Journal of Clinical Microbiology, Nov. 1997, p. 2715–2727 Vol. 35, No. 11
0095-1137/97/$04.00+0

MINIREVIEW

Laboratory Diagnosis of Rickettsioses: Current Approaches to Diagnosis of Old and New Rickettsial Diseases

BERNARD LA SCOLA and DIDIER RAOULT*

Unité des Rickettsies, UPRESA 6020, Faculté de Médecine, Université de la Méditerrannée, 13385 Marseille Cedex 05, France

INTRODUCTION

Members of the genera *Rickettsia* and *Orientia* are morphologically and biochemically similar to other gram-negative bacteria. They are, however, fastidious bacterial organisms that are obligate intracellular parasites. Although rickettsial species are arthropod-associated bacteria, they are also frequently capable of infecting vertebrates, including humans, usually as accidental hosts. They are short, rod-shaped, or coccobacillary organisms, usually 0.8 to 2.0 μm long and 0.3 to 0.5 μm in diameter. The order *Rickettsiales* has historically been divided into three families: *Rickettsiaceae*, *Bartonellaceae*, and *Anaplasmataceae*. Rickettsiae belong to the *Rickettsiae* tribe within the family *Rickettsiaceae* (180) and have long been subdivided into three genera: *Coxiella*, *Rickettsia*, and *Rochalimaea*. The advent of 16S rRNA gene analysis has enabled the determination of phylogenetic relationships among members of the order *Rickettsiales* (Fig. 1). *Coxiella burnetii* has been shown to be quite distinct from other rickettsiae, lying in the γ subgroup of *Proteobacteria*, whereas *Rickettsia* belongs to the α1 subgroup (178). Furthermore, the genus *Rochalimaea* has recently been united with the genus *Bartonella*, and the unified genus has been removed from the order *Rickettsiales* because phylogenetically, its members are in the α2 subgroup of the *Proteobacteria* (26). The species of the genus *Rickettsia* have been subdivided into three groups of antigenically related microorganisms, namely, the spotted fever, typhus, and scrub typhus groups (Table 1). This minireview is restricted to these organisms. Phylogenetic data have recently been used to support the reclassification of the agent of scrub typhus into a new genus, because it belongs to a unique and distinct clade within the rickettsia radius. *Rickettsia tsutsugamushi* is therefore now named *Orientia tsutsugamushi* (156). Rickettsiae are transmitted to humans by infected arthropod bites or feces. Several rickettsiae are considered to be nonpathogenic in humans because they have been isolated only from arthropods. This opinion may well be contradicted in the future, as in the case of *Rickettsia africae*, which was first isolated from ticks and subsequently from a patient's blood. We believe that every rickettsial species may have pathogenic potential, provided that its reservoir arthropod is capable of biting humans. The main symptoms of infection consist of fever and headache. Cutaneous eruption, which is sometimes associated with inoculation eschar, is reported in most cases. The pathogenesis of these diseases is vasculitis caused by the proliferation of organisms in the endothelial lining of small arteries, veins, and capillaries. New culture isolation techniques with shell vials have led to the isolation of an increasing number of strains over recent years. Such isolation is a prerequisite for the characterization of a new species and for the delineation of new rickettsial diseases, since serologic testing of sera from infected patients is unable to distinguish between different rickettsial species. Prior to 1984, only six spotted fever group rickettsioses were recognized, whereas in the last 12 years a further seven have been reported, including six since 1991. These new emerging diseases, with unique clinical manifestations and epidemiologic conditions (Table 2), include Japanese spotted fever due to *Rickettsia japonica*, first reported in 1984 (90); Flinders island spotted fever caused by *Rickettsia honei*, described in 1991 (147); Astrakhan spotted fever, reported in 1991 (159); African tick bite fever caused by *R. africae*, described in 1992 (85); the pseudotyphus of California due to *Rickettsia felis*, described in a patient in 1994 (78); and two new spotted fevers, the first due to "*Rickettsia mongolotimonae*," reported in 1996 (121), and the other due to *Rickettsia slovaca*, reported in 1997 (120). We suspect that infections due to several spotted fever group (SFG) rickettsiae, at present classified as nonhuman pathogens (Table 1), will increase the size of this group of new emerging diseases. Isolation of etiologic agents also allows for the recognition of rickettsial diseases in regions where they were not previously identified (antibodies against "new" rickettsiae are likely to cross-react with antigens from strains from areas where rickettsial diseases are endemic), as demonstrated by the isolation of *Rickettsia akari* in Croatia, an area where Mediterranean spotted fever (MSF) is endemic (117). Furthermore, the development of PCR amplification-based approaches and techniques for the analysis of amplified fragments, especially automatic DNA sequencing, allows convenient and rapid identification of rickettsiae, even in nonreference laboratories. To date, the diagnosis of a rickettsial illness has most often been confirmed by serologic testing. Serologic evidence of infection occurs no earlier than the second week of illness for any of the rickettsial diseases; thus, a specific diagnosis may not be available until after the patient has recovered or died. Severe forms of MSF and Rocky Mountain spotted fever (RMSF), with high mortality rates, have been described in patients with underlying infections such as diabetes mellitus, alcoholism, chronic liver diseases, or glucose-6-phosphate dehydrogenase deficiency (130, 167). Higher mortality rates are also correlated with delays in consulting a physician and delays in the administration of appropriate antibiotic therapy. In order to reduce the delay in diagnosis, new

* Corresponding author. Mailing address: Unité des Rickettsies, UPRESA 6020, Faculté de Médecine, Université de la Méditerrannée, 27 Boulevard Jean Moulin, 13385 Marseille Cedex 05, France. Phone: 33.91.38.55.17. Fax: 33.91.83.03.90. E-mail: Didier.Raoult@medecine.univ-mrs.fr.

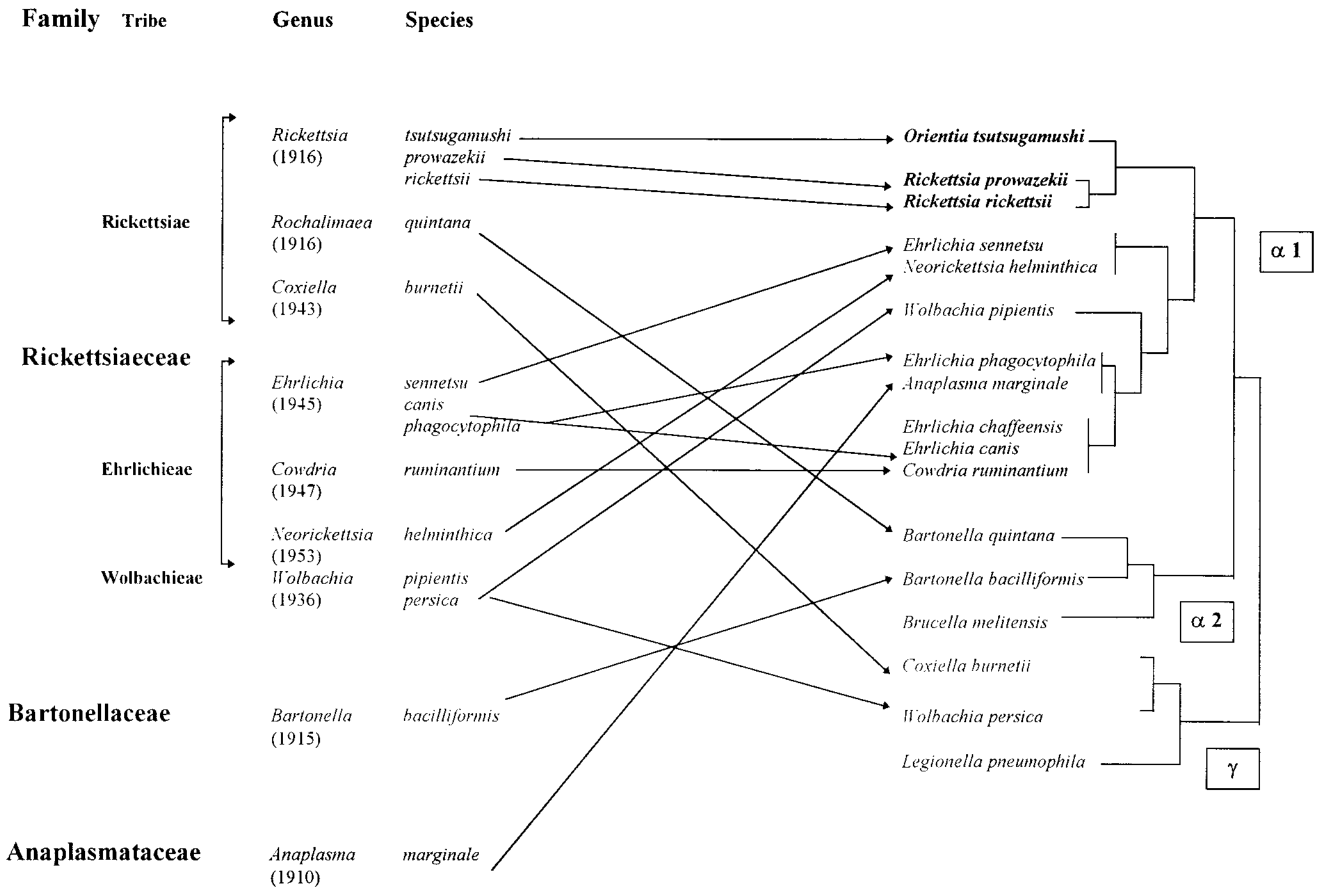

FIG. 1. Classification of rickettsiae. The left-hand side of the figure indicates the classification in Bergey's manual (180), and the classification on the right-hand side is based on a comparison of 16S rRNA gene sequences. Bacterial names in boldface type are representative of the rickettsiae studied in this minireview.

laboratory methods have been developed, including immunostaining of biopsy specimens or circulating endothelial cells, isolation on shell vial cell cultures, and PCR amplification of rickettsial DNA.

SEROLOGIC DIAGNOSIS OF RICKETTSIOSIS

Methods. The Weil-Felix test (177) is based on the detection of antibodies to various *Proteus* species which contain antigens with cross-reacting epitopes to antigens from members of the genus *Rickettsia* (36) with the exception of *R. akari*. Whole cells of *Proteus vulgaris* OX-2 react strongly with sera from persons infected with SFG rickettsiae with the exception of those with RMSF, and whole cells of *P. vulgaris* OX-19 react with sera from persons infected with typhus group rickettsiae as well as with RMSF. Subsequently, the OX-K strain of *Proteus mirabilis* was demonstrated to agglutinate with sera from scrub typhus patients and was further used in the diagnosis of *O. tsutsugamushi*-related infections. By the Weil-Felix test, agglutinating antibodies are detectable after 5 to 10 days following the onset of symptoms, with the antibodies detected being mainly of the immunoglobulin M (IgM) type (1, 2). Patients with Brill-Zinsser disease or infected with *R. akari* usually have no agglutinating antibodies detectable by the Weil-Felix test. Among the former case group of patients, patients occasionally have rising IgM antibody titers (53), therefore explaining a possible positive Weil-Felix test result (98). However, the Weil-Felix test may be positive without rising IgM antibody titers (102). The poor sensitivity and specificity of the Weil-Felix test are now well demonstrated for the diagnosis of RMSF (76, 81, 95, 168), MSF (128), murine typhus, epidemic typhus (102), and scrub typhus (29). Although a good correlation between the results of the Weil-Felix test and detection of IgM antibodies by an immunofluorescence assay (IFA) is often observed, with the development of techniques that are used to grow rickettsiae, this test should be used only as a first line of testing in rudimentary hospital laboratories.

With the development of techniques for growing rickettsiae, the complement fixation (CF) test was adapted for the detection of antibodies specific for rickettsiae. Washed particulate rickettsial antigens are species specific for the SFG and the typhus group, but cross-reacting antibodies among groups are observed (144). The CF test is strain specific for *O. tsutsugamushi*. This specificity, particularly with acute-phase sera, implies that all strains endemic to a region must be used to ensure the detection of every positive serum specimen (49). Antibody titers obtained by the CF test correlate better with IgG titers than with IgM titers obtained by immunofluorescence assay. Results vary according to the method of antigen production and the amount of antigen used in the assay (77). The use of 8 U of antigen increases the sensitivity of detection of the early IgM response but also increases the numbers of cross-reactions between antibodies to typhus group and SFG rickettsiae (144).

