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Rickettsioses as Paradigms of New or Emerging Infectious Diseases

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INTRODUCTION

Rickettsioses represent some of the oldest and most recently recognized infectious diseases. Epidemic typhus is suspected of being responsible for the Athens plague described by Thucydides during the 5th century BC, and the disease was certainly recognized during the 16th century, when the presence of an exanthema allowed its distinction, among fevers with tuphos, from typhoid (113, 264). Today, arthropod-borne rickettsial disease probably represents the most complete paradigm for understanding emerging diseases; of the 14 currently recognized rickettsioses, 6 have been described within the last 12 years. These newly described syndromes have resulted from several circumstances ranging from a single physician's curiosity and the introduction of new diagnostic tools to an improved knowledge of disease epidemiology, resulting in the demonstration of pathogenic roles for humans of rickettsiae previously found only in arthropods. Medical history is, however, full of stories in which pathogenic bacteria were first considered to be harmless "*Rickettsia*" species, the two most famous being *Coxiella burnetii*, the agent of Q fever, which was first isolated from an American tick (65) and *Legionella pneumophila*, whose association with Legionnaires' disease remained unknown from 1947 to 1976 (164).

For many years, rickettsiologists have had two reputations, one being that their scientific lives are potentially hazardous. Of the three prominent scientists studying epidemic typhus in the early part of this century, Ricketts and Von Prowazek died from rickettsial infections, and only Nicolle survived to collect the Nobel prize. The other reputation of rickettsiologists is one of having been involved in many recent discoveries of new infectious diseases because they are acquainted with techniques for growing strict intracellular parasites and because many physicians consider "unusual bacteria" to be rickettsiae. Rickettsiologists have played central roles in the discoveries of Legionnaire's disease (166), Lyme disease (49), ehrlichiosis (152), granulocytic ehrlichiosis (72), cat scratch disease (207), and new rickettsioses themselves.

Many new data on rickettsial diseases have been accumulated over recent years, and a comparison of the newly discovered diseases with previously known rickettsioses is of interest. Moreover, many other species must exist in arthropods without presently being associated with human disease. For *Rickettsia*, as with other genera of bacteria, it is hard to predict which are potential human pathogens. Furthermore, different isolates of the same species vary in virulence for the same host (161). For many years, the sole method for isolating rickettsiae was to inoculate animals, with guinea pigs being the most often used. This practice resulted in the selection of strains that were pathogenic for their experimental host. Only later did alternative isolation methods involving chicken embryos or cell culture become available to permit the characterization of new isolates. It is potentially misleading to rely on the results of studies of strains obtained from a specific animal model in making unbiased deductions regarding human pathogenicity. For example, the *Rickettsia rickettsii* T-type strain appears to be highly pathogenic for humans but induces only mild illness in guinea pigs (38). Indeed, since arthropod-transmitted rickettsiae are inoculated directly into the blood, one can suppose that potentially they can all cause disease if a sufficient inoculum is injected. Pathogenicity may, in fact, be linked to the ability of the host arthropod to bite humans; for example, new rickettsiae found in the ladybird beetle (AB bacterium) and in the pea aphid (pea aphid rickettsia) (61, 280) have not been implicated in human disease, probably because their hosts do not bite humans. Perhaps when a rickettsia is found in an arthropod capable of biting humans, it should be considered a potential human pathogen.

The precise classification within the genus *Rickettsia* is unclear, and more data are necessary to clarify the phylogenetic position of some bacteria. In this review, we will consider all rickettsiae of this genus. In addition to a general overview of the etiology of currently recognized rickettsioses, we will report on the modern tools used to identify new rickettsial pathogens, compare how previously and newly described rickettsial diseases were discovered, and theorize on which rickettsial species may be potential agents of future diseases.

BACTERIA

Bacteria of the order *Rickettsiales* were first described as short gram-negative bacillary microorganisms that retained basic fuchsin when stained by the method of Giménez (101) and grew in association with eukaryotic cells. Historically, the order *Rickettsiales* has been divided into three families, namely, *Rickettsiaceae*, *Bartonellaceae*, and *Anaplasmataceae*. The family *Rickettsiaceae* was composed of the tribes *Rickettsieae*, *Ehrlichieae*, and *Wolbachieae*, and the tribe *Rickettsieae* has long consisted of the genera *Coxiella*, *Rickettsia*, and *Rochalimaea* (277). This classification scheme continues to be modified as new information on these bacteria is uncovered.

The advent of molecular taxonomic methods, specifically 16S rRNA analysis, has enabled the determination of phylogenetic relationships between bacterial species (284). This methodology has been particularly useful in the study of intracellular bacteria that express few phenotypic characteristics traditionally used in taxonomy. Its application has exposed the shortfalls of traditional rickettsial taxonomy and provided a basis for reclassification of several species; *Coxiella burnetii* has now been removed from the order *Rickettsiales* following demonstration that its 16S rRNA sequence was most similar to those of members of the gamma subgroup of the *Proteobacteria*, rather than the alpha 1 subgroup to which *Rickettsia* spp. belong (274). Furthermore, the genus *Rochalimaea* has recently been placed in the genus *Bartonella*, which has been removed from the *Rickettsiales* since, phylogenetically, its members lie in the alpha 2 subgroup of the *Proteobacteria* (39). Hence, *Coxiella* and *Rochalimaea* no longer belong to the *Rickettsieae* tribe, leaving only the genus *Rickettsia*. This genus was subdivided into the typhus group (TG), whose members are *R. typhi*, *R. prowazekii*, and *R. canada*; the spotted fever group (SFG), which includes about 20 different species; and the scrub typhus group, which includes *R. tsutsugamushi*. Recent phylogenetic studies have demonstrated the evolutionary unity of the TG and the SFG rickettsiae. However, the position of *R. tsutsugamushi* has been found to be distinct enough to warrant transfer into a new genus *Orientia*, as *O. tsutsugamushi* (245).

Rickettsiae are strict intracellular parasites, requiring host cells in which to replicate. These bacteria lie exclusively intracellularly, although not enclosed by a vacuole (122, 252, 253). SFG rickettsiae can be observed in the nuclei of host cells, perhaps because they are able to move within the cell by means of actin polymerization (48, 122, 253). TG rickettsiae are observed exclusively in the cytoplasm (122, 253) (Fig. 1). Rickettsial genome sizes are small (1 to 1.6 Mb) and consist of a single circular chromosome (84, 220, 222). Rickettsiae are associated with arthropods which can transmit the microorganisms to vertebrates via salivary secretions or feces. The rickettsiae are transmitted to humans principally by infected arthropods, but contamination by aerosol (175) and blood transfusion (278) has also been described. Ixodid or hard ticks are the vectors or at least the hosts of SFG rickettsiae and *R.*

