CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, July 2000, p. 612–616 Vol. 7, No. 4
1071-412X/00/$04.00+0

Serological Differentiation of Murine Typhus and Epidemic Typhus Using Cross-Adsorption and Western Blotting

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Received 18 January 2000/Returned for modification 10 March 2000/Accepted 8 May 2000

Differentiation of murine typhus due to *Rickettsia typhi* and epidemic typhus due to *Rickettsia prowazekii* is critical epidemiologically but difficult serologically. Using serological, epidemiological, and clinical criteria, we selected sera from 264 patients with epidemic typhus and from 44 patients with murine typhus among the 29,188 tested sera in our bank. These sera cross-reacted extensively in indirect fluorescent antibody assays (IFAs) against *R. typhi* and *R. prowazekii*, as 42% of the sera from patients with epidemic typhus and 34% of the sera from patients with murine typhus exhibited immunoglobulin M (IgM) and/or IgG titers against the homologous antigen (*R. prowazekii* and *R. typhi*, respectively) that were more than one dilution higher than those against the heterologous antigen. Serum cross-adsorption studies and Western blotting were performed on sera from 12 selected patients, 5 with murine typhus, 5 with epidemic typhus, and 2 suffering from typhus of undetermined etiology. Differences in IFA titers against *R. typhi* and *R. prowazekii* allowed the identification of the etiological agent in 8 of 12 patients. Western blot studies enabled the identification of the etiological agent in six patients. When the results of IFA and Western blot studies were considered in combination, identification of the etiological agent was possible for 10 of 12 patients. Serum cross-adsorption studies enabled the differentiation of the etiological agent in all patients. Our study indicates that when used together, Western blotting and IFA are useful serological tools to differentiate between *R. prowazekii* and *R. typhi* exposures. While a cross-adsorption study is the definitive technique to differentiate between infections with these agents, it was necessary in only 2 of 12 cases (16.7%), and the high costs of such a study limit its use.

Epidemic typhus and murine typhus are arthropod-transmitted diseases caused by, respectively, *Rickettsia prowazekii* and *R. typhi*. The laboratory diagnoses of both diseases are based on serological reactions (24). Reactive antibodies in human sera cross-react extensively with species from the typhus group (18, 19). Recognition of the typhus agent is critical in cases of epidemic typhus, which is a body louse-transmitted disease (22, 33) responsible in the past for major epidemics and mortalities. The mortality rate for epidemic typhus is from 2 to 30%, whereas murine typhus is usually mild and, hence, mortality is uncommon. Due to the high epidemic potential of epidemic typhus, when a typhus case in a louse-harboring population is identified by serological testing, it must be rapidly differentiated from murine typhus, which is transmitted to humans by the rat flea *Xenopsylla cheopsis* (3–5), and public health networks must be notified. As an example, the outbreak of epidemic typhus that developed in Burundi in 1997 and ultimately affected 43,000 people (32) was preceded by a limited outbreak of the disease in a jail in that country (34) and, earlier, by a case involving a health care worker diagnosed in Switzerland (41). Moreover, in many countries, such as France, epidemic typhus is a disease for which public health authorities must be notified, whereas endemic typhus is not. The serological reference method is immunofluorescence analysis (29), but the Weil-Felix test (7, 26, 27), enzyme-linked immunosorbent assay (16), immunoperoxidase assay (21), latex agglutination test (17), dot blot assay (20), and Western immunoblotting (12) have also been used. Differentiation of etiological agents in the typhus group is difficult because differences in titer of less than one dilution are found in two-thirds of patients (32). In these patients, epidemiologic data may indicate the most likely etiological agent from a given biogroup. In areas where the etiological agents coexist, however, it may be impossible to make a definitive diagnosis by routine serological testing (28, 39).