The microagglutination test is based on the detection of interactions between antibodies and whole rickettsial cells (59). It has not been widely used because of the need for large

TABLE 1. Features of *Rickettsia* species classified in SFG rickettsiae pathogenic for humans, SFG rickettsiae never isolated from humans, the typhus group, and the genus *Orientia*

Group	Species	Disease	Associated arthropod	Distribution	Reference(s)
SFG rickettsiae (human pathogens)	*Rickettsia conorii* sensu stricto	MSF	*Rhipicephalus sanguineus*	Mediterranean countries, Africa, Black Sea, India	129
	Rickettsia conorii complex	Israeli spotted fever	*Rhipicephalus sanguineus*	Israel	68
	Rickettsia conorii complex	Astrakhan spotted fever	*Rhipicephalus pumilo*	Astrakhan (Russia)	158
	Rickettsia rickettsii	Rocky mountain spotted fever	*Dermacentor variabilis*, *Dermacentor andersoni*, *Rhipicephalus sanguineus*, *Amblyomma cajennense*	North and South America	166
	Rickettsia sibirica	Siberian tick typhus	*Dermacentor nuttalli*, *Dermacentor marginatus*, *Haemophysalis concinna*	Northern China, Pakistan, former USSR (Asian republics, Siberia, Armenia)	132
	Rickettsia akari	Rickettsialpox	*Allodermanyssus sanguineus*	United States, Ukraine, Croatia, Korea	27, 117
	Rickettsia africae	African tick bite fever	*Amblyomma hebraeum*	Southern Africa	84
	Rickettsia australis	Queensland tick typhus	*Ixodes holocyclus*	Australia (Queensland)	143
	Rickettsia japonica	Japanese tick typhus	*Haemophysalis longicornis*, *Dermacentor taiwanensis*	Japan (southwest)	91, 164
	Rickettsia honei	Finders Island tick typhus	Unknown	Finders Islands (Tasmania)	147
	"Rickettsia mongolotimonae"	Unnamed spotted fever	*Hyalomma asiaticum* (Inner Mongolia)	Inner Mongolia, France	8, 18, 121
	Rickettsia slovaca	Unnamed spotted fever	*Dermacentor marginatus*	Slovakia, Armenia, Russia, France, Switzerland, Portugal	16, 51, 120
SFG rickettsiae (never isolated from humans)	*Rickettsia massiliae*		*Rhipicephalus turanicus*, *Rhipicephalus sanguineus*, other *Rhipicephalus* spp.	France, Greece, Spain, Portugal, central Africa	12, 15
	Rickettsia rhipicephali		*Rhipicephalus sanguineus*	United States, France, Portugal, central Africa	33
	Rickettsia parkeri		*Amblyomma maculatum*	United States	105
	Rickettsia montana		*Dermacentor variabilis*	United States	20
	Rickettsia bellii		*Dermacentor spp.*	United States	109
	"Rickettsia aeschlimannii"		*Hyalomma marginatum*	Morocco	19
	Strain S		*Rhipicephalus sanguineus*	Armenia	50
	"Rickettsia amblyommii"		*Amblyomma americanum*	United States	149
	Unnamed rickettsia from Pakistan (JC 880)		*Rhipicephalus sanguineus*	Pakistan	133
	"Rickettsia heilongjiangi"		*Haemaphysalis concinna*	China	57
	Thai tick typhus rickettsia		*Ixodes* + *Rhipicephalus* pool	Thailand	133
	Rickettsia helvetica		*Ixodes ricinus*	Switzerland, France	32
	AB bacterium		*Adalia bipunctata* (ladybird beetle)	England, Russia, United States	14, 181
Typhus group	*Rickettsia prowazekii*	Epidemic typhus, recrudescent typhus (Brill-Zinsser disease)	*Pediculus humanus corporis*	Worldwide (most in highlands areas of South America, Asia, Africa)	172
	Rickettsia typhi	Murine typhus	*Xenopsylla cheopsis*	Worldwide	172
	Rickettsia felis	Pseudotyphus of California	*Ctenocephalides felis*	California, Texas, Oklahoma	78
Scrub typhus	*Orientia tsutsugamushi*	Scrub typhus	*Leptotrombidium deliense*	Eastern Asia, northern Australia, western Pacific Islands	156

amounts of purified rickettsial antigen in this test, and these antigens are not available commercially. An unspecific slide agglutination test, essentially used in France, should not be used because it led to numerous diagnostic errors (67).

The indirect hemagglutination test detects antibodies to an antigenic erythrocyte-sensitizing substance (ESS) used to coat human or sheep erythrocytes that are either fresh or fixed in glutaraldehyde (8). The ESS is rickettsial group specific with cross-reactivity among RMSF, rickettsialpox, and MSF rickettsiae (37). This test detects both IgG and IgM antibodies, but agglutination is more efficient with IgM antibodies (8).

In the latex agglutination test, ESS is used to coat latex beads (73). The reactivity is not exactly the same as that of the indirect hemagglutination test, because the ESS on latex beads probably contains more antigenic fractions than the ESS adsorbed onto erythrocytes (72). This test is rapid (15 min) and does not require elaborate instrumentation. Latex agglutination is reactive with IgG and IgM antibodies, but the agglutination efficiency of this test is greater when the antirickettsial IgM/IgG ratio is $\geq$1. This test allows the demonstration of antibodies within 1 week after the onset of illness. Significant antibody titers disappear after 2 months.

Enzyme-linked immunosorbent assay (ELISA) was first introduced for detection of antibodies against *Rickettsia typhi* and *Rickettsia prowazekii* (71). The use of this technique is highly sensitive and reproducible, allowing the differentiation of IgG and IgM antibodies. This technique was later adapted to the diagnosis of RMSF (38) and scrub typhus (42). An

TABLE 2. Clinical symptoms of rickettsiosis with emphasis on cutaneous manifestations[a]

Disease	Etiologic agent	Rash presence (% of subjects)	Rash specificity (%)	Eschar (% of subjects)	Multiple eschars	Enlarged local nodes	Mortality (%)
RMSF	*R. ricketsii*	90	Purpuric (45)	Very rare	No	No	1–5
MSF	*R. conorii* stricto sensu	97	Purpuric (10)	72	Very rare	Rare	1
Astrakhan spotted fever	*R. conorii* complex	100	None	23	No	No	No
Israeli spotted fever	*R. conorii* complex	100	Rarely purpuric	No	No	No	<1
Rickettsialpox	*R. akari*	100	Vesicular	83	Yes	Yes	Low
Queensland tick typhus	*R. australis*	100	Vesicular	65	No	Yes	Low
Flinders Island spotted fever	*R. honei*	85	Purpuric (8)	28	No	Yes	Low
Japanese spotted fever	*R. japonica*	100	None	48	No	No	Low
African tick bite fever	*R. africae*	30	Vesicular	100	Yes	Yes	Very low
Siberian tick typhus	*R. sibirica*	100	None	77	No	Yes	Low
Epidemic typhus	*R. prowazekii*	40	Purpuric	No	No	No	2–30
Murine typhus	*R. typhi*	50	None	No	No	No	Low
Scrub typhus	*O. tsutsugamushi*	50	None	Yes	No	Yes	2–5

[a] Data are from previous reports (28, 48, 68, 70, 85, 106, 129, 132, 138, 143, 159, 162, 163, 166, 173).

original approach, a "paper ELISA," was proposed for the detection of anti-*O. tsutsugamushi* antibodies (40). Its first steps are similar to those used for the IFA, but an anti-human IgG peroxidase conjugate and substrate-saturated filter paper, on which the reaction is visualized, are used. A modified ELISA technique designed as an inhibition ELISA has also been evaluated for use in the serodiagnosis of scrub typhus due to *O. tsutsugamushi* Kawasaki (61). This technique uses coated monoclonal antibodies and evaluates inhibition of antigen absorption by mixing test sera and crude antigen.

The rickettsial IFA adapted to a micromethod format is the test of choice for the serodiagnosis of rickettsial diseases (112). The micro-IFA has the advantage that it can simultaneously detect antibodies to a number of rickettsial antigens (up to nine antigens) with the same drop of serum in a single well containing multiple rickettsial antigen dots. IFA allows the detection of IgG and IgM antibodies or both. The identification by IFA of specific IgM antibodies to the various species of rickettsiae provides strong evidence of recent active infection, although the diagnosis may be obscured by a prozone phenomenon (111). This technique is, furthermore, affected by rheumatoid factor, thus requiring the use of a rheumatoid factor absorbent before IgM determination. In our laboratory, sera are diluted in phosphate-buffered saline (PBS) with 3% nonfat powdered milk in order to avoid nonspecific fixation of antibodies. For typhus and SFG rickettsial infections, the long-term persistence of detectable antibodies is usual (93), although cross-reacting antibodies between the two groups are not unusual (104). The persistence of antibodies in patients with scrub typhus remains controversial because old reports have demonstrated the persistence of antibodies over a period of many years (25), whereas more recent studies over a 2-year period have demonstrated an annual reversion rate from titers of greater than 1:50 to titers of less than 1:50 in 61% of subjects (140). Variable rates of reinfection and strain heterogeneity may be factors influencing these conflicting data. In cases of acute infections caused by SFG rickettsiae or primary infection with *O. tsutsugamushi*, a significant antibody titer is observed at the end of the first week, concomitant with the detection of IgM antibodies, whereas IgG antibodies appear at the end of the second week (24, 87, 127). In the case of reinfection with *O. tsutsugamushi*, IgG antibodies are detectable by day 6, with IgM antibody titers being variable (24).

An immunoperoxidase assay has been developed as an alternative to IFA (153) for the diagnosis of scrub typhus and was later evaluated for use in the diagnosis of infections due to *Rickettsia conorii* (125, 139) and *R. typhi* (83). The procedure is the same as IFA, but fluorescein is replaced by peroxidase. The advantage of the immunoperoxidase assay is that the results can be read with an ordinary light microscope. In addition, the immunoperoxidase assay provides a permanent slide record.

Western immunoblot assay with sodium dodecyl sulfate-gel-electrophoresed and electroblotted antigens is a powerful serodiagnostic tool for seroepidemiology and confirmation of serologic diagnoses obtained by conventional tests. It is especially useful in differentiating true-positive from false-positive results created by cross-reacting antibodies. These cross-reacting antibodies, observed both between biogroups (SFG and typhus group) and between species, appear to be directed against lipopolysaccharide (LPS) and to be of the IgM class, although IgG antibodies directed against both LPS and protein antigens (1–3, 122, 124) have also been observed. The line blot assay, which allows the testing of more than 45 antigens simultaneously (123), has been adapted to the diagnosis of MSF (122). It is a useful test for large-scale screening of sera when quantitative titers are not needed or when tests against a large number of agents are required. Finally, a commercially available dot blot immunoassay can be used to screen patients suspected of having scrub typhus (176). This assay tests for Karp, Kato, and Gilliam strain antigens.

Cross-absorption is used for the detection of antibodies cross-reacting with other species and within the rickettsial biogroups (124, 146). This cross-reactivity will vary depending on the technique used and on the host animal from which the antiserum is obtained. A mouse antiserum raised against a specific SFG rickettsia will not cross-react with other members of the SFG rickettsiae to any great extent. This peculiarity of mouse sera, related to the limited ability of mice to reflect by antibody synthesis the full range of antigenic determinants possessed by rickettsiae, is used as a tool for identification of rickettsiae (110, 114, 133). Conversely, human sera cross-react extensively with species of the same biogroup, between biogroups, and with other bacteria such as *Legionella* and *Proteus* species (124). Confirmation of antigenic cross-reactivity is made by Western immunoblotting. Treatment of antigens with proteinase K allows the distinction of cross-reacting antibodies specific to protein antigens of LPS epitopes. Western blotting must also be done after absorption of sera with cross-reacting antigens. A cross-adsorption study is performed by mixing separately the serum studied with the bacteria involved in the cross-reaction. Cross-adsorption of the serum studied results in the disappearance of homologous and heterologous antibodies when absorption is performed with the bacterium responsible for the disease, whereas disappearance of only homologous antibodies is observed when absorption is performed with the antigen of the bacterium responsible for the cross-reaction. The major limitation of this technique is the large amount of antigen needed.

The interrelationship of species within a rickettsial biogroup is so intimate that confirmation of their identity, and to a lesser extent of the rickettsial biogroup, is generally difficult. The geographical origin of the infection is one of the best indicators of species identity. The identification of the rickettsial species causing an infection by studying the patient's serum may be achieved by IFA or a cross-absorption test. For the former, multiple microimmunofluorescence assay titers of the sera against different species are required (104, 112). Usually homologous antibody titers are higher than heterologous antibody titers, and staining characteristics appear to be more specific against the infecting rickettsia. The differences in titers are usually large enough to differentiate between biogroups. On the contrary, among members of the same biogroup, heterologous antibody titers may be as high as homologous antibody titers, and as discussed above, cross-absorption studies may help in the differentiation of homologous and heterologous antibodies. The sera studied must be absorbed with different antigens, and then the titers must be determined.

Comparison among serologic tests. For a test to be useful in the diagnosis of an acute rickettsial infection, the most important criteria are sensitivity and the length of delay between the onset and appearance of detectable antibody titers. Conversely, when the test is to be used for seroepidemiologic studies, it should be highly specific to prevent false-positive results due to cross-reacting antibodies. Other criteria which need to be considered include the amount of antigens needed, their costs, and the minimal material required. Lastly, the commercial availability of a test is a major criterion for routine use. In the United States reagent kits for IFA and for latex agglutination are commercially available, but in Europe only reagent kits for IFA are commercially available.

