannii* strain MC16T, *Rickettsia massiliae* strain Mtu1T, *Rickettsia akari* strain MK ATCC VR-148T, and *Rickettsia felis* strain URRWXC2 ATCC VR-1525. IgG titers of $\geq 1:64$ and/or IgM titers of $\geq 1:32$ against any of the tested species were considered diagnostic for SFG rickettsial infection. We considered IgG and IgM titers against *R. africae* that were at least 2 dilutions higher than to any of the other species as serological evidence of *R. africae* infection (9). Conversely, samples with smaller differences in titer dilutions were classified as undetermined SFG rickettsial infection.

WB procedures were performed as described elsewhere (12), using 20 μ l of a 1-mg/ml suspension of *R. africae*, *R. conorii*, or *R. aeschlimannii* antigen per lane. We considered the detection of reactions against the broad washboard-like SFG rickettsia LPS in the <60-kDa regions or reactions to SFG rickettsia SPAs in the 110- to 140-kDa region, or both, as

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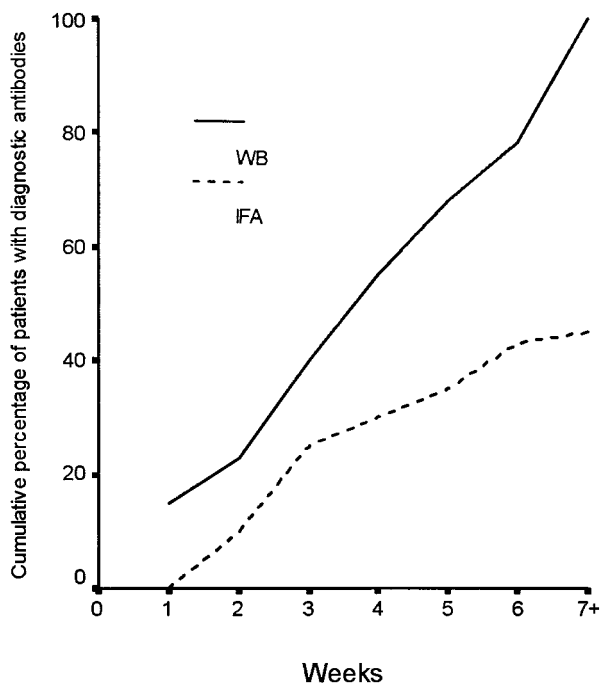


FIG. 1. Cumulative percentages of ATBF patients with diagnostic antibodies against SFG rickettsiae as detected by WB and IFA.

serological evidence of SFG rickettsial infection. The presence of antibodies against SPAs only in the 112- to 115-kDa range were considered diagnostic of *R. africae* infection, whereas demonstration of antibodies against SPAs of more than one

species or only against LPS were classified as undetermined SFG rickettsial infection.

The cross-adsorption assay using *R. africae* and *R. conorii* antigens and followed by WB on the resulting supernatant was performed as previously described (5). We considered assays where adsorption with *R. africae* antigen removed both homologous and heterologous antibodies and adsorption with *R. conorii* antigen removed homologous antibodies only as serological evidence of *R. africae* infection. If these criteria were not met, the result was classified as undetermined SFG rickettsial infection.

All 80 serum samples were examined with the seven-antigen IFA and the three-antigen WB assay. The cross-adsorption assay was performed on convalescent-phase sera from all cases classified as undetermined SFG rickettsial infection by IFA and WB and with IgG titers of >1:64 by IFA. All data were analyzed using a database software program (SPSS version 11.0; SPSS, Chicago, Ill.). Comparison of variables used the χ^2 test or Fisher's exact test where appropriate. Observed differences were considered significant when $P < 0.05$ for two-tailed tests.

Evidence of SFG rickettsial infection was detected in 18 (45%) patients by IFA and in 40 (100%) patients by WB ($P < 0.01$) (Fig. 1). All patients had WB reactions directed against SFG rickettsia SPAs, whereas 24 (60%) had antibodies against SFG rickettsia LPS. A specific diagnosis of *R. africae* infection was established by IFA in 6 (15%) patients and by WB in 22 (55%) patients ($P < 0.01$). Convalescent-phase samples obtained from 13 patients with undetermined SFG rickettsial infection were tested by the cross-adsorption assay, which confirmed *R. africae* infection in another seven cases (Fig. 2), resulting in identification of species-specific antibodies by WB in a total of 29 (73%) patients.