The reference test used to avoid such cross-reactions is the cross-adsorption procedure (31, 37). A cross-adsorption study is performed by incubating serum from a patient with the bacterium known to cross-react in serological tests. Cross-adsorption results in the disappearance of homologous and heterologous antibodies when adsorption is performed with the bacterium causing the disease. When it is performed with the bacterium not causing the disease (but responsible for the cross-reaction), antibodies reactive to this bacterium disappear whereas antibodies reactive to the bacterium causing the disease remain detectable. Antigenic cross-reactivity is confirmed by Western immunoblotting after adsorption of sera with the cross-reacting antigens.

The purpose of the present work was to compare the reactivities of sera from patients with epidemic typhus or murine typhus, in the largest series of sample results published to date, in order to evaluate the different methods available today.

MATERIALS AND METHODS

Patients and sera. Our center, located in Marseille (southern France), is the National Reference Center for Rickettsioses. Over the last 5 years we have received 29,188 sera for serological testing for *R. prowazekii* and/or *R. typhi*, including sera from patients infected during several outbreaks of epidemic typhus in Russia (444 sera), Peru (227 sera), and Burundi (373 sera) that were investigated by our laboratory. From this serum bank we selected sera with immunoglobulin G (IgG) titers of ≥128 and/or IgM titers of ≥32 against *R. typhi*

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and/or *R. prowazekii* (12, 27). Sera from serologically positive patients who had a history of a recent febrile illness, louse infestation, or clinical signs compatible with epidemic typhus, which were obtained during an identified epidemic typhus outbreak, were considered *R. prowazekii* positive. Patients with positive serological results who came from an area where murine typhus, but not epidemic typhus, was known to occur and who had clinical signs compatible with murine typhus were regarded to have had murine typhus. Serologically positive patients whose epidemiological evidence or clinical signs indicated that they could have had either epidemic or murine typhus were regarded as suffering from typhus of undetermined etiology. Sera from patients for whom there was no clinical and/or epidemiological data and patients who had evidence of other diseases (including other rickettsial diseases) were excluded from the study. Sera from five patients determined to have had epidemic typhus and from five patients determined to have had murine typhus were randomly selected for cross-adsorption and Western blotting studies. Sera from the two patients found to have had typhus of undetermined etiology were also studied by these methods. We did not test more sera for these complementary studies because they require, considerable amounts of purified antigen, especially for the cross-adsorption study.

Serological procedures. (i) Antigen preparation. The reference strains *R. typhi* (Wilmington) and *R. prowazekii* (Breinl) (27) were grown in confluent monolayers of HEL cells in 150-cm^2 culture flasks containing minimal essential medium supplemented with 2 mM L-glutamine and 10% fetal bovine serum. Rickettsial infection was monitored by microscopic examination of Gimenez-stained cells scraped from the flasks (16). When a heavy rickettsial infection was seen, the supernatants of 15 flasks were pelleted by centrifugation (5,000 × *g*, 15 min) and resuspended in 1 ml of phosphate-buffered saline (PBS; pH 7.3) with 0.1% formaldehyde. Intact cells were fragmented by sonication, and cellular debris was removed by two successive centrifugations (100 × *g*, 10 min each). After the supernatants were centrifuged through 20 ml of PBS with 25% sucrose (6,000 × *g*, 30 min), the resulting pellet was washed three times in PBS (6,000 × *g*, 10 min) and, using spectrophotometry, was resuspended in sterile distilled water at a concentration of 2 mg/ml prior to being frozen at −20°C.