The effectiveness of the microagglutination test for the diagnosis of RMSF has been compared with that of the CF test, immunofluorescence, and hemagglutination (111). It was shown to be less sensitive than hemagglutination and IFA and comparable in sensitivity to the CF test for both RMSF (87, 99) and, subsequently, epidemic typhus (102). The need for a large amount of purified antigen is the major limitation of this method.

The CF test is highly specific, with false-positive results occurring only very rarely at a serum dilution of 1/16 (144). However, the CF test has been reported in most studies to have poor sensitivity, especially in the relatively early stage of the disease, for the diagnosis of RMSF (81, 87, 99, 111, 145) or typhus group infections (102, 145). The poor sensitivity of this test in the early stage of the disease led to the low interest in its use for the diagnosis of acute cases of infection, but it remains useful for seroepidemiologic studies.

The hemagglutination assay is a very sensitive test that detects antibodies to SFG and typhus group earlier than any of the other tests studied (168). This high sensitivity for the diagnosis of RMSF with acute-phase sera has been reported in most studies (81, 87, 111, 168). A fourfold titer rise may be detected within the first week after the onset of RMSF but not MSF (87, 127). It is especially useful for the diagnosis of acute infections, but it should not be used for seroepidemiologic studies because only very low antibody titers are observed in late-convalescent-phase sera (182).

The latex agglutination assay has been developed as an immunoassay for the detection of *Rickettsia rickettsii* (73, 81), *R. conorii* (75, 127), *R. typhi*, and *R. prowazekii* (74). This assay is group specific, and its sensitivity is comparable to that of IFA. It has been proposed as an alternative to first-line testing of sera (especially as a replacement for the Weil-Felix test in laboratories not equipped to perform the Weil-Felix test). Its major drawback is the cost of reagents, although it does not require expensive equipment.

ELISA has been demonstrated to be as sensitive and as specific as IFA for the diagnosis of RMSF (38). Moreover, the ELISA is more sensitive than the IFA for the detection of the low levels of antibody that are present after vaccination and during late convalescence. ELISA was demonstrated to be as suitable as IFA for demonstrating rising antibody titers in patients with scrub typhus, but ELISA requires a complex and time-consuming antigen purification procedure (42). Although antibodies against any of the major prototype strains of scrub typhus could be detected with a single antigen, considerably higher titers were obtained when the homologous antigen was used. The paper ELISA gave results similar to those of IFA in the diagnosis of scrub typhus (40). The inhibition ELISA has only been tested for use in the diagnosis of scrub typhus and appears to be more sensitive than IFA, especially at the early stage of the disease (61).

IFA is the "gold standard" technique and is used as a reference technique in most laboratories. For the diagnosis of RMSF, sensitivity, as tested with 60 paired serum specimens, including specimens with stationary titers (5%) and fourfold rising titers (95%), was 100% (87). In another study with patients with no rickettsial diseases, a titer of $\geq$1:64 had a specificity of 100% and a sensitivity of 84.6%, and a titer of $\geq$1:32 had a specificity of 99.8% and a sensitivity of 97.4% (99). For the diagnosis of MSF, the sensitivity of a titer of $\geq$1:40 was demonstrated to increase with the length of delay between onset and sampling: only 46% between 5 and 9 days, 90% between 20 and 29 days, and 100% afterwards (128). For scrub typhus, the sensitivity of IFA is low if high specificity is required: for a titer of $\geq$1:100, sensitivity is 84% and specificity is 78%, for a titer of $\geq$1:200, sensitivity is 70% and specificity is 92%, and for a titer of $\geq$1:400, sensitivity is 48% and specificity is 96% (29). A fourfold increase to a titer of $\geq$1:200 is 98% specific and 54% sensitive.

The sensitivity and specificity obtained by immunoperoxidase assay for the serodiagnosis of scrub typhus (125, 154, 187), epidemic typhus (83), and MSF (125) resemble those obtained by IFA.

Western immunoblot assay was demonstrated to be more sensitive than IFA for the detection of early antibodies in MSF (160), with the first antigen detected being the nonspecific antigen LPS. Nevertheless, when considering only the reaction against the specific protein antigen on Western immunoblot assay, no difference in sensitivity from IFA could be demonstrated. By using samples from healthy blood donors, Western immunoblot assay was demonstrated to be more specific than IFA. In a study conducted in Greece, Western immunoblot assay revealed that both the specificity and the positive predictive value for a single serum tested by IFA were very low, especially when a low cutoff was used (11). The Western immunoblot assay is therefore the most specific tool when determining the true prevalence of rickettsial diseases. In a serologic survey of MSF in an area where the disease is not endemic, 53 IFA-positive serum specimens were tested by the Western immunoblot assay (119). Only 16 specimens reacted against the specific protein antigen, whereas only 17 specimens reacted with the nonspecific LPS. The true-positive specimens were obtained from individuals in a village with a unique sub-Mediterranean climate where the tick vector *Rhipicephalus sanguineus* can proliferate, whereas the false-positive specimens were obtained from individuals over a disseminated area with a colder climate. Further investigations allowed the demonstration of *R. sanguineus* ticks infected with *R. conorii* in the village whose population tested positive (unpublished data).

The line blot assay has been demonstrated to be almost as specific and sensitive as IFA for the diagnosis of MSF (122). The line blot immunoassay may be particularly useful for screening the many antigens that might be considered for patients with nonspecific or atypical clinical presentations. The commercially available dot blot immunoassay for the diagnosis of scrub typhus lacks both sensitivity and, especially, specificity. This test can be considered useful only as a first-line test, as an alternative to the Weil-Felix test, for the rapid diagnosis of acute cases of infection in areas with a high prevalence.

IMMUNODETECTION OF RICKETTSIAE IN BLOOD AND TISSUES

Detection of rickettsiae by using immunofluorescence allows the confirmation of infection in patients prior to their seroconversion. *R. typhi* has successfully been detected in the organs of a patient with a fatal case of murine typhus (175), although it is for RMSF and MSF that immunodetection has been the most widely used. Samples can be tested fresh (69, 82, 126, 170, 171, 185) or after formalin fixation and paraffin embedment (47, 48, 92, 169, 175). Biopsy specimens of the skin with a rash around the lesion, preferably petechial lesions, and tache noire specimens are the most common samples used (47, 82, 92, 126, 170, 171, 185). In animals or patients with fatal cases of infection, bacteria are detectable at autopsy in the tissues of numerous organs such as the liver, spleen, kidney, heart, meningeal membranes, or skin (48, 69). The immunofluorescence technique was first proposed by Woodward et al. (185). Later, an immunoperoxidase technique with increased sensitivity and specificity was described (47). Furthermore, this technique, which allows better microscopic definition of cells around the detected rickettsiae, can be used by laboratories without a fluorescence microscope.

Evaluations of these techniques in several cases have reported a specificity of 100% for the diagnosis of both RMSF (170) and MSF (126). Sensitivity remains low, between 53 and 75% (82, 126, 168, 170). We have recently described (46) and evaluated (89) a technique allowing the immunologic detection of *R. conorii* in circulating endothelial cells (CECs), which are isolated from whole blood by using immunomagnetic beads coated with an endothelial cell-specific monoclonal antibody. The average CEC count is 162 $\pm$ 454 cells/ml of whole blood before treatment (64). One milliliter of whole blood diluted 1:4 with phosphate-buffered saline is mixed with a suspension of monoclonal antibody-coated beads, and following incubation, magnetic beads and rosetted cells are divided into two aliquots. One aliquot is stained with acridine orange and the cells are counted with a hemocytometer, and the other is cytocentrifuged onto a glass slide. The smears are then fixed and bacteria are detected by immunofluorescence with a rabbit antiserum to *R. conorii*. The sensitivity of this method is 50% and does not appear to be influenced by the previous initiation of an antibiotic regimen or the presence of specific antibodies, as in the case of culture (89). Furthermore, it is a prognostic indicator because the level of CECs detected increases with the severity of infection (64). We have further developed a technique consisting of cutting tache noire specimens into small pieces, followed by collagenase treatment, for the recovery of endothelial cells, as described previously for umbilical veins (80). Endothelial cells are then recovered from this digestion mixture by using immunomagnetic beads as described above. This technique has allowed us to recover a strain of *R. conorii* from a tache noire biopsy specimen from an apyretic patient who had been treated for 3 days (unpublished data).

ISOLATION OF RICKETTSIAE

In the past, only research laboratories that had biosafety level 3 containment and personnel with extensive experience in cultivating rickettsiae were able to isolate rickettsiae from clinical specimens. During recent years, the development of cell culture systems for viral isolation has led to an increase in the number of laboratories suitably equipped to isolate rickettsiae. Since different rickettsial diseases may have indistinguishable clinical manifestations, the isolation of new isolates followed by their molecular characterization is critical for the discovery of new rickettsial diseases. The isolation of rickettsiae may be attempted with several samples: buffy coat of heparinized blood, defibrinated whole blood, triturated clot, plasma, necropsy tissue, skin biopsy, and arthropod samples.

Embryonated chicken egg yolk sacs and laboratory animals. Embryonated chicken egg yolk sacs have been widely used in the past (39), but they are now being replaced by cell culture systems. Inoculation into guinea pigs has also been widely used (66, 103). The mouse is the species of choice for the isolation of *R. akari*, *Rickettsia australis*, and especially *O. tsutsugamushi*. For this last species, great heterogeneity of virulence among strains is observed (155). Meadow voles (*Microtus pennsylvanicus*) are very susceptible to rickettsial infection, but they are not easily available. Recently, *R. felis* was reported to be initially grown in male Sprague-Dawley rats from fleas prior to successful cell culture (118). Inoculation into animals remains helpful in situations requiring isolation of the organism from postmortem tissues, which are usually contaminated with other bacteria. We have also used inoculation into animals in order to remove contaminating mycoplasmas from cell cultures for rickettsia (52).

Cell cultures. Cell culture, described for more than 60 years (100), is now the most widely used method for isolating rickettsiae from clinical samples. Isolation of *R. rickettsii* from blood has been achieved by using a primary monocyte culture (30, 44, 45). Later, an L929 mouse fibroblast cell monolayer in tube culture was also introduced for the isolation of *R. rickettsii* and *O. tsutsugamushi* from blood (82, 157). More recently, the shell vial assay, developed from a commercialized method for cytomegalovirus culture and early antigen detection, was adapted to the detection of *R. conorii*, with detection of the microorganism being possible in 48 to 72 h in most cases (94). The small surface area of the coverslip containing cells enhances the ratio of the numbers of rickettsia to the numbers of cells and allows better recovery. Inoculation should be made onto two types of cells. Vero or L929 cells have been shown to allow better and faster isolation of rickettsiae, especially from heavily infected samples, than HEL or MRC5 cells (86). Nevertheless, HEL or MRC5 cells have the advantage that once a monolayer is established, contact inhibition prevents further division and the cells can then be used for prolonged incubation. The shell vial technique is routinely used in our laboratory for the isolation of rickettsiae from human samples (decanted plasma or tissues) and hemolymphs from arthropods (13, 15, 16, 54, 56, 89). For an optimal yield, blood should be collected on heparin anticoagulant, avoiding EDTA or sodium citrate, which lead to detachment of the cell monolayer from coverslips. Erythrocytes should not be inoculated onto shell vials because they lead to high background levels at the time of examination with a UV microscope. The centrifugation step after inoculation of the shell vial is critical for the sensitivity of the technique, because it enhances rickettsial attachment to and penetration of cells (86, 108, 113, 179). We have evaluated this method for the diagnosis of MSF with 205 cultures of *R. conorii* from blood and skin samples from 157 patients (88). The bacterium was cultured from 29.8% of the samples. When patients were sampled prior to antibiotic therapy and when the concurrent IFA antibody titer was <1:32, the bacterium was cultured from 59% of the samples. Delay between the time of sample collection and inoculation onto shell vials also appeared to be critical, since no culture of samples not inoculated on the day of sampling but held at room temperature or 4°C was positive. Interestingly, for the 34 positive cultures, *R. conorii* was detected as soon as day 3 after inoculation, thus prior to seroconversion.

IDENTIFICATION OF RICKETTSIAE

Presumptive identification of a rickettsial isolate may be achieved by microscopic examination after staining. Rickettsiae appear as short rods which are not stained by staining with the Gram stain but which are visible after Giemsa or Gimenez staining (66).