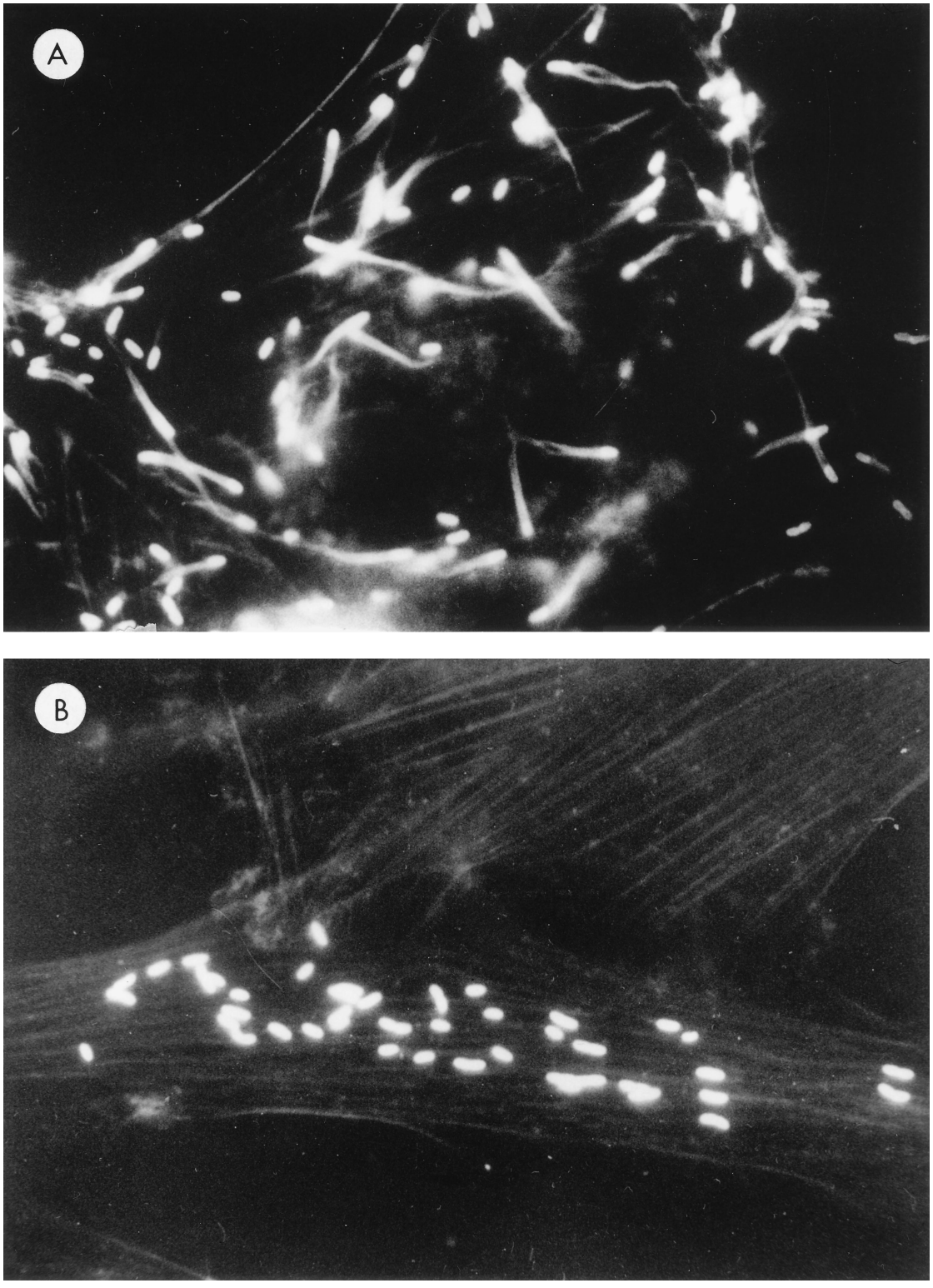

FIG. 1. Actin-based movements of rickettsiae as shown by double labelling. Rickettsiae were stained by immunofluorescence, and actin was labelled with phallacidin. (A) *R. conorii* induces an actin contraction. (B) *R. typhi* does not induce an actin contraction.

canada; mites are the vectors of *R. akari* and *O. tsutsugamushi*; lice are the vectors of *R. prowazekii*; and fleas are the vectors of *R. typhi* and *R. felis*. The ladybird beetle and pea aphid serve as hosts for the AB bacterium and pea aphid rickettsiae, respectively (61, 280), yet since these insects are not known to bite or feed on vertebrates, one would anticipate that they are endosymbionts incapable of being horizontally transmitted by their insect host.

Many rickettsiae are pathogenic for humans, although with the exception of *R. prowazekii*, the role of humans in the natural cycle of the rickettsiae is secondary. At present, 14 serotypes of rickettsiae have been isolated from patient specimens: *R. prowazekii*, the agent of epidemic typhus; *R. typhi*, the agent of murine typhus; *R. rickettsii*, the etiological agent of Rocky Mountain spotted fever (RMSF); *R. conorii*, which causes Mediterranean spotted fever (MSF); Astrakhan fever rickettsia, Israeli tick typhus rickettsia, *R. sibirica*, *R. africae*, *R. australis*, *R. akari*, *R. japonica*, *R. honei*, and *R. felis*, causing Astrakhan fever, Israeli spotted fever, Siberian tick typhus, African tick bite fever, Queensland tick typhus, rickettsialpox, Japanese fever, Flinders Island spotted fever, and "Californian flea rickettsiosis," respectively; and a rickettsia similar to *R. sibirica*, for which we propose the name "*R. mongolotimonae*" (200, 293). The other rickettsiae are supposedly nonpathogenic for humans because they have been isolated only from arthropods. This finding could change in the future, as in the case of *R. africae*, which was isolated first from ticks (185) and only subsequently from a patient's blood (137). The main symptoms of rickettsial infection consist of fever, headache, and cutaneous eruption. The target cell of rickettsiae is the endothelial cell, and proliferation of the rickettsiae in the vascular endothelium results in vasculitis.

The differentiation of the groups within the genus *Rickettsia* has historically been based on several factors (277): (i) the intracellular position of each species, which is thought to be related to the ability of a specific rickettsia to polymerize actin in the cytoplasm, allowing intracellular mobility (122, 253) in the nucleus and the cytoplasm for the SFG rickettsiae and *R. canada* and only in the cytoplasm for the others; (ii) an optimal growth temperature (32°C for the SFG rickettsiae and 35°C for the typhus group and *O. tsutsugamushi*); and (iii) the cross-reaction of sera from a patient with rickettsial infection with the somatic antigens of three strains of *Proteus*, OX19 (TG and *R. rickettsii*), OX2 (SFG), and OXK (*O. tsutsugamushi*) (273). Although the antigenic determinants of these immunological reactions were unknown, the distinctive immunogenic properties of rickettsial antigens were used in the first half of this century to distinguish between rickettsiae. Cross-immunity and vaccine protection tests (191) in guinea pigs and complement fixation (192) or toxin neutralization (33) tests were successfully applied to the differentiation of *R. rickettsii*, *R. sibirica*, and *R. conorii*. The indirect microimmunofluorescence (MIF) serologic typing test with mouse sera was developed in 1978 and remains the reference method for the identification of new SFG rickettsiae (189). Accordingly, the classically recognized SFG rickettsial species are in fact serotypes.

The advent of purification methods (276), enabling the separation of rickettsiae from host cell components, has allowed the study of rickettsial proteins and the understanding of the mechanisms on which these serological identification techniques have been based. With the development of a new cell culture isolation technique (the shell vial technique) (159), more and more strains have been isolated over the past few years. These strains have been characterized by a polyphasic approach involving phenotypic criteria (serotyping, protein analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) [6, 173], and genotypic criteria (restriction fragment length polymorphism (RFLP) analysis of PCR amplification products [85, 209] and macrorestriction analysis by pulsed-field gel electrophoresis [222]).

The exact taxonomic position of *R. canada* is unclear as, depending on the criteria used, this species belongs to either the SFG or the TG (170, 223, 224). The same problem exists with *R. bellii*. First it was considered a rickettsia of the SFG, based on its association with ixodid ticks. Then it was described as phenotypically different from rickettsiae of the SFG and the TG (188). The position of *R. felis* is also disputed, since it was originally considered a TG rickettsia whereas current data place it closer to the SFG when 16S rRNA sequences are considered (197) and closer to the TG when the citrate synthase gene is used (219).