(ii) Microimmunofluorescence assay. For the initial screening of sera submitted to our laboratory, antigens were prepared as described above, applied with a dip pen nib to each well of 30-well microscope slides (Dynatech Laboratories Ltd., Billingshurst, United Kingdom), air dried, and fixed in acetone for 10 min. Each antigen was applied at different sites in each well so that sera could be tested against all antigens simultaneously. Sera were diluted 1:32, 1:64, and 1:128 in PBS containing 3% nonfat dry milk and applied to the antigens on the slides, which were then incubated in a moist chamber for 30 min at 37°C prior to undergoing three 10-min washes in PBS. After the slides were air dried, bound antibody was detected by using a fluorescein isothiocyanate-conjugated goat anti-human total Ig (Fluoline H; Biomerieux, Marcy l'Etoile, France) diluted 1:300 in PBS. Incubation, washing, and drying were performed as described above. The slides were mounted in buffered glycerol (Fluoprep; Biomerieux) and examined under a Zeiss epifluorescence microscope at 400× magnification. After this screening for total Igs, serial twofold dilutions from 1:32 to 1:2,048, and higher if needed, of positive sera were made and the titers of reactive IgG and IgM were determined as described above, using goat anti-human IgG (Fluoline G; Biomerieux) or IgM (Fluoline M; Biomerieux). To remove IgG, rheumatoid factor adsorbant (RF-absorbent; Behringwerke AG, Marburg, Germany) was used in accordance with the manufacturer's instructions prior to IgM determination.

(iii) Serum cross-adsorption. Two aliquots of the 12 selected sera (100 μl each) were each diluted 1:10 separately in an *R. typhi* antigen suspension previously adjusted to contain 2 mg of protein/ml and in a *R. prowazekii* antigen suspension previously adjusted to contain 2 mg of protein/ml and shaken for 24 h at room temperature. After centrifugation at 10,000 × *g* for 10 min, the supernatant was removed and treated twice again as described above. *R. typhi* and *R. prowazekii* indirect fluorescent antibody assays (IFAs) were performed on all supernatants after the final adsorption. Therefore, 2.7-ml volumes of *R. prowazekii* and *R. typhi* antigen suspensions containing 2 mg of protein/ml were used to adsorb each serum specimen.

(iv) Western blot analysis. One volume of the *R. typhi* or *R. prowazekii* antigen suspension containing 2 mg of protein/ml was mixed with 1 volume of 1× Laemmli solubilizer (23), and the mixture was incubated at room temperature for 2 h. Aliquots (10 μl) of the preparations were electrophoresed at 20 mA for 1 h through 12% polyacrylamide separating gels with 4% polyacrylamide stacking gels, using a Mini-Protean II cell apparatus (Bio-Rad, Richmond, Calif.). A mixture of low-range molecular mass standards (Bio-Rad) was used to estimate the molecular masses of the separated antigens. Resolved antigens were electroblotted in a transblot cell onto nitrocellulose membranes at 50 V and 4°C for 3 h. The blots were blocked overnight at room temperature with 5% nonfat dry milk in Tris-buffered saline (TBS; 20 mM Tris-HCl [pH 7.5], 500 mM NaCl, 0.1% Merthiolate) and washed with distilled water. Sera, diluted 1:200 in TBS–3% nonfat dry milk, were applied to the blots, which were then incubated overnight at room temperature. After three washes in TBS (10 min each), the blots were incubated for 1 h with peroxidase-conjugated goat anti-human IgG and IgM (Diagnostics Pasteur, Marnes-la-coquette, France) diluted 1:100 in TBS–3% nonfat dry milk. The blots were again washed three times in TBS, and bound conjugate was revealed by incubation with a solution consisting of 0.015% 4-chloro-1-naphthol and 0.015% hydrogen peroxide in TBS–16.7% methanol for 15 min. Blinded reading of the Western blots was carried out by four research workers in our laboratory who had previous experience in interpreting Western blots.

RESULTS

Results of serological tests and cross-reactions. Of 29,188 sera tested, 452 had significant titers of antibody to *R. typhi* and/or *R. prowazekii*. After removing multiple serum samples from the same patient as well as sera of patients with evidence of spotted fever group rickettsial infections and lower titers of antibody to typhus group rickettsiae, we retained sera from 308 patients with evidence of typhus infection. Of these, 264 and 64 were considered to be from patients with epidemic typhus and 44 were from persons with murine typhus. The sera from 263 epidemic-typhus patients were collected during an outbreak of the disease that occurred in 1993 following a civil war in Burundi (32) and from louse-infested people in Peru (30) and Russia (38). The 43 patients with murine typhus were travelers returning from areas where murine typhus is endemic (Greece, Cyprus, Crete, Malta, Egypt, Malaysia, Indonesia, and Thailand) but epidemic typhus is not. Two patients were classified as having typhus of undetermined etiology. One traveler returning from Ethiopia (Table 1, patient 11) was initially classified as having typhus of undetermined etiology, since both endemic typhus and epidemic typhus are prevalent in that country and this patient had mild disease and no history of louse infestation. Cross-adsorption of the serum from this patient (see below) showed that he had murine typhus. Another traveller, returning from Algeria (Table 1, patient 12), where epidemic typhus has not been described for 30 years, was classified as having had typhus of undetermined etiology because the patient presented with severe disease and an IgG antibody titer to *R. prowazekii* higher than that to *R. typhi*. Following the completion of the study, *R. prowazekii* was isolated from a blood sample from this patient by using the shell vial assay (6).