Serological identification. Conventional serologic identification procedures require a laboratory equipped for cultivation of rickettsiae and a large panel of specific antisera. This approach is therefore usually possible only in reference laboratories. The first serologic technique to be described used the CF test with convalescent-phase guinea pig sera (115). A toxin neutralization test with mice (21, 22, 115) and a microimmunofluorescence method with mouse polyclonal antisera (110) were later described. The main problem with these techniques was the need for *Rickettsia* strain-infected reference sera, and each time a new isolate was tested, it and all other antigens need to be screened against all antisera. However, microimmunofluorescence remains the reference method for the identification of rickettsiae. Recently, monoclonal antibodies have been introduced in place of polyclonal antibodies. Monoclonal antibodies were first raised against *R. rickettsii* (4–7, 88) and were later raised against *R. akari* (96), *R. conorii* (174), *R. prowazekii* (23), *R. japonica* (165), *O. tsutsugamushi* (142), and *R. africae* (186) epitopes. By using group-specific and strain-specific monoclonal antibodies, the identification of a rickettsial isolate is easy (provided, of course, that one has an exhaustive collection of monoclonal antibodies). Protein analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis has also been used to differentiate rickettsial species of the SFG, with the major distinctive proteins lying in the high-molecular-mass range of >90 kDa (15). When studied by Western blot assay, the major antigenic protein or species-specific protein antigens are among these high-molecular-mass proteins, corresponding to the outer membrane proteins OmpA and OmpB (5, 6, 41, 43, 58, 122). These proteins determine the serologic specificity for *R. rickettsii*, *R. conorii* sensu stricto, and Israeli spotted fever rickettsia (18).

Molecular biology-based identification. The first proposed molecular biology-based identification method was based on PCR-restriction fragment length polymorphism (RFLP) analysis of the gene encoding the OmpA protein, which allowed the differentiation of the nine SFG rickettsiae studied (131). Later, by using a combination of this approach with a method based on PCR-RFLP analysis of a fragment of the gene encoding the OmpB protein, all 36 SFG strains except *R. africae* and *Rickettsia parkeri* could be differentiated (55). Many studies have proven that these approaches are sensitive, accurate, and reproducible (13, 15, 51, 54, 134, 188). The careful choice of the appropriate endonuclease for PCR-RFLP analysis (134) and the storage of species-specific RFLP profiles in databases have greatly simplified the identification of SFG rickettsiae. Pulsed-field gel electrophoresis has also been shown to be a good interspecies identification tool for the SFG rickettsiae (135). By this approach distinctive patterns were obtained for the 16 species studied, whereas 10 isolates of *R. conorii* all exhibited the same profile. Macrorestriction analysis is, however, time-consuming and requires large amounts of cultivated bacteria (about 10 150-cm^2 flasks of cell cultures for each strain). Furthermore, as with serotyping, it is necessary to include all rickettsial species in the gel to obtain a precise comparison of the profiles. With the development of automatic nucleotide sequencers, nucleotide base sequence analysis of PCR products is now a rapid, convenient, and sensitive technique for the identification of rickettsiae. About 20 genes have been sequenced to date, mainly among members of the typhus group. Five of these genes have been proposed for use in the identification of rickettsia, namely, those encoding 16S rRNA (136, 148, 149), a protein of 17 kDa (10), citrate synthase (14, 137, 184), OmpA (9, 134), and OmpB (34, 65). Nucleotide sequence analysis of the 16S rRNA gene is useful for identification to the genus level, but since several species share similar 16S rRNA gene sequences, study of this gene does not provide accurate identification to the species level (136). The gene encoding the 17-kDa protein has not yet been studied enough to become an identification tool, although nucleotide sequence comparison revealed homologies of 99.8, 88.1, and 88.7% between *R. rickettsii* and *R. conorii*, *R. typhi*, and *R. prowazekii*, respectively (10), indicating its potential. The citrate synthase gene (*gltA*) of all rickettsiae with the exception of *O. tsutsugamushi* has now been sequenced. Species-specific sequences can be recognized in a 1,234-bp fragment of this gene, which is bordered by conserved regions which act as suitable hybridization sites for consensus primers. Nevertheless, this gene is not divergent enough to allow one to distinguish among all rickettsial species (137). The *ompA* gene is specific for the SFG rickettsiae and exhibits enough heterogeneity to ensure accurate identification of bacteria from this group by comparison of a 632-bp region at the 5′ end of the gene. Indeed, the gene is polymorphic enough in this region to allow the differentiation of some strains of *R. conorii* (134). Unsurprisingly, this differentiation is in accordance with the previously described antigenic diversity among strains of this species (174). However, this approach does not allow the identification of *Rickettsia bellii*, *R. akari*, *Rickettsia helvetica*, *R. australis*, *Rickettsia canada*, *R. typhi*, *R. prowazekii*, or *O. tsutsugamushi*, either because of an absence of this gene or because the primers used do not hybridize (especially to *R. canada*). In our laboratory, the identification of SFG rickettsiae is achieved by PCR amplification and sequencing of the *gltA* and *ompA* genes. Any laboratory with facilities for gene PCR amplification and sequencing and access to a sequence database can differentiate all species of rickettsiae.

In the absence of amplifiable fragments of the *gltA* and *ompA* genes, the molecular identification of *O. tsutsugamushi* has been achieved by a nested PCR which allows the differentiation of strains to the serotype level (62, 97). The first primer pair allows the amplification of a fragment of the gene that encodes a 56-kDa protein, which is responsible for type strain antigenic specificity (101, 150), and the second primer pair allows the determination of the serotype strain.

TABLE 3. Main characteristics of laboratory diagnostic tests available for the diagnosis of rickettsiosis

Technique	Indications	Advantages	Drawbacks	Conclusion
Shell vial assay	Isolation of rickettsiae from blood and tissues of infected patients and from arthropods	Characterization of etiologic agent, positive result 3 days after sampling, positive result before antibody titer rise	Limited to laboratories with biohazard facilities, vials need to be inoculated the day of sampling, negative for patients with prior antibiotic therapy	Essential technique for identification of new rickettsial pathogens, allows early diagnosis before seroconversion
PCR-based detection	Detection and identification of rickettsiae from blood and tissues of infected patients and from arthropods	Not limited to laboratories with biohazard facilities or reference centers, positive result 24 h after sampling, may be positive for patients with prior antibiotic therapy	Needs facilities for molecular biology-based tests	Probably the technique of choice for early diagnosis before seroconversion in most laboratories, useful for screening arthropods
Immunodetection	Detection of rickettsiae from tissues of infected patients and arthropods	Available in most pathology laboratories, positive result 2 days after sampling, may be positive for patients with prior antibiotic therapy	Requires experienced personnel	Useful technique for early diagnosis before seroconversion, especially in patients with inoculation eschar
Circulating endothelial cells	Detection of rickettsiae from blood and tissues of infected patients	Available in most laboratories, positive result 3 h after sampling, may be positive for patients with prior antibiotic therapy	Technique limited by quantity and quality of circulating endothelial cells	Quickest technique for early diagnosis before seroconversion, level of CEC detection correlates with severity of infection
Weil-Felix test	Serodiagnosis	Inexpensive test	Lacks both sensitivity and specificity	Should be used only in very poor countries for diagnosis of acute cases
CF test	Serodiagnosis	High specificity (good species specificity)	Lack of sensitivity early in the disease	Should be used only for seroepidemiologic studies
Indirect hemagglutination	Serodiagnosis	Both specific and sensitive, early detectable antibodies	Low antibody titers in late-convalescent-phase sera	Should be used only for the diagnosis of acute cases
Latex agglutination	Serodiagnosis	Simple, no expensive material required, commercially available	Expensive kit	Should be used in non-equipped laboratory
ELISA	Serodiagnosis	Both specific and sensitive		Useful for both diagnosis of acute cases and seroepidemiology
Microimmunofluorescence	Serodiagnosis	Both specific and sensitive, commercially available	Requires fluorescence microscope	Reference technique in most laboratories, useful for both diagnosis of acute cases and seroepidemiology
Immunoperoxidase	Serodiagnosis	Both specific and sensitive, does not require fluorescence microscope	Except for scrub typhus, cannot be used for large-scale evaluation	Alternative technique to IFA that allows permanent slide records
Line blot	Serodiagnosis	Both specific and sensitive, large number of antigens tested simultaneously	No quantitative titers available	Large-scale screening for seroepidemiologic studies
Western immunoblot	Serodiagnosis	Most specific and sensitive serologic test, earliest detectable antibodies	Time-consuming	Probably best serologic tool for seroepidemiologic studies

PCR-BASED DETECTION OF RICKETTSIAE FROM CLINICAL SPECIMENS

Several clinical samples are suitable for use in PCR amplification of rickettsial DNA. Skin biopsy specimens and peripheral blood mononuclear cells are routinely used in specialized laboratories but can be used in any laboratory with PCR facilities (79, 97, 183). CECs concentrated in the buffy coat obtained after decantation of heparinized blood, like the CECs used for the inoculation of shell vials, can be used, but the cells must be treated with heparinase prior to PCR amplification. We therefore recommend that blood be collected in tubes containing EDTA or sodium citrate. Blood clots, whole blood, or serum has also been successfully used in several studies (35, 60, 62, 141, 151, 152, 161) for the detection of *O. tsutsugamushi*, *R. rickettsii*, *R. typhi*, and *R. prowazekii*, but *R. conorii* DNA could not be amplified when serum was used (89). Rickettsial DNA used as a template for PCR amplification can also be extracted from tache noire specimens (when present) (183), cerebrospinal fluid (141), or paraffin-embedded tissues (151). PCR-based detection in published reports has been based on amplification of the gene encoding the 56-kDa antigen for *O. tsutsugamushi* (62, 79, 97, 151, 152) and the gene encoding the 17 kDa protein for *R. rickettsii*, *R. prowazekii*, and *R. japonica* (35, 60, 161). In our laboratory, amplification of the *ompA* and *gltA* genes, as outlined above, is used. If the former is amplified it is further sequenced for identification. The *gltA* gene is sequenced for identification only when the *ompA* gene fragment is not successfully amplified.

ISOLATION AND DETECTION OF RICKETTSIAE FROM ARTHROPODS

Ticks collected for use in attempts to isolate rickettsiae should be kept alive in a box which retains moisture prior to testing (63). Ticks may also be frozen (86). The hemolymph test should be performed while the ticks are still alive (31). The

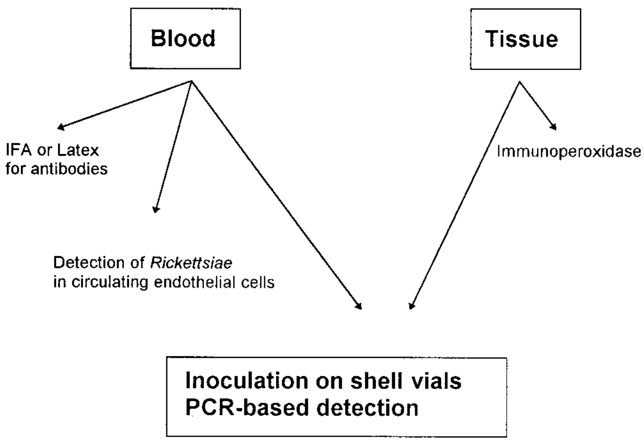

FIG. 2. Practical approach to the laboratory diagnosis of rickettioses.

distal portion of one leg is amputated, allowing the collection of a drop of hemolymph, which can be spread onto a slide and then subjected to either Gimenez staining or immunodetection methods. The tick should then undergo surface disinfection, and another drop can be inoculated onto a shell vial (15, 16, 54, 107). The tick can also be disinfected with iodinated alcohol and then crushed in 1 ml of cell culture medium before inoculation onto a shell vial (86). All the methods described above for the PCR-based detection of rickettsiae from clinical samples may be applied to arthropods (16, 17). Immunodetection methods may also be applied to arthropods. A drop of hemolymph can be placed onto a slide, air dried, and fixed in acetone before being treated with fluorescein isothiocyanate-labelled immunoglobulins against the various rickettsial groups (31). An antigen capture immunoenzyme assay with a cross-reactive monoclonal antibody directed against the major surface antigen of 135-kDa has been demonstrated to be a good alternative for the primary screening of tick samples (116).

SUMMARY AND STRATEGY IN USING DIFFERENT DIAGNOSTIC TESTS

The most efficient tests for the diagnosis of acute cases of rickettsial infection are those which directly detect rickettsiae (Table 3 and Fig. 2). The test that is most appropriate for use during the acute phase is that which detects rickettsiae in endothelial cells, followed by specific gene amplification by PCR, immunodetection with tissue biopsy specimens, and the shell vial assay. Because the most important considerations in the choice of a serologic assay in this situation are its sensitivity and the length of delay between the onset and appearance of detectable antibody titers, laboratories so equipped should use IFA (especially tests specific for IgM). For laboratories without a UV microscope, the indirect immunoperoxidase assay should be considered, although this test requires further evaluation. The latex agglutination test appears to be a good alternative for screening sera in a laboratory not equipped with a UV microscope, but it remains too expensive for use in very poor countries, in which case the Weil-Felix test is probably the best alternative. In the case of acute infections, a case should be confirmed if testing reveals an IFA titer greater than or equal to the cutoff (which should be defined for each rickettsial disease and each area) or a fourfold rise in titer by the CF test, IFA, the microagglutination test, the latex agglutination test, or the hemagglutination assay. Doubtful cases should be investigated by Western immunoblot assay.

Although the IFA has been used for seroepidemiologic studies, it has been demonstrated by Western immunoblotting to lack sensitivity when diagnosing MSF (11, 119). IFA should be considered a technique for seroepidemiology only in areas where the seroprevalence of rickettsial disease has already been established. The line blot assay should be considered as a seroepidemiologic tool since it allows the large-scale screening of sera on numerous agents in the same assay. The Western immunoblot assay is probably the most specific tool for determining the real prevalence of rickettsial diseases.