Comparison of sequences from different genes allows significant phylogenetic inferences to be made at different taxonomic levels, ranging from those between closely related species to those between more distantly related organisms. Phylogenetic analysis of the rickettsiae, based on 16S rRNA gene sequence comparison, has been carried out by Stothard and Fuerst (243) and in our laboratory (223). These studies have confirmed the evolutionary unity of the genus (Fig. 2), but, since the sequences were almost identical, significant inferences about intragenus phylogeny were not possible. We have recently studied two fast-mutating genes that encode the enzyme citrate synthase (224) and the outer membrane protein, rOmpA (90), to find more sensitive and significant phylogenetic relationships among rickettsiae. Some species were, however, not included in these analyses, because they were not available (*R. felis*, *R. honei*, and "*R. amblyommii*"). The results of the comparison demonstrated that (i) *R. canada*, *R. bellii*, and the AB bacterium lie outside both the TG and the SFG on an evolutionary lineage, which diverged before the separation of these two groups; (ii) *R. prowazekii* and *R. typhi* cluster together; (iii) the tick-borne *R. helvetica* and *R. australis* and the mite-borne *R. akari* are associated with the SFG cluster; and (iv) the SFG rickettsiae can be subdivided into two groups, one including *R. massiliae*, Bar 29, *R. rhipicephali*, "*R. aeschlimannii*" (MC16), and *R. montana*, and the second being a larger subgroup and including all the other described SFG rickettsiae. Comparison of phylogenetic inferences derived from either *gltA* or *ompA* sequences indicates similar evolutionary models, but it is best if both genes are analyzed during the characterization of putative new species, since phylogenetic analysis must stem from identical results obtained with different tools. The precise organization within the genus *Rickettsia* remains unclear, although these phylogenetic studies have demonstrated that a simple division of species into either the TG or the SFG is not evolutionarily accurate (Fig. 3).

The traditional identification methods used in bacteriology cannot be applied to rickettsiae because of their strictly intracellular nature. At present, serological typing by MIF with mouse antisera remains the reference method for the differentiation of rickettsiae and the identification of a new species (189). The antigenic determinants for this serotyping scheme are two high-molecular-weight outer membrane proteins, rOmpA and rOmpB. Over the past few years, several new species have been described on the basis of pathogenic, ecological, genotypic, and/or antigenic observations, and thus no consensus criteria for the definition of rickettsial species exist. With the development of molecular approaches, MIF can no longer be used alone as a reference method. The obligate intracellular nature of the members of the genus *Rickettsia* sets them apart from the free-living bacteria, and thus their taxonomic definition requires a specialized set of as yet unagreed on criteria. The establishment and implementation of such steps are essential.

ARTHROPODS AND RICKETTSIAE

Rickettsiae are associated with arthropods, which may act as vectors, reservoirs, and/or amplifiers in the life cycles of the

FIG. 2. Phylogenetic tree derived from the 16S rRNA gene of bacteria belonging to the *Rickettsia* genus. Sequences extracted from GenBank were aligned with the multisequence alignment program CLUSTAL, which is a part of the BISANCE software package. Phylogenetic relationships were inferred with version 3.4 of the PHYLIP software package. The evolutionary distance values were determined by the method of Jukes and Cantor. These values were used to construct a dendrogram by the neighbor-joining method. The scale bar (lower left) represents a 0.5% difference in nucleotide sequences. Bootstrap values are not indicated at the nodes because they are not significant.

bacteria. Ticks are the main vectors and reservoirs of SFG rickettsiae (Fig. 4; Table 1). The typical life cycle of an SFG member is as follows. Rickettsiae infect and multiply in almost all organs of their invertebrate hosts. When the ovaries and oocytes of an adult female tick are infected, rickettsiae may be transmitted transovarially to at least some of its offspring. The percentage of infected eggs obtained from females of the same tick species infected with the same rickettsial strain may vary, depending on factors that have yet to be elucidated (50, 55). Once an egg is infected, all subsequent life stages of the tick will be infected (the rate of transstadial transmission is therefore 100%). Ixodid ticks are bloodsucking arthropods through-

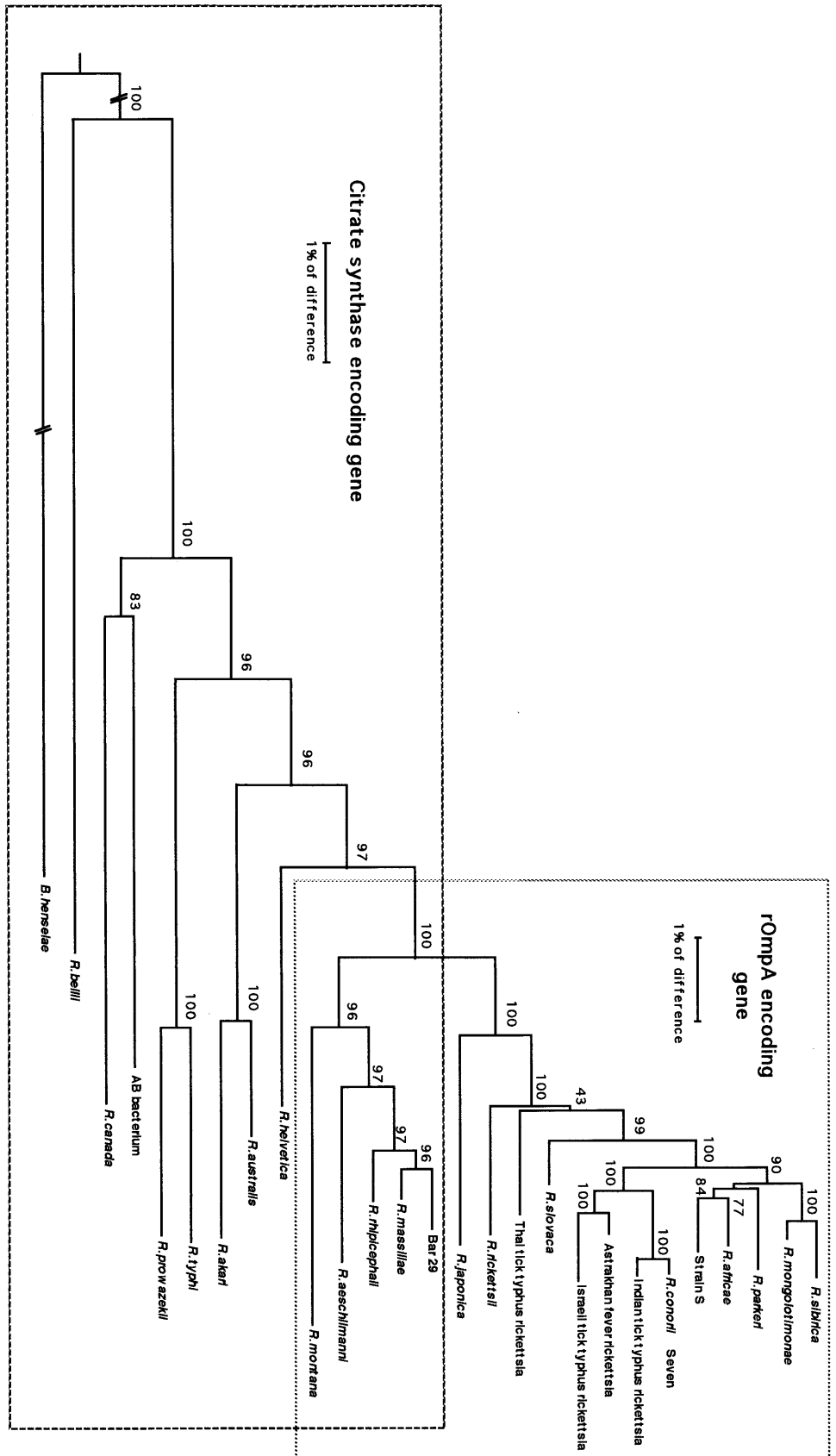

FIG. 3. Dendrogram representing phylogenetic relationships between *Rickettsia* species. The tree includes data determined from analysis of the *gltA* and *ompA* genes. The dendrogram was constructed as described in the legend to Fig. 2. Bootstrap values are indicated at the nodes.