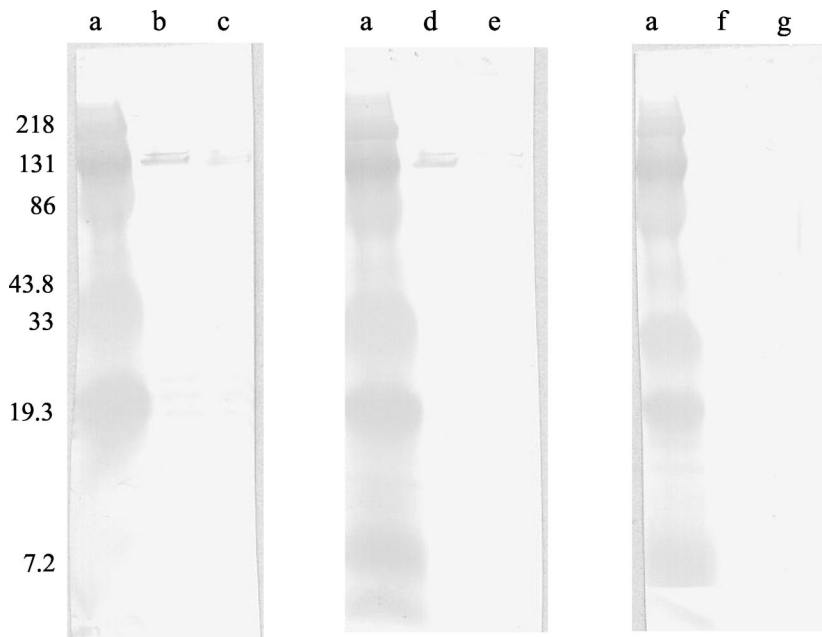


FIG. 2. Western immunoblotting of serum samples from a patient with ATBF before and after cross-adsorption assay with *R. conorii* or *R. africae*. Lanes: a, molecular mass markers; b, d, and f, *R. africae* antigen; c, e, and g, *R. conorii* antigen. Serum samples were either untreated (lanes b and c) or adsorbed with *R. conorii* (lanes d and e) or *R. africae* (lanes f and g). The positions of molecular mass markers (in kilodaltons) are shown to the left of the gel.

The present report constitutes one of the few serological evaluations of consecutive patients with rickettsioses. In contrast to most previous studies, which are retrospective and based on clinical samples submitted to reference laboratories (1, 6, 13, 15), our series of patients contains many cases with uncomplicated and mild presentation (e.g., not necessitating antibiotic therapy or hospitalization). With a sensitivity rate of only 45%, the present data indicate that IFA, the considered reference method in rickettsial infections (10) and a commercially available test used by laboratories worldwide, may miss many cases of ATBF. This limitation, which was also reported from an outbreak among U.S. soldiers deployed to Botswana (14), adds to other recently identified problems associated with IFA, including a poor antibody response in ATBF patients treated with doxycycline (3). In contrast, WB appears to be highly sensitive in evaluating consecutive cases of ATBF and detected antibodies against SFG rickettsia SPAs in all our patients. Thus, if serological diagnosis is regarded important in IFA-negative cases of assumed ATBF, serum samples could be shipped to a reference laboratory for WB. For unknown reasons and in contrast to other reports (11, 15), antibodies against SFG rickettsia LPS were detected in less than two-thirds of our patients. Although the antibodies were directed against nonspecific antigens shared by most rickettsial species, the demonstration of these antibodies is of diagnostic importance, since they are frequently the first serological evidence of a rickettsiosis (15).

A specific serological diagnosis of ATBF vis-à-vis other SFG rickettsioses is sometimes of clinical and epidemiological importance. Whereas the present seven-antigen IFA detected species-specific reactions in only a small proportion of our patients, WB established a diagnosis of *R. africae* infection in 55% of the cases, with a further increase to 73% if the cross-adsorption assay, a sophisticated serological tool designed to neutralize cross-reacting antibodies, was added (5). The major limitations with the cross-adsorption assay are the large amounts of antigen needed, high costs, and the fact that conclusive reactions are usually only seen in samples with pronounced immune response (e.g., with IgG titers of >1:64) (11).

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