The techniques that allow the direct detection of bacteria should be used with arthropods. The hemolymph test or detection by direct immunofluorescence should be used to screen large numbers of ticks, followed by specific gene analysis. The shell vial assay incorporating two types of cells, as explained above, should then be used to isolate rickettsiae from infected arthropods.

Several approaches should be taken in the search for new rickettsial diseases. In areas where rickettsial diseases have not been described, the first step should be the recovery of rickettsiae from resident arthropods, in order to characterize the strains and to have "resident" antigens for seroepidemiologic testing. In such regions cross-reactivity among rickettsiae of different groups can also be exploited as a first line for serologic testing. In areas where a rickettsial disease is endemic, a physician's curiosity should be triggered by atypical cases of rickettsiosis, such as the occurrence of cases out of season or with atypical clinical presentations. Under such conditions, Western immunoblot assay is a useful tool with which to explore a possible cross-reaction with the endemic strain. Recovery of rickettsiae from resident arthropods by the shell vial assay must also be attempted in order to identify potential pathogens for humans. In all cases, the isolation and characterization of the causative pathogen from clinical samples is the definitive test. This test can be augmented with specific PCR amplifications with skin biopsy specimens, which allows the detection of rickettsiae in patients who have received antibiotic therapy.

In conclusion, with the widespread availability of the new tools with which one can confirm the diagnosis of rickettsial diseases, a rickettsiologist accustomed to the study of old diseases is now, more than ever, equipped to investigate new emerging rickettsial diseases.

ACKNOWLEDGMENT

We thank Richard Birtles for reviewing the manuscript.

REFERENCES

1. **Amano, K., N. Suzuki, H. Hatakeyama, Y. Kasahara, S. Fujii, K. Fukushi, T. Suto, and F. Mahara.** 1992. The reactivity between rickettsiae and Weil-Felix test antigens against sera of rickettsial disease patients. Acta Virol. **36:**67–72.
2. **Amano, K. I., H. Hatakeyama, M. Okutta, T. Suto, and F. Mahara.** 1992. Serological studies of antigenic similarity between Japanese spotted fever rickettsiae and Weil-Felix test antigens. J. Clin. Microbiol. **30:**2441–2446.
3. **Amano, K. I., K. Kyohno, S. Aoki, and T. Suto.** 1995. Serological studies of the antigenic similarity between typhus group rickettsiae and Weil-Felix test antigens. Microbiol. Immunol. **39:**63–65.
4. **Anacker, R. L., R. H. List, R. E. Mann, S. F. Hayes, and L. A. Thomas.** 1985. Characterization of monoclonal antibodies protecting mice against *Rickettsia rickettsii*. J. Infect. Dis. **151:**1052–1060.
5. **Anacker, R. L., R. H. List, R. E. Mann, and D. L. Wiedbrauk.** 1986. Antigenic heterogeneity in high- and low-virulence strains of *Rickettsia rickettsii* revealed by monoclonal antibodies. Infect. Immun. **51:**653–660.
6. **Anacker, R. L., R. E. Mann, and C. Gonzales.** 1987. Reactivity of monoclonal antibodies to *Rickettsia rickettsii* with spotted fever and typhus group rickettsiae. J. Clin. Microbiol. **25:**167–171.
7. **Anacker, R. L., G. A. McDonald, R. H. List, and R. E. Mann.** 1987. Neutralizing activity of monoclonal antibodies to heat-sensitive and heat-resistant epitopes of *Rickettsia rickettsii* surface proteins. Infect. Immun. **55:**825–827.

8. **Anacker, R. L., R. N. Philip, L. A. Thomas, and E. A. Casper.** 1979. Indirect hemagglutination test for detection of antibody to *Rickettsia rickettsii* in sera from humans and common laboratory animals. J. Clin. Microbiol. **10:**677–684.
9. **Anderson, B. E., G. A. MacDonald, D. C. Jones, and R. L. Regnery.** 1990. A protective protein antigen of *Rickettsia rickettsii* has tandemly repeated, near-identical sequences. Infect. Immun. **58:**2760–2769.
10. **Anderson, B. E., R. L. Regnery, G. M. Carlone, T. Tzianabos, J. E. McDade, Z. Y. Fu, and W. J. Bellini.** 1987. Sequence analysis of the 17-kilodalton-antigen gene from *Rickettsia rickettsii*. J. Bacteriol. **169:**2385–2390.
11. **Babalis, T., H. Tissot-Dupont, Y. Tselentis, C. Chatzichristodoulou, and D. Raoult.** 1993. *Rickettsia conorii* in Greece: comparison of a microimmunofluorescence assay and Western blotting for seroepidemiology. Am. J. Trop. Med. Hyg. **48:**784–792.
12. **Babalis, T., Y. Tselentis, V. Roux, A. Psaroulaki, and D. Raoult.** 1994. Isolation and identification of a rickettsial strain related to *Rickettsia massiliae* in Greek ticks. Am. J. Trop. Med. Hyg. **50:**365–372.
13. **Bacellar, F., R. L. Regnery, M. S. Nuncio, and A. R. Filipe.** 1995. Genotypic evaluation of rickettsial isolates recovered from various species of ticks in Portugal. Epidemiol. Infect. **114:**169–178.
14. **Balayeva, N. M., M. E. Eremeeva, H. Tissot-Dupont, I. A. Zakharov, and D. Raoult.** 1995. Genotype characterization of the bacterium expressing the male-killing trait in the ladybird beetle *Adalia bipunctata* with specific rickettsial molecular tools. Appl. Environ. Microbiol. **61:**1431–1447.
15. **Beati, L., J. P. Finidori, B. Gilot, and D. Raoult.** 1992. Comparison of serologic typing, sodium dodecyl sulfate-polyacrylamide gel electrophoresis protein analysis, and genetic restriction fragment length polymorphism analysis for identification of rickettsiae: characterization of two new rickettsial strains. J. Clin. Microbiol. **30:**1922–1930.
16. **Beati, L., P. F. Humair, A. Aeschlimann, and D. Raoult.** 1994. Identification of spotted fever group rickettsiae isolated from *Dermacentor marginatus* and *Ixodes ricinus* ticks collected in Switzerland. Am. J. Trop. Med. Hyg. **51:**138–148.
17. **Beati, L., P. Kelly, L. Matthewman, P. Mason, and D. Raoult.** 1995. Prevalence of Rickettsia-like organisms and spotted fever group Rickettsiae in ticks (Acari: Ixodidae) from Zimbabwe. J. Med. Entomol. **32:**787–792.
18. **Beati, L., P. J. Kelly, P. R. Mason, and D. Raoult.** 1994. Species-specific BALB/c mouse antibodies to rickettsiae studied by Western blotting. FEMS Microbiol. Lett. **119:**339–344.
19. **Beati, L., M. Meskini, B. Thiers, and D. Raoult.** 1997. *Rickettsia aeschlimannii* sp. nov., a new spotted fever group rickettsia associated with *Hyalomma marginatum* ticks. Int. J. Syst. Bacteriol. **47:**548–554.
20. **Bell, E. J., G. M. Kohls, H. G. Stoenner, and D. B. Lackman.** 1963. Nonpathogenic rickettsias related to the spotted fever group isolated from ticks, *Dermacentor variabilis* and *Dermacentor andersoni* from eastern Montana. J. Immunol. **90:**770–781.
21. **Bell, E. J., and E. G. Pickens.** 1953. A toxic substance associated with the rickettsias of the SFG. J. Immunol. **70:**461–472.
22. **Bell, E. J., and H. G. Stoenner.** 1960. Immunologic relationships among the spotted fever group of rickettsias determined by toxin neutralisation tests in mice with convalescent animal serums. J. Immunol. **84:**171–182.
23. **Black, C. M., T. Tzianabos, L. F. Roumillat, M. A. Redus, J. E. McDade, and C. B. Reimer.** 1983. Detection and characterization of mouse monoclonal antibodies to epidemic typhus rickettsiae. J. Clin. Microbiol. **18:**561–568.
24. **Bourgeois, A. L., J. G. Olson, R. C. Fang, J. Huang, C. L. Wang, L. Chow, D. Bechthold, D. T. Dennis, J. C. Coolbaugh, and E. Weiss.** 1982. Humoral and cellular responses in scrub typhus patients reflecting primary infection and reinfection with Rickettsia tsutsugamushi. Am. J. Trop. Med. Hyg. **31:**532–540.
25. **Bozeman, F. M., and B. L. Elisberg.** 1967. Studies of the antibody response in scrub typhus employing indirect immunofluorescence. Acta Med. Biol. **15:**105–111.
26. **Brenner, D. J., S. P. O'Connor, H. H. Winkler, and A. G. Steigerwalt.** 1993. Proposals to unify the genera *Bartonella* and *Rochalimaea*, with descriptions of *Bartonella quintana* comb. nov., *Bartonella vinsonii* comb. nov., *Bartonella henselae* comb. nov., and *Bartonella elizabethae* comb. nov., and to remove the family *Bartonellaceae* from the order *Rickettsiales*. Int. J. Syst. Bacteriol. **43:**777–786.
27. **Brettman, L. R., S. Lewin, R. S. Holzman, W. D. Goldman, J. S. Marr, P. Kechijian, and R. Schinella.** 1981. Rickettsialpox: report of an outbreak and a contemporary review. Medicine (Baltimore) **60:**363–372.
28. **Brouqui, P., J. R. Harle, J. Delmont, C. Frances, P. J. Weiller, and D. Raoult.** 1997. African tick bite fever: an imported spotless rickettsiosis. Arch. Intern. Med. **157:**119–124.
29. **Brown, G. W., A. Shirai, C. Rogers, and M. G. Groves.** 1983. Diagnostic criteria for scrub typhus: probability values for immunofluorescent antibody and Proteus OXK agglutinin titers. Am. J. Trop. Med. Hyg. **32:**1101–1107.
30. **Buhles, W. C., D. L. Huxsoll, G. Ruch, R. H. Kenyon, and B. L. Elisberg.** 1975. Evaluation of primary blood monocyte and bone marrow cell culture for the isolation of *Rickettsia rickettsii*. Infect. Immun. **12:**1457–1463.
31. **Burgdorfer, W.** 1970. Hemolymph test. A technique for detection of rickettsiae in ticks. Am. J. Trop. Med. Hyg. **19:**1010–1014.
32. **Burgdorfer, W., A. Aeschlimann, O. Peter, S. F. Hayes, and R. N. Philip.** 1979. *Ixodes ricinus*: vector of a hitherto undescribed spotted fever group agent in Switzerland. Acta Trop. **36:**357–367.
33. **Burgdorfer, W., L. P. Brinton, W. L. Krinsky, and R. N. Philip.** 1978. *Rickettsia rhipicephali*: a new spotted fever group rickettsia from the brown dog tick *Rhipicephalus sanguineus*, p. 307–316. *In* J. Kazar, R. A. Ormsbee, and I. V. Tarasevich (ed.), Rickettsiae and rickettsial diseases. Veda, Publishing House of the Slovak Academy of Sciences, Bratislava, Slovakia.
34. **Carl, M., M. E. Dobson, W. M. Ching, and G. A. Dasch.** 1990. Characterization of the gene encoding the protective paracrystalline-surface-layer protein of *Rickettsia prowazekii*: presence of a truncated identical homolog in *Rickettsia typhi*. Proc. Natl. Acad. Sci. USA **87:**8237–8241.
35. **Carl, M., C. W. Tibbs, M. E. Dobson, S. Paparello, and G. A. Dasch.** 1990. Diagnosis of acute typhus infection using the polymerase chain reaction. J. Infect. Dis. **161:**791–793.
36. **Castaneda, M. R., and S. Zia.** 1933. The antigenic relationship between proteus X-19 and typhus rickettsiae. J. Exp. Med. **58:**55–62.
37. **Chang, R. S., E. S. Murray, and J. C. Snyder.** 1954. Erythrocyte-sensitizing substances from rickettsiae of the Rocky Mountain spotted fever group. J. Immunol. **73:**8–15.
38. **Clements, M. L., J. S. Dumler, P. Fiset, C. L. Wisseman, Jr., M. J. Snyder, and M. M. Levine.** 1983. Serodiagnosis of Rocky Mountain spotted fever: comparison of IgM and IgG enzyme-linked immunosorbent assays and indirect fluorescent antibody test. J. Infect. Dis. **148:**876–880.
39. **Cox, H. R.** 1938. Use of yolk sac of developing chick embryo as medium for growing rickettsiae of Rocky Mountain spotted fever and typhus group. Public Health Rep. **53:**2241–2247.
40. **Crum, J. W., S. Hanchalay, and C. Eamsila.** 1980. New paper enzyme-linked immunosorbent technique compared with microimmunofluorescence for detection of human serum antibodies to *Rickettsia tsutsugamushi*. J. Clin. Microbiol. **11:**584–588.
41. **Dasch, G. A.** 1981. Isolation of species-specific protein antigens of *Rickettsia typhi* and *Rickettsia prowazekii* for immunodiagnosis and immunoprophylaxis. J. Clin. Microbiol. **14:**333–341.
42. **Dasch, G. A., S. Halle, and A. L. Bourgeois.** 1979. Sensitive microplate enzyme-linked immunosorbent assay for detection of antibodies against the scrub typhus rickettsia, *Rickettsia tsutsugamushi*. J. Clin. Microbiol. **9:**38–48.
43. **Dasch, G. A., J. P. Burans, M. E. Dobson, F. M. Rollwagen, and J. Misiti.** 1984. Approaches to subunit vaccines against the typhus rickettsiae, *Rickettsia typhi* and *Rickettsia prowazekii*, p. 251–256. *In* L. Leive and D. Schlessinger (ed.), Microbiology—1984. American Society for Microbiology, Washington, D.C.
44. **Davis, J. P., W. Burgdorfer, L. T. Gutman, P. Melvin, R. N. Philip, and C. M. Wilfert.** 1978. Primary human monocyte culture for diagnosis of Rocky Mountain spotted fever (RMSF). Pediatr. Res. **12:**490.
45. **DeShazo, R. D., J. R. Boyce, J. V. Osterman, and E. H. Stephenson.** 1976. Early diagnosis of Rocky Mountain spotted fever. Use of primary monocyte culture technique. JAMA **235:**1353–1355.
46. **Drancourt, M., F. Georges, P. Brouqui, J. Sampol, and D. Raoult.** 1992. Diagnosis of Mediterranean spotted fever by indirect immunofluorescence of *Rickettsia conorii* in circulating endothelial cells isolated with monoclonal antibody-coated immunomagnetic beads. J. Infect. Dis. **166:**660–663.
47. **Dumler, J. S., W. R. Gage, G. L. Pettis, A. F. Azad, and F. P. Kuhadja.** 1990. Rapid immunoperoxidase demonstration of *Rickettsia rickettsii* in fixed cutaneous specimens from patients with Rocky Mountain spotted fever. Am. J. Clin. Pathol. **93:**410.
48. **Dumler, J. S., J. P. Taylor, and D. H. Walker.** 1991. Clinical and laboratory features of murine typhus in South Texas, 1980 through 1987. JAMA **266:**1365–1370.
49. **Elisberg, B. L., J. M. Campbell, and F. M. Bozeman.** 1968. Antigenic diversity of *Rickettsia tsutsugamushi*: epidemiologic and ecologic significance. J. Hyg. Epidemiol. Microbiol. Immunol. **12:**18–25.
50. **Eremeeva, M., N. M. Balayeva, V. Roux, V. Ignatovich, M. Kotsinjan, and D. Raoult.** 1995. Genomic and proteinic characterization of strain S, a rickettsia isolated from *Rhipicephalus sanguineus* ticks in Armenia. J. Clin. Microbiol. **33:**2738–2744.
51. **Eremeeva, M. E., N. M. Balayeva, V. F. Ignatovich, and D. Raoult.** 1993. Proteinic and genomic identification of spotted fever group rickettsiae isolated in the former USSR. J. Clin. Microbiol. **31:**2625–2633.
52. **Eremeeva, M. E., N. M. Balayeva, and D. Raoult.** 1994. Purification of rickettsial cultures contaminated by mycoplasmas. Acta Virol. **38:**231–233.
53. **Eremeeva, M. E., N. M. Balayeva, and D. Raoult.** 1995. Serological response of patients suffering from primary and recrudescent typhus: comparison of complement fixation reaction, Weil-Felix test, microimmunofluorescence, and immunoblotting. Clin. Diagn. Lab. Immunol. **1:**318–324.
54. **Eremeeva, M. E., L. Beati, V. A. Makarova, N. F. Fetisova, I. V. Tarasevich, N. M. Balayeva, and D. Raoult.** 1994. Astrakhan fever rickettsiae: antigenic and genotypic analysis of isolates obtained from human and *Rhipicephalus pumilio* ticks. Am. J. Trop. Med. Hyg. **51:**697–706.
55. **Eremeeva, M. E., X. Yu, and D. Raoult.** 1994. Differentiation among spotted fever group rickettsiae species by analysis of restriction fragment length