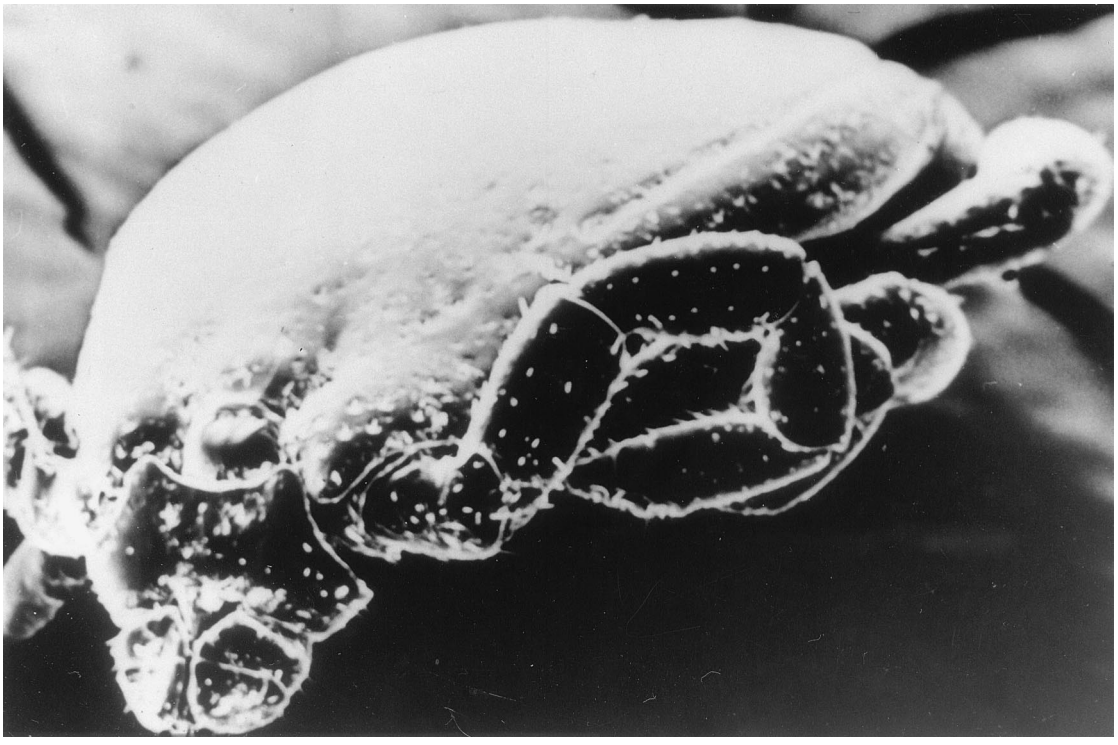

FIG. 4. *Rhipicephalus sanguineus* tick. Magnification, ×5,000.

out all their developmental stages, apart from some of the adult male ticks in some *Ixodes* species. Rickettsiae infecting the ticks' salivary glands can be transmitted to vertebrate hosts during feeding. Therefore, since larvae, nymphs, and adults may all be infective for susceptible vertebrate hosts, the ticks must be regarded as the main reservoir host of rickettsiae. Sexual transmission from male to female ticks has been described in *Ixodes ricinus* and *Dermacentor andersoni* ticks (116, 186). Uninfected, immature *D. andersoni* ticks were allowed to feed simultaneously with adults infected with *R. rickettsii* on the same uninfected guinea pigs. The rickettsiae were transmitted both to the guinea pigs and to the uninfected immature ticks, showing that a rickettsemic blood meal is a mode of uptake (184). Long-term starvation of a tick does not kill its infecting rickettsiae, although it may alter some of their properties. For example, *R. rickettsii* in *D. andersoni* ticks loses its virulence for guinea pigs when the ticks are subjected to physiological stress, such as long starvation. However, subsequent exposure of these ticks to a temperature of 37°C for 24 to 48 h or refeeding them on laboratory animals restores the original virulence of the bacteria. This long-recognized phenomenon is known as reactivation (240). While there is wide consensus about this part of the rickettsial cycle, the role of vertebrate reservoirs in maintaining zoonotic foci has yet to be agreed upon. For vertebrates to be efficient reservoirs of rickettsiae, they need to be normal hosts of the vector and be susceptible to the rickettsiae and should develop a relatively long duration of rickettsiaemia. If they did not fulfill these criteria, ticks would not be able to acquire rickettsiae from the bloodstream of their hosts. Humans are not a good reservoir for rickettsiae, since they are seldom infested with large numbers of ticks for a long period and rickettsiaemia is usually of only short duration, especially with antibiotic intervention.

Although yet to be demonstrated, there is potentially another method by which rickettsiae may be transmitted between ticks. The social behavior of ticks is determined mainly by the effects of different pheromones (112, 180). Some pheromones are responsible for the aggregation of ticks on the host, enhancing the chance for meeting and copulation. Under such circumstances, ticks would also feed, and thus the mouthparts of several different ticks would be in the skin of the host in very close proximity. Under such feeding conditions, direct spread of rickettsiae to uninfected ticks might be possible without causing infection of the animal which is being fed upon.

Little is known about the effects of rickettsial infection on ticks, although Burgdorfer et al. reported that rickettsial infection lowered tick fertility (50, 55). Ixodid ticks are highly adapted to maintaining a favorable water balance in arid environments, often feeding on a specific host only seasonally and surviving for months or years when these hosts are absent (125). They are slow-feeding ticks, taking days to engorge. It is difficult to determine the association between rickettsial and tick species because specific characterization of species and subspecies in both phyla lacks sensitivity. Consequently, it is difficult to determine how long a tick species has been associated with a rickettsial species and therefore if coevolution has occurred. The range of host specificity of a tick varies greatly from one species to another, although larval and nymph stages are usually less specific in their choice of host and bite humans more often than adult ticks do. Some species, such as the brown dog tick *Rhipicephalus sanguineus*, are very host specific and rarely bite humans (99), whereas others, such as *I. ricinus* in Europe or *Amblyomma* species in Africa, will bite any mammal. Indeed, tick ecology determines all the epidemiological aspects of tick bite fevers.

The geographical distribution of *Rickettsia* spp. is determined by the incidence of its tick host, and the seasonal incidence of diseases parallels tick activity. It should be remem-

TABLE 1. Association of tick genera and rickettsial species

Tick genus	Rickettsia
Rhipicephalus	*R. conorii*
	Astrakhan fever rickettsia
	Israeli tick typhus rickettsia
	R. rhipicephali
	Strain S
	Thai tick typhus rickettsia
	R. massiliae
	Bar 29
	JC880
	Thai tick typhus rickettsia?
Dermacentor	*R. rickettsii*
	R. sibirica
	R. japonica
	"*R. slovaca*"
	R. bellii
	R. montana
	R. rhipicephali
	R. peacockii
	Unnamed rickettsiae
Amblyomma	*R. africae*
	R. parkeri
	"*R. amblyommii*"
	R. rickettsii
	R. texiana
	Unnamed rickettsia
Haemaphysalis	*R. japonica*
	HL-93
	R. rickettsii
	R. canada
	R. conorii
	R. sibirica
	R. bellii
Hyalomma	"*R. mongolotimonae*"
	"*R. aeschlimanni*"
Ixodes	*R. helvetica*
	R. australis
	R. rickettsii
	R. japonica
	Thai tick typhus rickettsia?
	Unnamed rickettsia
Argas	*R. bellii*
Ornithodoros	*R. bellii*

bered that immature stages of ticks can be involved in disease transmission and that their incidence differs from that of the adult population. For example, MSF, caused by *R. conorii*, is transmitted by *Rhipicephalus sanguineus*, whose adult population peaks in May. Most MSF cases, however, occur in August, 3 months later (204), suggesting that larvae or nymphs are responsible, particularly since the immature stages of numerous tick species bite humans (99) and their highest incidence occurs in August. The infecting tick bite is painless, and the tick is not usually observed, especially when smaller larvae or nymphs are involved. When not engorged, these stages are smaller than a pinhead. A history of tick bite is an important finding but is often absent. In several cases of MSF, ticks have been found at the site of a bite on patients who have been ill for several days. The patients had simply not noticed the presence of the ticks, which must have been attached for more than 10 days, since the incubation period for the disease is usually 7 days. In other locales, such as areas of the United States or Africa, huge numbers of ticks are found and patients with rickettsiosis can easily identify attached ticks.