Sera from patients with epidemic typhus and from those with murine typhus cross-reacted extensively, as all sera showed significant titers of antibodies against both the homologous and heterologous antigens. Differences in titers between *R. typhi* and *R. prowazekii* antigens were easily determined, even when they differed by only one serum dilution, because both antigens were measured simultaneously on the same spot on the IFA slides. Most patients had the same titers of antibodies against *R. typhi* and *R. prowazekii* antigens, since of the 44 patients with murine typhus, 13 (29%) had IgG titers and 1 (2%) had an IgM titer which were more than a one dilution higher against *R. typhi* antigen than against *R. prowazekii* antigen. Fifteen (34%) differed by more than one dilution when both IgG and IgM titers were considered (at least more than one dilution in IgG or IgM, or at least one dilution in both). Of the 264 patients with epidemic typhus, 65 (24%) had IgG titers and 42 (15%) had IgM titers which were more than a one dilution higher against *R. prowazekii* antigen than against *R. typhi* antigen; 113 (42%) differed by more than one dilution when both IgG and IgM titers were considered.

Western blot analysis. Similar reaction profiles were observed for both epidemic-typhus patients and patients with murine typhus when their sera were reacted against the homologous antigens (Fig. 1). Two major groups of reactive antigens were observed: a strong reaction was detected against a 100-kDa antigen, and another pattern of reactivity was observed against several low-molecular-mass antigens (LMA) of from 17 to 50 kDa. This reactivity was stronger for antigens of less than 30 kDa and around 50 kDa. In Western blot analyses

TABLE 1. The IgG and IgM titers of sera from patients with murine typhus, epidemic typhus, or typhus of undetermined etiology

Patient no.	Diagnosis	Antibody titer obtained											
		Before adsorption				After *R. typhi* adsorption				After *R. prowazekii* adsorption			
		R. typhi		*R. prowazekii*		*R. typhi*		*R. prowazekii*		*R. typhi*		*R. prowazekii*	
		IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM
1	Murine typhus	512	1,024	256	2,048	<32	<32	<32	<32	256	32	<32	<32
2	Murine typhus	2,048	1,024	2,048	1,024	<32	<32	<32	<32	512	64	<32	<32
3	Murine typhus	256	512	128	128	<32	<32	<32	<32	256	<32	<32	<32
4	Murine typhus	256	256	128	128	<32	<32	<32	<32	64	<32	<32	<32
5	Murine typhus	2,048	1,024	512	512	<32	<32	<32	<32	256	64	<32	<32
6	Epidemic typhus	512	256	2,048	2,048	<32	<32	256	128	<32	<32	<32	<32
7	Epidemic typhus	64	32	128	512	<32	<32	128	<32	<32	<32	<32	<32
8	Epidemic typhus	256	512	1,024	1,024	<32	<32	512	<32	<32	<32	<32	<32
9	Epidemic typhus	256	32	256	32	<32	<32	128	<32	<32	<32	<32	<32
10	Epidemic typhus	256	1,024	1,024	2,048	<32	<32	256	128	<32	<32	<32	<32
11	Undetermined[a]	256	256	64	256	<32	<32	<32	<32	64	<32	<32	<32
12	Undetermined	2,048	128	4,096	128	<32	<32	512	<32	<32	<32	<32	<32