polymorphism of PCR-amplified DNA. J. Clin. Microbiol. **32:**803–810.

56. **Espejo-Arenas, E., and D. Raoult.** 1989. First isolates of *Rickettsia conorii* in Spain using a centrifugation-shell vial assay. J. Infect. Dis. **159:**1158–1159. (Letter.)
57. **Fan, M. Y., D. H. Walker, S. R. Yu, and Q. H. Liu.** 1987. Epidemiology and ecology of rickettsial diseases in the People's Republic of China. Rev. Infect. Dis. **9:**823–840.
58. **Feng, H. M., D. H. Walker, and J. G. Wang.** 1987. Analysis of T-cell-dependent and -independent antigens of *Rickettsia conorii* with monoclonal antibodies. Infect. Immun. **55:**7–15.
59. **Fiset, P., R. A. Ormsbee, R. Silberman, M. Peacock, and S. H. Spielman.** 1969. A microagglutination technique for detection and measurement of rickettsial antibodies. Acta Virol. **13:**60–66.
60. **Furuya, Y., T. Katayama, Y. Yoshida, and I. Kaiho.** 1995. Specific amplification of *Rickettsia japonica* DNA from clinical specimens by PCR. J. Clin. Microbiol. **33:**487–489.
61. **Furuya, Y., S. Yamamoto, M. Otu, Y. Yoshida, N. Ohashi, M. Murata, N. Kawabata, A. Tamura, and A. J. Kawamura.** 1991. Use of monoclonal antibodies against *Rickettsia tsutsugamushi* Kawasaki for serodiagnosis by enzyme-linked immunosorbent assay. J. Clin. Microbiol. **29:**340–345.
62. **Furuya, Y., Y. Yoshida, T. Katayama, S. Yamamoto, and A. Kawamura, Jr.** 1993. Serotype-specific amplification of *Rickettsia tsutsugamushi* DNA by nested polymerase chain reaction. J. Clin. Microbiol. **31:**1637–1643.
63. **Garcia, L. S., and D. A. Bruckner.** 1993. Diagnostic medical parasitology. American Society for Microbiology, Washington, D.C.
64. **George, F., P. Brouqui, M. C. Boffa, M. Mutin, M. Drancourt, C. Brisson, D. Raoult, and J. Sampol.** 1993. Demonstration of *Rickettsia conorii*-induced endothelial injury in vivo by measuring circulating endothelial cells, thrombomodulin and Von Willebrand factor in patients with Mediterranean spotted fever. Blood **82:**2109–2116.
65. **Gilmore, R. D., W. Cieplak, P. F. Policastro, and T. Hackstadt.** 1991. The 120 kilodalton outer membrane protein (rOmpB) of *Rickettsia rickettsii* is encoded by an unusually long open reading frame. Evidence for protein processing from a large precursor. Mol. Microbiol. **5:**2361–2370.
66. **Gimenez, D. F.** 1964. Staining rickettsiae in yolk-sac cultures. Stain Technol. **39:**135–140.
67. **Giroud, P.** 1973. Syndromes cliniques non classiques provoqués par des rickettsies et des agents proches. Med. Mal. Infect. **3:**241–245.
68. **Goldwasser, R. A., Y. Steiman, W. Klingberg, T. A. Swartz, and M. A. Klingberg.** 1974. The isolation of strains of rickettsiae of the spotted fever group in Israel and their differentiation from other members of the group by immunofluorescence methods. Scand. J. Infect. Dis. **6:**53–62.
69. **Green, W. R., D. H. Walker, and B. G. Cain.** 1978. Fatal viscerotropic Rocky Mountain spotted fever. Report of a case diagnosed by immunofluorescence. Am. J. Med. **64:**523–528.
70. **Gross, E. M., and P. Yagupsky.** 1987. Israeli rickettsial spotted fever in children. A review of 54 cases. Acta Trop. **44:**91–96.
71. **Halle, S., G. A. Dasch, and E. Weiss.** 1977. Sensitive enzyme-linked immunosorbent assay for detection of antibodies against typhus rickettsiae, *Rickettsia prowazekii* and *Rickettsia typhi*. J. Clin. Microbiol. **6:**101–110.
72. **Hechemy, K. E., R. L. Anacker, N. L. Carlo, J. A. Fox, and H. A. Gaafar.** 1983. Absorption of *Rickettsia rickettsii* antibodies by *Rickettsia rickettsii* antigens in four diagnostic tests. J. Clin. Microbiol. **17:**445–449.
73. **Hechemy, K. E., R. L. Anacker, R. N. Philip, K. T. Kleeman, J. N. MacCormack, S. J. Sasowski, and E. E. Michaelson.** 1980. Detection of Rocky Mountain spotted fever antibodies by a latex agglutination test. J. Clin. Microbiol. **12:**144–150.
74. **Hechemy, K. E., J. V. Osterman, C. S. Eisemann, L. B. Elliott, and S. J. Sasowski.** 1981. Detection of typhus antibodies by latex agglutination. J. Clin. Microbiol. **13:**214–216.
75. **Hechemy, K. E., D. Raoult, C. Eisemann, Y. S. Han, and J. A. Fox.** 1986. Detection of antibodies to *Rickettsia conorii* with a latex agglutination test in patients with Mediterranean spotted fever. J. Infect. Dis. **153:**132–135.
76. **Hechemy, K. E., R. W. Stevens, S. Sasowski, E. E. Michaelson, E. A. Casper, and R. N. Philip.** 1979. Discrepancies in Weil-Felix and microimmunofluorescence test results for Rocky Mountain spotted fever. J. Clin. Microbiol. **9:**292–293.
77. **Hersey, D. F., M. C. Colvin, and C. C. Shepard.** 1957. Studies on the serologic diagnosis of murine typhus and Rocky Mountain spotted fever. J. Immunol. **79:**409–415.
78. **Higgins, J. A., S. Radulovic, M. E. Schriefer, and A. F. Azad.** 1996. *Rickettsia felis*: a new species of pathogenic rickettsia isolated from cat fleas. J. Clin. Microbiol. **34:**671–674.
79. **Horinouchi, H., K. Murai, A. Okayama, Y. Nagatomo, N. Tachibana, and H. Tsubouchi.** 1996. Genotypic identification of *Rickettsia tsutsugamushi* by restriction fragment length polymorphism analysis of DNA amplified by the polymerase chain reaction. Am. J. Trop. Med. Hyg. **54:**647–651.
80. **Jaffe, E. A., R. L. Nachman, C. G. Becker, and C. R. Minick.** 1973. Culture of human endothelial cells derived from umbilical veins. Identification by morphologic and immunologic criteria. J. Clin. Invest. **52:**2745–2756.
81. **Kaplan, J. E., and L. B. Schonberger.** 1986. The sensitivity of various serologic tests in the diagnosis of Rocky Mountain spotted fever. Am. J. Trop. Med. Hyg. **35:**840–844.
82. **Kaplowitz, L. G., J. V. Lange, J. J. Fischer, and D. H. Walker.** 1983. Correlation of rickettsial titers, circulating endotoxin, and clinical features in Rocky Mountain spotted fever. Arch. Intern. Med. **143:**1149–1151.
83. **Kelly, D. J., P. W. Wong, E. Gan, and G. E. Lewis, Jr.** 1988. Comparative evaluation of the indirect immunoperoxidase test for the serodiagnosis of rickettsial disease. Am. J. Trop. Med. Hyg. **38:**400–406.
84. **Kelly, P. J., L. Beati, P. R. Mason, L. A. Matthewman, V. Roux, and D. Raoult.** 1996. *Rickettsia africae* sp. nov., the etiological agent of African tick bite fever. Int. J. Syst. Bacteriol. **46:**611–614.
85. **Kelly, P. J., L. Matthewman, L. Beati, D. Raoult, P. Mason, M. Dreary, and R. Makombe.** 1992. African tick-bite fever—a new spotted fever group rickettsiosis under an old name. Lancet **340:**982–983.
86. **Kelly, P. J., D. Raoult, and P. R. Mason.** 1991. Isolation of spotted fever group rickettsias from triturated ticks using a modification of the centrifugation-shell vial technique. Trans. R. Soc. Trop. Med. Hyg. **85:**397–398.
87. **Kleeman, K. T., J. L. Hicks, R. L. Anacker, R. L. Philip, E. A. Casper, K. E. Hechemy, C. M. Wilfert, and J. N. MacCormack.** 1996. Early detection of antibody to *Rickettsia rickettsii*: a comparison of four serological methods: indirect hemagglutination, indirect fluorescent antibody, latex agglutination, and complement fixation, p. 171–178. *In* J. Kazar (ed.), Rickettsiae and rickettsial diseases. Veda, Publishing House of the Slovak Academy of Sciences, Bratislava, Slovakia.
88. **Lange, J. V., and D. H. Walker.** 1984. Production and characterization of monoclonal antibodies to *Rickettsia rickettsii*. Infect. Immun. **46:**289–294.
89. **La Scola, B., and D. Raoult.** 1996. Diagnosis of Mediterranean spotted fever by cultivation of *Rickettsia conorii* from blood and skin samples using the centrifugation-shell vial technique and by detection of *R. conorii* in circulating endothelial cells: a 6-year follow-up. J. Clin. Microbiol. **34:**2722–2727.
90. **Mahara, F.** 1984. Three Weil-Felix reaction OX2 positive cases with skin eruptions and high fever. J. Anan Med. Assoc. **68:**4–7.
91. **Mahara, F.** 1987. Japanese spotted fever. A new disease named for spotted fever group rickettsiosis in Japan. Ann. Rep. Ohara Hosp. **30:**83–89.
92. **Mansueto, S., G. Tringali, R. Di Leo, M. Maniscalco, M. R. Montenegro, and D. H. Walker.** 1984. Demonstration of spotted fever group rickettsiae in the tache noire of a healthy person in Sicily. Am. J. Trop. Med. Hyg. **33:**479–482.
93. **Mansueto, S., G. Vitale, M. Bentivegna, G. Tringali, and R. Di Leo.** 1985. Persistence of antibodies to *Rickettsia conorii* after an acute attack of boutonneuse fever. J. Infect. Dis. **151:**377. (Letter.)
94. **Marrero, M., and D. Raoult.** 1989. Centrifugation-shell vial technique for rapid detection of Mediterranean spotted fever rickettsia in blood culture. Am. J. Trop. Med. Hyg. **40:**197–199.
95. **Marx, R. S.** 1983. Rocky mountain spotted fever. Compr. Ther. **9:**43–48.
96. **McDade, J. E., C. M. Black, L. F. Roumillat, M. A. Redus, and C. L. Spruill.** 1988. Addition of monoclonal antibodies specific for *Rickettsia akari* to the rickettsial diagnostic panel. J. Clin. Microbiol. **26:**2221–2223.
97. **Murai, K., A. Okayama, H. Horinouchi, T. Oshikawa, N. Tachibana, and H. Tsubouchi.** 1995. Eradication of *Rickettsia tsutsugamushi* from patients' blood by chemotherapy, as assessed by the polymerase chain reaction. Am. J. Trop. Med. Hyg. **52:**325–327.
98. **Murray, E. S., G. Baehr, R. A. Mandelbaum, N. Rosenthal, J. C. Doane, L. B. Weiss, S. Cohen, and J. C. Snyder.** 1950. Brill's disease. JAMA **142:**1059–1066.
99. **Newhouse, V. F., C. C. Shepard, M. D. Redus, T. Tzianabos, and J. E. McDade.** 1979. A comparison of the complement fixation, indirect fluorescent antibody, and microagglutination tests for the serological diagnosis of rickettsial diseases. Am. J. Trop. Med. Hyg. **28:**387–395.
100. **Nigg, C., and K. Landsteiner.** 1932. Studies on the cultivation of the typhus fever rickettsia in the presence of live tissue. J. Exp. Med. **55:**563–576.
101. **Ohashi, N., H. Nashimoto, H. Ikeda, and A. Tamura.** 1990. Cloning and sequencing of the gene (tsg56) encoding a type-specific antigen from *Rickettsia tsutsugamushi*. Gene **91:**119–122.
102. **Ormsbee, R., M. Peacock, R. Philip, E. Casper, J. Plorde, T. Gabre-Kidan, and L. Wright.** 1977. Serologic diagnosis of epidemic typhus fever. Am. J. Epidemiol. **105:**261–271.
103. **Ormsbee, R. A., M. G. Peacock, R. Gerloff, G. Tallent, and D. Wike.** 1978. Limits of rickettsial infectivity. Infect. Immun. **19:**239–245.
104. **Ormsbee, R. A., M. G. Peacock, R. Philip, E. Casper, J. Plorde, T. Gabre-Kidan, and L. Wright.** 1978. Antigenic relationships between the typhus and spotted fever groups of rickettsiae. Am. J. Epidemiol. **108:**53–59.
105. **Parker, R. R., G. M. Kohls, G. W. Cox, and G. E. Davis.** 1939. Observations on an infectious agent from *Amblyomma maculatum*. Public Health Rep. **54:**1482–1484.
106. **Perine, P. L., B. P. Chandler, D. K. Krause, P. McCardle, S. Awoke, E. Habte-Gabr, C. L. Wisseman, and J. E. McDade.** 1992. A clinico-epidemiological study of epidemic typhus in Africa. Clin. Infect. Dis. **14:**1149–1158.
107. **Peter, O., D. Raoult, and B. Gilot.** 1990. Isolation by a sensitive centrifugation cell culture system of 52 strains of spotted fever group rickettsiae from ticks collected in France. J. Clin. Microbiol. **28:**1597–1599.