The risk of ticks transmitting rickettsiae and consequently the prevalence of a specific disease is dependent on several parameters. (i) The prevalence of rickettsia-infected ticks, which can vary greatly, is important. For example, up to 12% of *Rhipicephalus sanguineus* ticks are infected with *R. conorii* in southern France (183), whereas only 0.5% of *D. variabilis* ticks in North Carolina are infected by *R. rickettsii* (270). (ii) The affinity of a specific tick for human beings also varies. For example, in Mediterranean countries, although nearly everybody is in contact with the dog tick *Rhipicephalus sanguineus*, the prevalence of MSF is only 50 per 100,000 inhabitants. The reason is the low affinity of this tick for hosts other than the dog. (iii) The abundance of the tick itself is important and is influenced by many factors, including climatic and ecologic conditions (115, 158).

R. akari is responsible for rickettsialpox, which is an urban disease involving mites of the genus *Allodermanyssus*, the house mouse *Mus musculus*, and, accidentally, humans. Humans are typically attacked by mites after mouse extermination campaigns. The nymphal stages and both the female and the male adult stages of the mite feed mainly on mice, which are highly susceptible to infection with *R. akari*. Mice can be considered the natural reservoirs of *R. akari*; however, this organism can be transmitted transovarially, the mite may act not only as a vector but also as a reservoir of *R. akari.*

R. prowazekii is transmitted by the human body louse (*Pediculus hominis corporis*), and its main reservoir is in humans (277). Lice are extremely host specific, spending their entire life cycle on the same host. The body louse is not well adapted to *R. prowazekii* infection and invariably succumbs to infection within 1 to 2 weeks. The human head louse has not been implicated as a vector of epidemic typhus. During the 1960s and the 1970s, the identification of nonhuman reservoirs (52, 53) in ticks and mammals caused a major controversy. However, Bozeman et al. (37) were able to isolate *R. prowazekii* from *Glaucomys volans volans*, the Eastern flying squirrel, in the United States. Fleas and lice from flying squirrels were also shown to be infected. These arthropods are apparently only vectors, acquiring *R. prowazekii* from a rickettsemic host during the feeding process and becoming infected with rickettsiae 5 to 7 days later. Transmission of the bacteria does not occur directly via a bite but, rather, by contamination of bite sites by the feces or the crushed bodies of infected lice. When rickettsiae are ingested as part of a blood meal, they infect the midgut epithelial cells of the louse and undergo rapid multiplication. As a result of the excessive growth of *R. prowazekii*, infected epithelial cells enlarge and eventually burst to release the rickettsiae into the gut lumen. Massive quantities of rickettsiae are discharged in the feces and can remain infective for up to 100 days. As ruptured epithelial cells are not replaced, infection with *R. prowazekii* leads to the death of the louse.

R. typhi is transmitted by several flea species as well as other arthropod vectors (lice, mites, and ticks) (256). It can rarely be transmitted transovarially in fleas (88). However, fleas are usually solely vectors, with *Rattus norvegicus* and *Rattus rattus* acting as the primary reservoirs (13). Infection with *R. typhi* in rats is not fatal, but the persistence of rickettsiae in the circulating blood of infected rats is limited (days 7 to 12 after inoculation) (11). When ingested with an infectious blood meal, the rickettsiae enter the epithelium of the flea midgut, the only part of the intestine that lacks a cuticular lining. Here, the bacteria propagate and are subsequently excreted in the

feces. *R. typhi* in feces remains viable for several years (256). Once infected, fleas remain infected for life, but their life span is unaffected by the presence of rickettsiae. Transmission to the host occurs by contamination of the skin or respiratory tract by aerosols of dust containing infective material or via contamination of the conjunctivae of the host with infected flea feces.

DIAGNOSTIC TOOLS

Clinical Presentation and Observations

The advent of novel diagnostic tools such as the microculture assay (159, 183) and molecular biological assays has dramatically improved the efficiency of diagnosing rickettsioses and of recognizing new rickettsial species. However, it is important to remember that diseases such as RMSF and MSF have long been described solely on the basis of clinical evidence. Careful clinical examination and epidemiologic investigation of patients with potential rickettsioses is critical. Clinically, the mainstay of the diagnosis has always been the presence of a characteristic rash. The typical clinical picture during rickettsiosis is high fever (39.5 to 40°C), headache, and rash. The disease can be mild or severe but will usually last for 2 to 3 weeks. This very basic knowledge should always be borne in mind.

Conor and Bruch in 1909 characterized MSF from only two cases, because it was different from viral eruptions, which are usually milder and of shorter duration (62). They concluded that the two cases were equivalent to RMSF. In our experience in Astrakhan, an exanthematic infection was mistakenly suspected of being due to an echovirus, enterovirus, or arbovirus infection when in fact it was a rickettsiosis. The physicians making the diagnosis were not aware that the clinical presentation that they were facing was pathognomonic for a rickettsial eruption. A diagnosing physician must therefore have a good knowledge of the literature when diagnosing rickettsial diseases.

Serology

Serological assays are the simplest diagnostic tests to perform, since serum can readily be sent to a reference laboratory. The Weil-Felix test was the first such assay to be used and involves antigens from three *Proteus* strains: *P. vulgaris* OX2, *P. vulgaris* OX19, and *P. mirabilis* OXK. This test is used to diagnose rickettsiosis based on serological cross-reactions (202). Although the test lacks sensitivity and specificity, it has historically been used for laboratory diagnosis and provides evidence of newly encountered rickettsioses.

Today, the most commonly used serological test is the MIF. The test is reliable but does not allow differentiation of infection among the SFG rickettsiae (120, 121). The enzyme-linked immunosorbent assay was first introduced for detection of antibodies against *R. typhi* and *R. prowazekii* (111). This technique is highly sensitive and reproducible, allowing differentiation of immunoglobulin G (IgG) and IgM antibodies. The method was later adapted to the diagnosis of RMSF (265). The Western blot immunoassay (201) allows differentiation among the SFG, provided that acute-phase sera are used. The test detects two types of antigens, lipopolysaccharide and two high-molecular-weight proteins (rOmpA and rOmpB). These proteins are species specific (28, 202) and provide the basis for rickettsial serotyping (189). However, although inoculated mice produce a predominance of antibodies against these proteins, human beings do not, and cross-reactions between rickettsial proteins make it difficult to identify the infecting rickettsia to the species level (120, 121). If sera are collected very early in infection, strong homologous reactions are often observed, making a specific diagnosis possible. However, as this rarely occurs, more specific methods are needed. Cross-absorption studies are useful, especially if complemented by Western blotting (42). This is the case for typhus because in 50% of patients, the sera had the same level of antibodies to both *R. prowazekii* and *R. typhi*. Unfortunately, although this technique is accurate, it is also very expensive and time-consuming, since a large number of rickettsiae is required for each absorption. Radulovic et al. have recently proposed an alternative immunoassay involving epitope saturation by specific monoclonal antibodies (198).

It must be emphasized that at present, serological testing can be considered only the first step towards diagnosing or recognizing a rickettsial disease. For example, although Rehacek has reported cases of meningoencephalitis associated with seroconversion to "*R. slovaca*" in Slovakia (210), it will be difficult to accept that "*R. slovaca*" is a human pathogen until clinical isolates have been obtained. In general, direct evidence of the identity of a rickettsial pathogen is required before purported new syndromes, new manifestations, or new areas of endemic infection can be defined. This evidence should be based on a combination of culture or microscopic or genetic detection techniques and not solely on serology. In fact, the literature is full of serologically based evidence of new diseases or new clinical forms of disease that must be viewed with some degree of scepticism. The occurrence of so much rickettsial disease in France in the 1970s suggests that diagnostic errors were made through the use of the nonspecific slide agglutination test and incorrect interpretation of test results. Multiple sclerosis, myocardial infarction, and schizophrenia have all been falsely reported to be related to rickettsioses on the basis of serological tests and have led to the prescription of incorrect therapeutic regimens (102, 148). Recently, cross-reactions were identified between *Legionella* and *Rickettsia* species (202). Misuse of serology has also been observed for other infections, for example, Lyme disease (16). In this instance, such problems led to the formation of an international lobbying group for an alternative interpretation of Lyme disease serology.