[a] Undetermined, diagnosis of typhus of undetermined etiology.

using heterologous antigens, the intensities of reactions against the LMA were essentially the same as those against the homologous LMA. The reactions against the heterologous 100-kDa antigen either were undetectable or were weaker than or indistinguishable from those against the homologous 100-kDa antigen (Fig. 1). When the 12 Western blots were interpreted in a blinded fashion by four members of the laboratory, the workers were unable to determine if the Western blot reactions for sera from six patients (patients 3, 7, 8, 9, 10, and 12) and were due to exposure to *R. prowazekii* or to *R. typhi*. They could, however, correctly determine the etiology of the infection for six sera. In contrast, with the same sera, but using a microimmunofluorescence assay, a difference of more than onefold dilution when both IgG and IgM titers were considered (at least 2 dilutions in IgG or IgM, or at least 1 dilution in both) was evident for 8 of 12 sera. Among the four undetermined remaining sera (patients 1, 2, 9, and 12), the causal agent in two could be identified by Western blotting. Therefore, when both IFA and Western blot results were considered, exposure to *R. prowazekii* or *R. typhi* was reliably determined for 10 of the 12 patients.

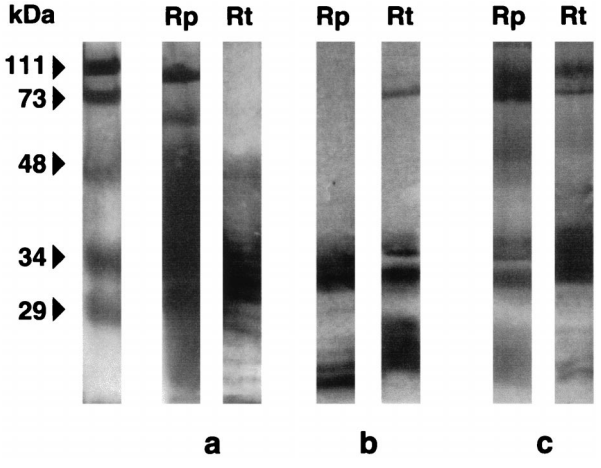

FIG. 1. Western immunoblot for distinguishing between *R. typhi* and *R. prowazekii* exposures. Shown are typical Western blot patterns of sera from a patient with epidemic typhus (patient 2 in Table 1) (a), a patient with murine typhus (patient 6) (b), and a patient with typhus of undetermined etiology (patient 12) (c). Molecular weight markers were run in the lane on the left. Rp, *R. prowazekii* antigen; Rt, *R. typhi* antigen.

Serum adsorption studies. When the sera from the five patients with murine typhus and the five patients with epidemic typhus were adsorbed with their homologous antigens (*R. typhi* and *R. prowazekii*, respectively), IgG and IgM antibodies against either antigen were no longer detected in any sera by IFA. When these sera were adsorbed with heterologous antigens, IgG and IgM antibodies against these heterologous antigens were longer detected by IFA. In IFAs against homologous antigens, however, reactive IgG were still detected in all sera and reactive IgM was still detected in 5 of the 10 sera. Following the adsorption of the serum from one patient with typhus of undetermined etiology (patient 11) against *R. typhi*, IgG and IgM antibodies against both *R. typhi* and *R. prowazekii* were no longer detected by IFA. When this serum was adsorbed with *R. prowazekii*, IgG and IgM antibodies against *R. prowazekii* were no longer detected but the IgG titer against *R. typhi* was 64. We concluded then that this patient was suffering from murine typhus. When the serum from the other patient with typhus of undetermined etiology (patient 12) was adsorbed with *R. prowazekii*, IgG and IgM antibodies against *R. typhi* and *R. prowazekii* were no longer detected, while when the sera was adsorbed with *R. typhi*, IgG against *R. prowazekii* was still detected at a dilution of 1:512. We concluded, therefore, that this patient was suffering from epidemic typhus, and this was subsequently confirmed when we isolated *R. prowazekii* from the patient's blood (6). Adsorption studies then enabled us to determine the etiological agent of the infection for each patient.