108. **Philip, R. N., and E. A. Casper.** 1981. Serotypes of spotted fever group rickettsiae isolated from *Dermacentor andersoni* (Stiles) ticks in western Montana. Am. J. Trop. Med. Hyg. **30:**230–238.
109. **Philip, R. N., E. A. Casper, R. L. Anacker, J. Cory, S. F. Hayes, W. Burgdorfer, and E. Yunker.** 1983. *Rickettsia bellii* sp. nov.: a tick-borne rickettsia, widely distributed in the United States, that is distinct from the spotted fever and typhus biogroups. Int. J. Syst. Bacteriol. **33:**94–106.
110. **Philip, R. N., E. A. Casper, W. Burgdorfer, R. K. Gerloff, L. E. Hugues, and E. J. Bell.** 1978. Serologic typing of rickettsiae of the spotted fever group by micro immunofluorescence. J. Immunol. **121:**1961–1968.
111. **Philip, R. N., E. A. Casper, J. N. MacCormack, D. Sexton, L. A. Thomas, R. L. Anacker, W. Burgdorfer, and S. Vick.** 1977. A comparison of serologic methods for diagnosis of Rocky Mountain spotted fever. Am. J. Epidemiol. **105:**56–67.
112. **Philip, R. N., E. A. Casper, R. A. Ormsbee, M. G. Peacock, and W. Burgdorfer.** 1976. Microimmunofluorescence test for the serological study of Rocky Mountain spotted fever and typhus. J. Clin. Microbiol. **3:**51–61.
113. **Philip, R. N., R. S. Lane, and E. A. Casper.** 1981. Serotypes of tick-borne spotted fever group rickettsiae from western California. Am. J. Trop. Med. Hyg. **30:**722–727.
114. **Pickens, E. G., E. J. Bell, D. B. Lackman, and W. Burgdorfer.** 1965. Use of mouse serum in identification and serologic classification of *Rickettsia akari* and *Rickettsia australis*. J. Immunol. **94:**883–889.
115. **Plotz, H., R. L. Reagan, and K. Wertman.** 1944. Differentiation between "Fièvre boutonneuse" and Rocky Mountain spotted fever by means of complement fixation. Proc. Soc. Exp. Biol. Med. **55:**173–176.
116. **Radulovic, S., H. M. Feng, P. Crocquet-Valdes, M. Morovic, B. Dzelalija, and D. H. Walker.** 1994. Antigen-capture enzyme immunoassay: a comparison with other methods for the detection of spotted fever group rickettsiae in ticks. Am. J. Trop. Med. Hyg. **50:**359–364.
117. **Radulovic, S., H. M. Feng, M. Morovic, D. Djelalija, V. Popov, P. Crocquetvaldes, and D. H. Walker.** 1996. Isolation of *Rickettsia akari* from a patient in a region where Mediterranean spotted fever is endemic. Clin. Infect. Dis. **22:**216–220.
118. **Radulovic, S., J. A. Higgins, D. C. Jaworski, G. A. Dasch, and A. F. Azad.** 1995. Isolation, cultivation, and partial characterization of the ELB agent associated with cat fleas. Infect. Immun. **63:**4826–4829.
119. **Raoult, D., J. P. Arzouni, M. C. Jambon, J. Beytout, and O. Ramousse.** 1994. Western blot as a seroepidemiologic tool for detecting foci of Mediterranean spotted fever (MSF). Eur. J. Epidemiol. **10:**37–40.
120. **Raoult, D., P. Berbis, V. Roux, W. Xu, and M. Maurin.** A new tick-transmitted disease due to *Rickettsia slovaca*. Lancet **350:**112–113.
121. **Raoult, D., P. Brouqui, and V. Roux.** 1996. A new spotted-fever-group rickettsiosis. Lancet **348:**412.
122. **Raoult, D., and G. A. Dasch.** 1989. Line blot and Western blot immunoassays for diagnosis of Mediterranean spotted fever. J. Clin. Microbiol. **27:** 2073–2079.
123. **Raoult, D., and G. A. Dasch.** 1989. The line blot: an immunoassay for monoclonal and other antibodies. Its application to the serotyping of gram-negative bacteria. J. Immunol. Methods **125:**57–65.
124. **Raoult, D., and G. A. Dasch.** 1995. Immunoblot cross-reactions among *Rickettsia*, *Proteus* spp. and *Legionella* spp. in patients with Mediterranean spotted fever. FEMS Immunol. Med. Microbiol. **11:**13–18.
125. **Raoult, D., C. De Micco, H. Chaudet, and J. Tamalet.** 1985. Serological diagnosis of Mediterranean spotted fever by the immunoperoxidase reaction. Eur. J. Clin. Microbiol. Infect. Dis. **4:**441–442. (Letter.)
126. **Raoult, D., C. De Micco, H. Gallais, and M. Toga.** 1984. Laboratory diagnosis of Mediterranean spotted fever by immunofluorescent demonstration of *Rickettsia conorii* in cutaneous lesions. J. Infect. Dis. **150:**145–148.
127. **Raoult, D, K. E. Hechemy, and H. Chaudet.** 1985. Serology of Mediterranean boutonneuse fever. Kinetics of antibodies detected by 3 methods: indirect immunofluorescence, indirect hemagglutination and latex agglutination. Pathol. Biol. **33:**839–841.
128. **Raoult, D., S. Rousseau, B. Toga, C. Tamalet, H. Gallais, P. de Micco, and P. Casanova.** 1984. Serological diagnosis of Mediterranean boutonneuse fever. Pathol. Biol. **32:**791–794.
129. **Raoult, D., P. J. Weiller, A. Chagnon, H. Chaudet, H. Gallais, and P. Casanova.** 1986. Mediterranean spotted fever: clinical, laboratory and epidemiological features of 199 cases. Am. J. Trop. Med. Hyg. **35:**845–850.
130. **Raoult, D., P. Zuchelli, P. J. Weiller, C. Charrel, J. L. San Marco, H. Gallais, and P. Casanova.** 1986. Incidence, clinical observations and risk factors in the severe form of Mediterranean spotted fever among patients admitted to hospital in Marseilles 1983–1984. J. Infect. **12:**111–116.
131. **Regnery, R. L., C. L. Spruill, and B. D. Plikaytis.** 1991. Genomic identification of rickettsiae and estimation of intraspecies sequence divergence for portions of two rickettsial genes. J. Bacteriol. **173:**1576–1589.
132. **Rehacek, J., and I. V. Tarasevich.** 1988. Acari-borne rickettsiae and rickettsioses in Eurasia, p. 128–145. Veda, Publishing House of the Slovak Academy of Sciences, Bratislava, Slovakia.
133. **Robertson, R. G., and C. L. Wisseman, Jr.** 1973. Tick-borne rickettsiae of the spotted fever group in west Pakistan. II. Serological classification of isolates from west Pakistan and Thailand: evidence for two new species. Am. J. Epidemiol. **97:**55–64.
134. **Roux, V., P.-E. Fournier, and D. Raoult.** 1996. Differentiation of spotted fever group rickettsiae by sequencing and analysis of restriction fragment length polymorphism of PCR-amplified DNA of the gene encoding the protein rOmpA. J. Clin. Microbiol. **34:**2058–2065.
135. **Roux, V., and D. Raoult.** 1993. Genotypic identification and phylogenetic analysis of the spotted fever group rickettsiae by pulsed-field gel electrophoresis. J. Bacteriol. **175:**4895–4904.
136. **Roux, V., and D. Raoult.** 1995. Phylogenetic analysis of the genus Rickettsia by 16S rDNA sequencing. Res. Microbiol. **146:**385–396.
137. **Roux, V., E. Rydkina, M. Eremeeva, and D. Raoult.** 1997. Citrate synthase gene comparison, a new tool for phylogenetic analysis, and its application for the rickettsiae. Int. J. Syst. Bacteriol. **47:**252–261.
138. **Saah, A. J., and R. B. Hornick.** 1985. Rickettsiosis, *Rickettsia akari* (rickettsialpox), p. 1087–1088. *In* G. L. Mandell, R. G. Douglas, and J. E. Bennett (ed.), Principles and practice of infectious diseases. John Wiley & Sons, Inc., New York, N.Y.
139. **Sarov, B., E. Manor, N. Hanuka, E. Sikuler, A. Galil, and I. Sarov.** 1992. Comparison of structural polypeptides, detected by immunoblotting technique, in the sera of spotted fever group Rickettsia positive cases—symptomatic versus asymptomatic. Acta Virol. **36:**57–61.
140. **Saunders, J. P., G. W. Brown, A. Shirai, and D. L. Huxsoll.** 1980. The longevity of antibody to Rickettsia tsutsugamushi in patients with confirmed scrub typhus. Trans. R. Soc. Trop. Med. Hyg. **74:**253–257.
141. **Schattner, A., M. Leitner, A. Keysary, and D. Geltner.** 1992. Fatal seronegative rickettsial infection diagnosed by the polymerase chain reaction. Am. J. Med. Sci. **303:**392–394.
142. **Seung Kang, J., and W. H. Chang.** 1996. Serological classification of *Rickettsia tsutsugamushi* by indirect immunofluorescent test using monoclonal antibodies against eight prototype strains, p. 91–96. *In* J. Kazar and R. Toman (ed.), Rickettsiae and rickettsial diseases. Veda, Publishing House of the Slovak Academy of Sciences, Bratislava, Slovakia.
143. **Sexton, D. J., B. W. Dwyer, R. Kemp, and S. Graves.** 1991. Spotted fever group rickettsial infections in Australia. Rev. Infect. Dis. **13:**876–886.
144. **Shepard, C. C., M. A. Redus, T. Tzianabos, and D. T. Warfield.** 1976. Recent experience with the complement fixation test in the laboratory diagnosis of rickettsial diseases in the United States. J. Clin. Microbiol. **4:**277–283.
145. **Shirai, A., J. W. Dietel, and J. V. Osterman.** 1975. Indirect hemagglutination test for human antibody to typhus and spotted fever group rickettsiae. J. Clin. Microbiol. **2:**430–437.
146. **Sompolinsky, D., I. Boldur, R. A. Goldwasser, H. Kahana, R. Kazak, A. Keysary, and A. Pik.** 1986. Serological cross-reactions between *Rickettsia typhi*, *Proteus vulgaris* OX19, and *Legionella bozemanii* in a series of febrile patients. Isr. J. Med. Sci. **22:**745–752.
147. **Stewart, R. S.** 1991. Flinders Island spotted fever: a newly recognised endemic focus of tick typhus in Bass Strait. Part 1. Clinical and epidemiological features. Med. J. Aust. **154:**94–99.
148. **Stothard, D. R., J. B. Clark, and P. A. Fuerst.** 1994. Ancestral divergence of *Rickettsia bellii* from the spotted fever and typhus groups of *Rickettsia* and antiquity of the genus *Rickettsia*. Int. J. Syst. Bacteriol. **44:**798–804.
149. **Stothard, D. R., and P. A. Fuerst.** 1995. Evolutionary analysis of the spotted fever and typhus groups of *Rickettsia* using 16S rRNA gene sequences. Syst. Appl. Microbiol. **18:**52–61.
150. **Stover, C. K., D. P. Marana, J. M. Carter, B. A. Roe, E. Mardis, and E. V. Oaks.** 1990. The 56-kilodalton major protein antigen of *Rickettsia tsutsugamushi*: molecular cloning and sequence analysis of the *sta*56 gene and precise identification of a strain-specific epitope. Infect. Immun. **58:**2076–2084.
151. **Sugita, Y., T. Nagatani, K. Okuda, Y. Yoshida, and H. Nakajima.** 1992. Diagnosis of typhus infection with *Rickettsia tsutsugamushi* by polymerase chain reaction. J. Med. Microbiol. **37:**357–360.
152. **Sugita, Y., Y. Yamakawa, K. Takahashi, T. Nagatani, K. Okuda, and H. Nakajima.** 1993. A polymerase chain reaction system for rapid diagnosis of scrub typhus within six hours. Am. J. Trop. Med. Hyg. **49:**636–640.
153. **Suto, T.** 1985. Rapid and accurate serologic diagnosis of tsutsugamushi disease in Japan employing the immunoperoxidase reaction, p. 444–452. *In* J. Kazar (ed.), Rickettsiae and rickettsial diseases. Veda, Publishing House of the Slovak Academy of Sciences, Bratislava, Slovakia.
154. **Suto, T.** 1991. A ten years experience on diagnosis of rickettsial diseases using the indirect immunoperoxidase methods. Acta Virol. **35:**580–586.
155. **Tamura, A.** 1996. Overview of *Orientia tsutsugamushi* based on recent findings, p. 62–63. *In* J. Kazar and R. Toman (ed.), Rickettsiae and rickettsial diseases. Veda, Publishing House of the Slovak Academy of Sciences, Bratislava, Slovakia.
156. **Tamura, A., N. Ohashi, H. Urakami, and S. Miyamura.** 1995. Classification of *Rickettsia tsutsugamushi* in a new genus, *Orientia* gen. nov., as *Orientia tsutsugamushi* comb. nov. Int. J. Syst. Bacteriol. **45:**589–591.
157. **Tamura, A., K. Takahashi, T. Tsuruhara, H. Urakami, S. Miyamura, H. Sekikawa, M. Kenmotsu, M. Shibata, S. Abe, and H. Nezu.** 1984. Isolation of *Rickettsia tsutsugamushi* antigenically different from Kato, Karp, and