Isolation of Rickettsiae

Rickettsiae are characterized by Giménez staining, although some other bacteria also retain the basic carbol fuchsin stain and must be distinguished from rickettsiae on the basis of culture requirements. For example, coinfection of ticks with "*Wolbachia*-like" organisms is possible, and these organisms may appear as rickettsiae in nonspecific stains. *Rickettsia* has been isolated by several different methods. Animal inoculation has been widely used, originally with guinea pigs and subsequently with rats and voles. Recently, *R. felis* was reported to have grown from cat fleas (*Ctenocephalis felis*) in Sprague-Dawley male rats (197) prior to successful cell culture. Embryonated eggs have also been widely used. However, cell culture is currently the most widely used system for primary isolation. It differs from that used for viral isolation, since antibiotics, with the exception of co-trimoxazole, cannot be used during rickettsial isolation (142). Tick or mammalian cell lines can be used. We have used a microculture system to isolate rickettsiae from human blood and other sources (17, 22, 83, 159). The shell vial assay was adapted from a commercially available method for cytomegalovirus culture and early antigen detection. Isolation of rickettsiae by cell culture is now performed routinely in our laboratory from heparinized blood (leukocytic cell buffy coat), skin biopsy samples before antibiotic therapy, or arthropods (86, 159). Many rickettsiae, including *R. conorii*,

R. rickettsii, *R. massiliae*, "*R. aeschlimannii*," "*R. slovaca*," *R. helvetica*, "*R. mongolotimonae*," and *R. africae*, have been isolated by this method. It has also been used in Zimbabwe (141) and in Portugal (17). This procedure involves the centrifugation-shell vial technique with human embryonic lung (HEL) fibroblasts. Each sample is assayed in triplicate. Rickettsiae are detected directly inside the shell vial by immunofluorescence staining and microscopic examination of coverslips. After fixation with acetone, the coverslips are incubated with anti-*R. conorii* rabbit antibodies or with anti-*R. prowazekii* human antibodies. The culture is kept for 2 weeks with examination of one shell vial each week for the SFG and is kept for 3 weeks with examination of one shell vial each 10 days for the TG. After this time, if immunofluorescence is negative, the culture is considered negative. If immunofluorescence is positive, parallel shell vials are inoculated onto confluent monolayers of HEL cells in culture flasks in an attempt to obtain isolates of *Rickettsia* spp. Although this assay is useful, about one-third of the isolates are lost on passage for unknown reasons. The importance of culture cannot, however, be underestimated, since obtaining an isolate from a tick or a patient is the ultimate goal in rickettsial disease description.

Immunological Detection of Rickettsiae

Skin biopsy specimens have been used in the diagnosis of both RMSF and MSF since the early work of Woodward (268). Samples can be tested fresh or after fixation and paraffin embedding. The "tâche noire" rash, when present, should definitely be biopsied, because it contains huge numbers of rickettsiae (169). We have recently developed a technique of cutting biopsy samples of tâche noire into small pieces and subjecting them to collagenase treatment. Endothelial cells are then recovered from these digestion mixtures with immunomagnetic beads as described above. This technique allows rickettsiae to be recovered with relative ease, even in patients receiving antibiotic therapy. Other clinical samples obtained at autopsy can be tested in the same manner as skin biopsy specimens (269, 271).

The use of methods incorporating specific polyclonal antibodies or monoclonal antibodies allows the detection of rickettsiae in blood or other tissues. This diagnostic approach allows the confirmation of infection in patients before their seroconversion and thus permits early prescription of specific treatment. The method can also be used to diagnose rickettsial infection in fixed tissues retrospectively. We have recently described an adaptation of this technique allowing the immunologic detection of rickettsiae in circulating endothelial cells, which are isolated from whole blood with immunomagnetic beads coated with an endothelial cell-specific monoclonal antibody (96). A 1-ml volume of whole blood diluted 1:4 with phosphate-buffered saline is mixed with a suspension of monoclonal antibody-coated beads. Following incubation, the magnetic beads and rosetted cells are separated from other blood constituents with a magnetic particle extractor. After being washed, the rosetted cells are divided into two aliquots. One is stained with acridine orange and counted in a hemocytometer, and the other is cytocentrifuged onto a glass slide. These smears are then fixed, and bacteria are detected by immunofluorescence with polyclonal *R. conorii* antiserum. The sensitivity of this method is estimated to be 50% for acutely ill patients (147). Moreover, it has a prognostic use, because the number of circulating endothelial cells detected is directly proportional to the severity of the infection (97).

Ticks collected for attempted isolation of rickettsiae should be kept alive before being tested. If they need to be transported or kept for long periods, a humidifier box is useful. While the ticks are still alive, the hemolymph test should be performed following surface sterilization (45). In this procedure, one tick leg is severed, allowing the collection of a drop of hemolymph, which can be spread onto a slide and then subjected either to Giménez staining (101) or to immunodetection methods. The tick should then be dissected (142, 183). Organs, including the reproductive tissue, can be carefully dissected and separated for further testing. Immunological detection methods can incorporate polyclonal or monoclonal antibodies, the latter of which can be used to determine the infecting species. Immunofluorescent labels have been widely used in conjunction with these antibodies, but immunoperoxidase labels and detection systems appear to allow a better microscopic definition of cells around the detected rickettsiae (73). *R. typhi* can also be detected in infected fleas by an enzyme-linked immunosorbent assay (68).

PCR-Based Detection of Rickettsiae

Rickettsiae may be detected by PCR amplification from an array of samples that include blood, skin biopsy samples, and arthropod tissues. Specific procedures must be used prior to testing samples. Blood is held at ambient temperature until cells are sedimented and rickettsiae are sought in the leukocytic cell buffy coat. Although heparinized blood is used for cell culture, it is necessary to use blood collected in EDTA or sodium citrate for PCR amplification, because heparin inhibits PCR and is difficult to neutralize. The PCR amplification must be performed before initiation of antibiotic treatment and before antibody becomes detectable. The tâche noire is the most useful biopsy sample to assay (283), although this sign is not always present. Fresh tissues are preferred for this procedure, but paraffin-embedded tissues and even slide-fixed specimens may be used (241). Tâche noire samples are the best to detect SFG rickettsiae because more bacteria are present than in blood; several such isolates were characterized in our laboratory by this approach (42, 200). PCR amplification of tâche noire or blood samples can be very useful because infection can be detected before cell culture is positive or seroconversion has occurred.

PCR-based methods for the detection of rickettsiae are attractive as they not only circumvent the need for culture but also possibly offer more sensitive and specific alternatives. Rickettsial DNA can also be detected in ticks (91, 92), fleas, and lice by PCR-based amplification methods (123). However, at present, very few rickettsial genes have been studied; therefore, the choice of suitable hybridization sites for specific PCR primers is limited. Detection strategies based on recognition of sequences within the 16S rRNA gene (223, 243), and those encoding a 17-kDa protein (9, 19, 22), citrate synthase (202a, 231, 285), and the rOmpB (98, 202a) and rOmpA (for SFG rickettsiae) (171, 200, 221) outer membrane proteins have been described. Since none of the PCR assays to date are specific for individual rickettsial species, reaction products must be further analyzed to identify the species being detected. Approaches involving either restriction endonuclease analysis or base sequence determination have been described and are discussed further below.