DISCUSSION

We report here the first systematic study of cross-reactions among the typhus group in a large series. Despite recent developments in cell culture and antigen and molecular detection methods for the diagnosis of rickettsial diseases (24), serological assays remain the simplest diagnostic tests to perform. Furthermore, sera can be readily sent to a reference laboratory for serological testing, even on filter paper (13). Serological cross-reactions between rickettsiae and other bacteria—for example, *Proteus* in the Weil-Felix test (7, 40)—have long been used for the diagnosis of rickettsial diseases. The development of techniques for growing rickettsiae has enabled the replace-

ment of the Weil-Felix test, which lacks both sensitivity and specificity, by more reliable tests; these include an enzyme-linked immunosorbent assay (16), a complement fixation test (36), an immunoperoxidase assay (21), a latex agglutination test (17), and an IFA which has now become the most commonly used test (29). As observed in our study, human sera against members of the typhus biogroup cross-react extensively in IFAs, and it is difficult to use differences in antibody titers against the two organisms to determine the species to which the patients had been exposed. The occurrence of cross-reacting antibodies in sera from typhus patients tested by IFA was described as early as 1959 by Goldwasser and Shepard (15). While some authors maintain that differences in IFA titers and staining patterns allow the differentiation of the diseases (27, 29), others have concluded that this is not possible because these differences are generally insignificant (15, 17). Halle et al. (16) demonstrated the efficacy of the enzyme-linked immunosorbent assay in detecting antibodies against *R. typhi* and *R. prowazekii* and noted differences in the species specificities of the reactive antibodies. Although this variation was higher than that observed in complement fixation tests (36), they were unable to reliably differentiate between sera from epidemic typhus patients and sera from murine typhus patients (16). A latex test for the detection of antibodies to murine and epidemic typhus rickettsiae and a commercial enzyme immunoassay for the detection of antibody to *R. typhi* were also unable to differentiate between sera from epidemic typhus patients and those from murine typhus patients (17, 20).

Two major groups of antigens are involved in the serological response to typhus group rickettsiae. The first is a heat-labile, species-specific surface protein antigen (SPA) which appears in Western blots as a 100-kDa antigen and is identified as rOmpB (14). Antibodies against this SPA have been shown to have a protective effect against infections with typhus group rickettsiae (8). When the antigen is boiled before Western blotting, the 100-kDa antigen is modified and appears as a 135-kDa protein with exposed group-specific epitopes (12). The second group of antigens comprises proteinase K-resistant low-molecular-mass antigens related to lipopolysaccharides (8, 9, 11, 12). They are mostly, but not exclusively, responsible for the observed cross-reactivity. As observed for the Western blots prepared with heterologous antigens in our study, the specificity of human antisera to SPA is lower than that observed with antisera from mice (10), and this is consistent with the findings of previous studies using enzyme-linked immunosorbent assays (16).

The differentiation of *R. typhi* and *R. prowazekii* infections is very important in cases in which dissemination of epidemic typhus is possible—for example, when cases appear in a louse-infested population or in people returning from such an area, especially health care workers (41). When a *R. prowazekii*-positive serological result is confirmed in such populations, the World Health Organization must be alerted and measures to control the epidemic must be immediately instituted. Moreover, in many countries, epidemic typhus cases must be reported to the proper public health agency. Such control measures can theoretically be based on delousing the affected population and implementing extensive use of antibiotics, in particular doxycycline (32). In this study, we were able to determine that the sporadic case observed in Algeria, where no case of epidemic typhus had been diagnosed in the previous 30 years, was epidemic typhus and that the case from Ethiopia, where epidemic typhus is prevalent, was in fact murine typhus.