Gilliam strains from patients. Microbiol. Immunol. **28:**873–882.

158. **Tarasevich, I. V., V. Makarova, N. F. Fetisova, A. Stepanov, E. Mistkarova, N. M. Balayeva, and D. Raoult.** 1991. Astrakhan fever: new spotted fever group rickettsiosis. Lancet **337:**172–173.
159. **Tarasevich, I. V., V. A. Makarova, N. F. Fetisova, A. V. Stepanov, E. D. Miskarova, and D. Raoult.** 1991. Studies of a "new" rickettsiosis: "Astrakhan" spotted fever. Eur. J. Epidemiol. **7:**294–298.
160. **Teysseire, N., and D. Raoult.** 1992. Comparison of Western immunoblotting and microimmunofluoresence for diagnosis of Mediterranean spotted fever. J. Clin. Microbiol. **30:**455–460.
161. **Tzianabos, T., B. E. Anderson, and J. E. McDade.** 1989. Detection of *Rickettsia rickettsii* DNA in clinical specimens by using polymerase chain reaction technology. J. Clin. Microbiol. **27:**2866–2868.
162. **Uchida, T.** 1993. *Rickettsia japonica*, the etiologic agent of oriental spotted fever. Microbiol. Immunol. **37:**91–102.
163. **Uchida, T., F. Mahara, Y. Tsuboi, and A. Oya.** 1985. Spotted fever group rickettsiosis in Japan. Jpn. J. Med. Sci. Biol. **38:**151–153.
164. **Uchida, T., T. Uchiyama, K. Kumano, and D. H. Walker.** 1992. *Rickettsia japonica* sp. nov., the etiological agent of spotted fever group rickettsiosis in Japan. Int. J. Syst. Bacteriol. **42:**303–305.
165. **Uchiyama, T., T. Uchida, and D. H. Walker.** 1990. Species-specific monoclonal antibodies to *Rickettsia japonica*, a newly identified spotted fever group rickettsia. J. Clin. Microbiol. **28:**1177–1180.
166. **Walker, D. H.** 1989. Rocky Mountain spotted fever: a disease in need of microbiological concern. Clin. Microbiol. Rev. **2:**227–240.
167. **Walker, D. H.** 1990. The role of host factors in the severity of spotted fever and typhus rickettsioses. Ann. N. Y. Acad. Sci. **590:**10–19.
168. **Walker, D. H., M. S. Burday, and J. D. Folds.** 1980. Laboratory diagnosis of Rocky Mountain spotted fever. South. Med. J. **73:**1443–1449.
169. **Walker, D. H., and B. G. Cain.** 1978. A method for specific diagnosis of Rocky Mountain spotted fever on fixed, paraffin-embedded tissue by immunofluorescence. J. Infect. Dis. **137:**206–209.
170. **Walker, D. H., B. G. Cain, and P. M. Olmstead.** 1978. Laboratory diagnosis of Rocky Mountain spotted fever by immunofluorescent demonstration of *Rickettsia* in cutaneous lesions. Am. J. Clin. Pathol. **69:**619–623.
171. **Walker, D. H., B. G. Cain, and P. M. Olmstead.** 1978. Specific diagnosis of Rocky Mountain spotted fever by immunofluorescence demonstration of *Rickettsia rickettsii* in biopsy of skin. Public Health Lab. Rep. **36:**96–100.
172. **Walker, D. H., and D. B. Fishbein.** 1991. Epidemiology of rickettsial diseases. Eur. J. Epidemiol. **7:**237–245.
173. **Walker, D. H., R. M. Gay, and M. Valdes-Dapena.** 1981. The occurrence of eschars in Rocky Mountain spotted fever. J. Am. Acad. Dermatol. **4:**571–576.
174. **Walker, D. H., Q. H. Liu, X. J. Yu, H. Li, C. Taylor, and H. M. Feng.** 1992. Antigenic diversity of *Rickettsia conorii*. Am. J. Trop. Med. Hyg. **47:**78–86.
175. **Walker, D. H., F. M. Parks, T. G. Betz, J. P. Taylor, and J. W. Muehlberger.** 1989. Histopathology and immunohistologic demonstration of the distribution of *Rickettsia typhi* in fatal murine typhus. Am. J. Clin. Pathol. **91:**720–724.
176. **Weddle, J. R., T. C. Chan, K. Thompson, H. Paxton, D. J. Kelly, G. Dasch, and D. Strickman.** 1995. Effectiveness of a dot-blot immunoassay of anti-*Rickettsia tsutsugamushi* antibodies for serologic analysis of scrub typhus. Am. J. Trop. Med. Hyg. **53:**43–46.
177. **Weil, E., and A. Felix.** 1916. Zur serologischen Diagnose des Fleckfiebers. Wien. Klin. Wochenschr. **29:**33–35.
178. **Weisburg, W. G., M. E. Dobson, J. E. Samuel, G. A. Dasch, L. P. Mallavia, O. Baca, L. Mandelco, J. E. Sechrest, E. Weiss, and C. R. Woese.** 1989. Phylogenetic diversity of the rickettsiae. J. Bacteriol. **171:**4202–4206.
179. **Weiss, E., and H. R. Dressler.** 1960. Centrifugation of rickettsiae and viruses into cells and its effect on infection. Proc. Soc. Exp. Biol. Med. **103:**691–695.
180. **Weiss, E., and J. W. Moulder.** 1984. Order I *Rickettsiales*, Gieszczkiewicz 1939, p. 687–703. *In* N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology. The Williams & Wilkins Co., Baltimore, Md.
181. **Werren, J. H., G. D. Hurst, W. Zhang, J. A. J. Breeuwer, R. Stouthamer, and M. E. N. Majerus.** 1994. Rickettsial relative associated with male killing in the ladybird beetle (*Adalia bipunctata*). J. Bacteriol. **176:**388–394.
182. **Wilfert, C. M., E. Austin, V. Dickinson, K. Kleeman, J. L. Hicks, J. N. MacCormack, R. L. Anacker, E. A. Casper, and R. N. Philip.** 1981. The incidence of Rocky Mountain spotted fever as described by prospective epidemiologic surveillance and the assessment of persistence of antibodies to *R. rickettsii* by indirect hemagglutination and microimmunofluorescence tests, p. 179–189. *In* W. Burgdorfer and R. L. Anacker (ed.), Rickettsiae and rickettsial diseases. Academic Press, Inc., New York, N.Y.
183. **Williams, W. J., S. Radulovic, G. A. Dasch, J. Lindstrom, D. J. Kelly, C. N. Oster, and D. H. Walker.** 1994. Identification of *Rickettsia conorii* infection by polymerase chain reaction in a soldier returning from Somalia. Clin. Infect. Dis. **19:**93–99.
184. **Wood, D. O., L. R. Williamson, H. H. Winkler, and D. C. Krause.** 1987. Nucleotide sequence of the *Rickettsia prowazekii* citrate synthase gene. J. Bacteriol. **169:**3564–3572.
185. **Woodward, T. E., C. E. Pedersen, Jr., C. N. Oster, L. R. Bagley, J. Romberger, and M. J. Snyder.** 1976. Prompt confirmation of Rocky Mountain spotted fever: identification of rickettsiae in skin tissues. J. Infect. Dis. **134:**297–301.
186. **Xu, W., L. Beati, and D. Raoult.** 1997. Characterization of and application of monoclonal antibodies against *Rickettsia africae*, a newly recognized species of spotted fever group rickettsia. J. Clin. Microbiol. **35:**64–70.
187. **Yamamoto, S., and Y. Minamishima.** 1982. Serodiagnosis of tsutsugamushi fever (scrub typhus) by the indirect immunoperoxidase technique. J. Clin. Microbiol. **15:**1128–1132.
188. **Yu, X., M. Fan, G. Xu, Q. Liu, and D. Raoult.** 1993. Genotypic and antigenic identification of two new strains of spotted fever group rickettsiae isolated from China. J. Clin. Microbiol. **31:**83–88.