Identification and Differentiation of Rickettsiae

A rickettsial isolate can be identified by several tools including staining, SDS-PAGE, electrophoresis, and DNA analysis. Rickettsiae are poorly stained by Gram stain but retain basic fuchsin when stained by the Giménez method (101). For many years, the differentiation of rickettsiae was based solely on

TABLE 2. Symptoms of SFG rickettsioses

Disease	Bacterium responsible	% of patients with:			Multiple eschar	Enlarged local nodes	% of patients with purpuric rash
		Rash	Headache	Eschar			
RMSF	*R. rickettsii*	90	80	0	No	No	45
MSF	*R. conorii*	97	56	72	Very rare	Rare	10
Siberian tick typhus	*R. sibirica*	100	100	77	No	Yes	No
Israeli spotted fever	Israeli tick typhus rickettsia	100	Yes	0	No	No	Rare
Rickettsialpox	*R. akari*	100[a]	100	83	Yes	Yes	No
Queensland tick typhus	*R. australis*	100[a]		65	No	Yes	
Japanese spotted fever	*R. japonica*	100	22	48	No	No	No
Astrakhan fever	Astrakhan fever rickettsia	100	92	23	No	No	No
African tick bite fever	*R. africae*	30[a]	Yes	100	Yes	Yes	No
Flinders Island spotted fever	*R. honei*	85	73	25	No	Yes	8
California flea rickettsiosis	*R. felis*	Variable	?	?	?	?	?
Unnamed spotted fever	"*R. mongolotimonae*"	Yes	Yes	Yes	No	No	No

[a] Vesicular.

immunologic methods. Initially, the toxin neutralization test in mice was used (33, 216); this was followed by complement fixation (192) and later MIF (189). The main problems with these techniques are that reference sera are needed and that each time a new isolate is tested, the test sample and all other antigens need to be screened against all antisera. Monoclonal antibodies against *R. rickettsii* (5, 7), *R. akari* (163), *R. conorii* (272), and *R. japonica* (259) have been introduced. Although these are useful tools, a complete collection, organized in pools, is required to identify all rickettsiae.

Protein analysis by SDS-PAGE has also been used to differentiate rickettsial species (181). The molecular masses of the two major protein antigens, rOmpA and rOmpB are estimated to be 115 and 155 kDa for *R. rickettsii*, although their precise size varies among rickettsial species. These proteins determine the serospecificity in mice (28). However, since the reproducibility of PAGE is never perfect and depends on gel conditions and temperature solubilization, it is usually necessary to include all species when attempting to identify a new isolate. Furthermore, since comparison with other species or strains is needed, it is necessary to introduce all purified rickettsiae; the technique is time-consuming and laborious.

Macrorestriction analysis of rickettsiae by pulsed-field gel electrophoresis is also a sensitive method for differentiating species (222). It has been a useful approach for identifying rickettsiae, but much biomass is required and it is necessary to include other rickettsiae on the gel to obtain a precise comparison of the profiles. Thus, applying this approach each time a strain is isolated is almost impossible.

Regnery et al. (209) described the usefulness of RFLP analysis of PCR-amplified fragments of the citrate synthase and rOmpA-encoding genes. This technique has proven to be sensitive and practical, and when it was coupled with RFLP analysis of a rOmpB gene fragment (24, 85, 221), all Russian and European and many Chinese isolates were identified (17, 80, 83, 293). RFLP analysis of the gene coding for a 17-kDa protein has also been used (22, 197). Species-specific RFLP profiles can be stored in a database, simplifying subsequent identifications.

By sequencing the PCR amplification product, it is easy to obtain a precise identification of a new isolate. Since a databank of sequences exists, the determined sequence can be compared with those previously obtained. Sequencing part of the genes coding for 16S rRNA, citrate synthase, a 17-kDa protein, rOmpA, or rOmpB was used to characterize rickettsia. At present, the identification of the rickettsiae in our laboratory is based on PCR amplification followed by sequencing of a fragment of the citrate synthase-encoding gene (*gltA*) or the rOmpA-encoding gene (*ompA*). However, the use of broad-spectrum 16S rRNA gene primers allows PCR to be used to detect rickettsiae in unexpected conditions.

The 5′ end of the *ompA* gene demonstrates marked heterogeneity as well as conserved regions. We have found that amplification and then sequencing of a 590-bp fragment of this region of the gene allows a clear differentiation of most of the SFG members. Unfortunately, at present, the primers used for amplification do not hybridize to *ompA* sequences of *R. akari*, *R. australis*, *R. helvetica*, *R. bellii*, *R. canada*, *R. typhi*, and *R. prowazekii*, so that analysis of the gene in these species has not yet been possible. However, these seven species showed a specific *gltA* sequence when a fragment of 341 bp was studied. Interestingly, *ompA* sequence differences have been detected among *R. conorii* isolates, which demonstrate genotypic diversity (221) and confirm the antigenic diversity described previously (272). It is therefore possible that analysis of this gene will form a basis for an epidemiological study of the species.

DISEASES

The following is a brief description and comparison of newly and previously described rickettsial diseases, including the eight recognized before 1984 and six recognized thereafter. The signs and symptoms of rickettsioses are listed in Table 2.

Previously Described Diseases

Epidemic typhus. "The incidence of typhus may serve as an indicator of man's follies" (251). The origin of typhus is controversial. Some authors consider that it is an old European disease, which caused the Athens plague. Others believe that the reservoir is extrahuman and is of American origin, as shown by disease in flying squirrels. However, as Zinsser stated, epidemic typhus has caused more deaths than all the wars in history (295). Epidemic typhus is transmitted by the body louse (109), as Nicolle demonstrated in 1928. The main reservoir outside the United States appears to be in humans, since lice die of the infection. Humans who contract typhus retain some rickettsiae for the rest of their lives. Under certain stressful conditions, they may relapse and suffer from Brill-Zinsser disease, a milder form of typhus. The bacteremia may then allow feeding lice to become infected and to start a new epidemic. Body lice live in clothes, and typhus is therefore observed in situations where poverty, lack of hygiene, and cold

weather favor louse proliferation. Currently, the disease is limited to highlands in South America, Asia, and Africa. Typhus is a reemerging disease, with more than 30,000 infected people in Burundi during the current civil war (289), the biggest outbreak since World War II. The onset of disease is severe. Patients present with high fever, headaches, and severe myalgias. A rash, which can become purpuric, is observed 5 to 7 days after the onset of symptoms. The rash is observed more rarely in Africa (20 to 40% of patients) on dark skin. Cough and pneumonia are observed in two-thirds of patients. In Africa the disease can be differentiated from typhoid because diarrhea is rare during typhus and from malaria because splenomegaly and chills are rarely observed during typhus. The disease is fatal in 10 to 30% of patients, depending on underlying diseases and on the nutritional state of the host. Since a single dose of 200 mg of doxycycline will save the patient, any suspected case should be treated.

In the United States, a search for a nonhuman reservoir of *R. prowazekii*, the agent of epidemic typhus, was carried out in African ticks (212) and in flying squirrels (37). The isolated strains were recovered from fleas, lice, and the spleens of the flying squirrels (239). The strains differ only slightly from the reference worldwide strains (208). Cases of indigenous flying squirrel-acquired typhus have been described since 1980 (1, 59, 162, 227). The disease is found in the eastern and southern United States, where the southern flying squirrel is distributed. Although cases of epidemic typhus are rare (33 cases reported in 1984), the disease is diagnosed in winter and patients frequently report that they have handled squirrels. The fact that a new reservoir of epidemic typhus was found in the United States prompted a team from the Centers for Disease Control to search for indigenous cases, which were found. The diagnosis is performed by serology. The antibodies cross-react with those to *R. typhi*, but in more than half of the cases, IgG antibody titers are higher for *R. prowazekii*. When this is not the case, cross-absorption will determine which rickettsia is responsible for the disease. This is critical when sporadic cases are observed, since the epidemic potentials of the two rickettsiae differ greatly. In our experience, indirect immunofluorescence and immunoblot tests cannot differentiate between Brill-Zinsser disease (recrudescent typhus) and primary epidemic typhus (82). Epidemic typhus remains a very serious threat for humans. Outbreaks could occur in Russia in the coming years, because a large louse outbreak has been observed in Moscow's homeless people (228).