As demonstrated in our study, cross-adsorption studies of sera are the most effective technique to differentiate between cases of epidemic typhus and cases of endemic typhus as they allow the diagnosis of all cases. For all 12 sera that we tested by this method, we were able to correctly differentiate between exposure to *R. typhi* and exposure to *R. prowazekii*. The technique was first described by Goldwasser and Shepard, who demonstrated that it was a reliable means of differentiating between sera from patients with epidemic typhus and those from persons with murine typhus (15). In their study, only one serum specimen gave an unexpected result; a serum sample from a patient with murine typhus reacted as if it had come from an epidemic-typhus patient. This serum was classified as coming from a patient with murine typhus because it was from a patient in the United States, a country in which epidemic typhus was not known to occur at that time. Twenty years later, however, epidemic typhus was shown to occur in the United States (1, 2, 25, 35), and it would appear, then, that Goldwasser and Shepard provided the first evidence of an indigenous case of epidemic typhus in the United States. A major drawback of cross-adsorption studies is that they require relatively large amounts of serum, more than what is routinely submitted for serological testing. Moreover, large amounts of purified antigens (2.7-ml volumes of *R. prowazekii* and *R. typhi* antigen suspensions containing 2 mg of protein/ml) are required for the studies. Based on the price of commercially available antigens for IFA in France, the cost of performing a cross-adsorption study of a serum specimen would be about $600. Cross-adsorption studies would appear, then, to be restricted to laboratories with facilities for safe culturing of rickettsiae and to be limited to the study of only a few sera from a population in which an outbreak of epidemic typhus is suspected. In fact, we now need to develop a micromethod for cross-adsorption in order to reduce the amount of antigen needed.

In our study, Western blotting was found to be reliable in differentiating between *R. typhi* and *R. prowazekii* infections in half of the 12 patients tested (sensitivity, 50%). When more than one-dilution differences in anti-*R. typhi* and anti-*R. prowazekii* IgG and IgM titers were considered, IFAs enabled the differentiation of the etiological agent in two-thirds of the selected sera (sensitivity, 66%). However, in the general population, 34% (murine typhus) and 42% (epidemic typhus) of the patients were identified by this technique. When the results of both IFA and Western blotting were considered, it was possible to correctly determine the etiological agent of the typhus infection in 10 of the 12 selected patients (sensitivity, 83%). Note that in our study we were able to reliably detect onefold differences in titers of antibodies against *R. typhi* and *R. prowazekii*. This was possible because both antigens were present in each well of our IFA slide, and the reactivities of the sera at different dilutions against each antigen could therefore be read simultaneously. In commercially available IFA slides, there is only a single antigen in each well and, hence, the simultaneous reading of IFA titers is therefore not possible.

The results of our study indicate that when an outbreak of a disease resembling epidemic typhus occurs, an IFA serological test should be performed to determine if specific antibodies to the typhus group rickettsiae are present in the population. If these antibodies are found to be present, differentiation between epidemic typhus and endemic typhus should be carried out by considering differences in IgG and IgM titers against *R. typhi* and *R. prowazekii*. The sensitivity of differentiating between exposure to these organisms will be increased if Western blot assays are also performed. When the results of these studies are inconclusive, cross-adsorption studies should be carried out to provide a definitive diagnosis. The major limitation will be the fact that outbreaks of typhus usually occur in countries where techniques such as IFAs and Western blotting

procedures are not available. Nevertheless, patients' sera can be sampled onto blotting paper as previously described (13), and the samples can be sent by mail to a reference laboratory. However, this technique allows only the performance of an IFA because the serum sample is too small for the additional Western blotting or cross-adsorption analysis. A cross-adsorption study is highly contributive to differentiation and should be used in cases in which an etiological diagnosis is needed for a single patient, as in the case of the *R. prowazekii*-infected health care worker, returning to Switzerland from Burundi, who was diagnosed too late to prevent death (41). It is likely that the immediate recognition of this sentinel case would have limited the gigantic outbreak that later involved more than 43,000 people in Burundi.

ACKNOWLEDGMENTS

We are indebted to M. Drancourt, P. E. Fournier, P. Brouqui, and V. Roux for interpretation of Western blots and to P. Kelly for reviewing the manuscript.

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