Murine typhus. Murine typhus is caused by *R. typhi* (formerly named *R. mooseri*). During investigations of the etiology of typhus fever, it was recognized that rats are the reservoirs of a rickettsia that produced a milder form of typhus in humans (275). Murine typhus is most prevalent in warmer countries, and epidemic typhus is prevalent in colder countries. Murine typhus is a zoonosis (110) maintained in rodents and transmitted to humans by the rat flea *Xenopsylla cheopis* (13). Humans are infected by contamination of disrupted skin or the respiratory tract with infected flea feces. *R. typhi* strains have a worldwide distribution, but the number of reported cases does not reflect the current prevalence. The fact that the disease is mild and nonspecific suggests that its incidence is probably largely underestimated in tropical countries. However, the disease is prevalent in Texas (74, 249), in Africa (254), in Europe, and in Asia (44). Patients present with a fever, headaches, and rash. The rash is nonspecific and does not appear in half of the patients. *R. typhi* has been detected in a number of fleas (263). Diagnosis is made by serology; the reference method is MIF, although a latex test (119) and a dot blot enzyme-linked immunosorbent assay (236) have been used.

Rocky Mountain spotted fever. Between 1906 and 1910, Ricketts demonstrated that RMSF was transmissible to guinea pigs and incriminated the wood tick, *D. andersoni*, as the vector (267). Several other tick species have been found to be naturally infected with rickettsiae. They include *Hemaphysalis leporispalustris*, *Dermacentor parumapertus*, *Ixodes dentatus*, *I. brunneus*, and *I. texanus*. These ticks rarely attack humans and therefore are of little significance in the epidemiology of spotted fever. Nevertheless, they are important in maintaining and disseminating rickettsiae in nature. Rickettsiae that are closely related or identical to *R. rickettsii* have also been recovered from *Amblyomma americanum*, *A. maculatum*, *D. occidentalis*, *I. scapularis*, *I. pacificus*, and *I. cookei*. These ticks attack humans and must be considered potential vectors of *R. rickettsii* (103). RMSF occurs in North American (the United States and Canada), Central American (Mexico, Panama, and Costa Rica) and South America (Brazil and Columbia). A series of case reports collected in the United States showed that the disease is most frequently observed in young, white, male patients. There is a wide variation in the incidence of RMSF across the United States (270). The number of reported cases of the disease increased during the 1970s but has subsequently decreased from more than 1,000 to about 500 per annum (60, 66). Fewer cases are reported in the west and midwestern states, with most cases now occurring on the Atlantic seaboard, a region where the disease was once relatively rare. Increased incidence has, however, been reported in Oklahoma, Texas, and Arkansas (250). Some new foci of the disease have appeared, including in urban areas such as the Bronx in New York City (229). The onset of 92% of cases was between April and August (146), although in Oklahoma, Texas, and Arkansas, 11% of cases occurred between October and March, with 17% of the cases in Texas occurring during these months (250). The factors that influence the numbers of ticks that are infected with *R. rickettsii* have yet to be determined.

It is difficult to determine exactly when RMSF was first described, although the first clinical report of RMSF was made in 1899 by Maxey in Idaho: "A febrile disease, characterized clinically by a continuous moderately high fever, and a profuse or purpuric eruption in the skin, appearing first on ankles, wrists, and forehead, but rapidly spreading to all parts of body" (213, 214). This disease has the reputation of being the most severe SFG rickettsiosis, since it can be lethal even in previously healthy and young patients (114, 267, 270). Fatalities are, however, usually associated with delay or failure in giving a specific antibiotic therapy (143), delayed or absence of rash, black race, old age, absence of tick exposure history, and winter onset, but a possible diagnosis of RMSF should not be neglected (67). A rickettsiosis should always be suspected when both rash and high fever are present. The incubation period of RMSF is 6 to 8 days following the tick bite and is usually characterized by malaise, chills, headache, fever, and myalgia (266). These nonspecific signs and symptoms may be followed by nonspecific digestive disorders such as nausea, anorexia, vomiting, or diarrhea. A rash appears on about day 3 after the onset of clinical symptoms and may develop into purpura and become necrotic or gangrenous in severe forms of the disease. Interestingly, in some areas of the United States, up to 11% of patients with RMSF have no rash (233). RM "spotless" fever, with an erythematous rash around the site of a tick bite that resembles erythema migrans, can be mistaken for Lyme disease (129). Eschars are not often reported but may be present (271). Mildly affected patients may recover after 2 weeks. Patients with severe forms of the disease have prolonged signs characterized by pulmonary and peripheral edema, renal failure, hemorrhagic purpura, hypovolemia, and

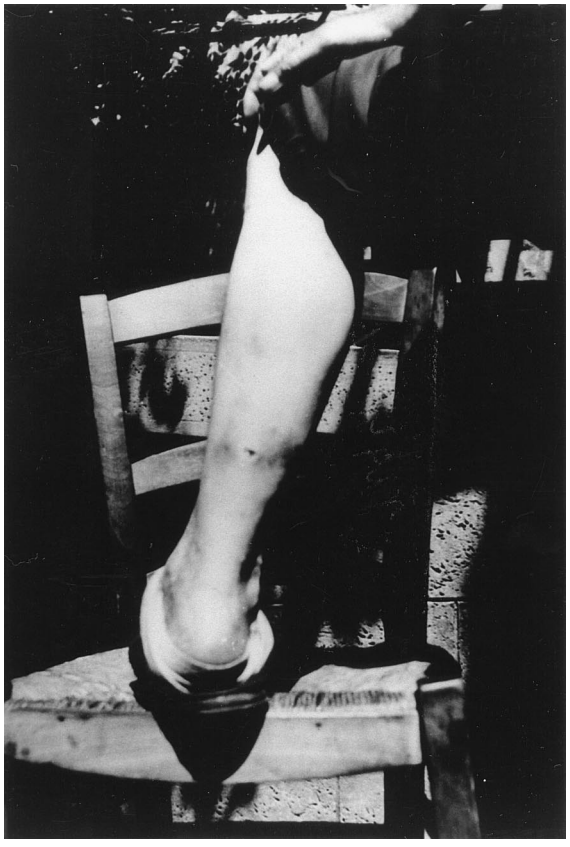

FIG. 5. Pieri's first patient with a tâche noire (left leg) (courtesy of J. Pieri).

hypotension. Neurological signs include delirium, seizures, and coma. Long-term sequelae of RMSF have been described (12). If the disease is recognized in its early phase and treated appropriately, defervescence usually follows in 2 to 3 days. An important remaining question is the spectrum of the various rickettsial strains in terms of pathogenicity. The early works of Ricketts (213) showed a wide difference in the severity of disease between patients from Montana and Idaho, associated with strains of different virulence in guinea pigs. The questions posed in 1909 are still unanswered, and only speculation can explain why 65 to 80% of the untreated people infected in Montana die compared with 5% in Idaho. The correlation between strain variability of *R. rickettsii* and the severity of the disease has been investigated by comparing the pathogenicity of the strains in guinea pigs, but it is not yet clear whether the structural differences found were indeed related to variations in strain virulence (8). Although some cases without spots are definitely due to *R. rickettsii* (culture confirmed), many others are diagnosed solely by serology or immunologic detection of rickettsiae in biopsy specimens. The limitations on species identification by these methods are discussed above, and thus it would be unwise to exclude other SFG agents as potential alternative agents on this basis.

Mediterranean spotted fever. MSF was first described in Tunisia in 1909 (62). As characteristic skin eruptions were papular rather than macular, the disease was referred to as "boutonneuse" fever. The eschar at the site of the tick bite was described in Marseille in 1925 (Fig. 5) by Boinet and Pieri (35). The disease is encountered all around the Mediterranean, in sub-Saharan Africa (254), in India, around the Black Sea (80), and even in Vladivostock in the eastern part of Russia close to Japan. An increase in the number of cases of MSF in France, Italy, Spain, and Portugal paralleled that for RMSF in the United States during the 1970s (158). This increase in incidence was correlated in Spain with higher temperatures and lower rainfall and in France with a decrease in the number of days of frost during the preceding year (203). Sporadic cases are observed in areas without endemic infection, such as Belgium (144) and Switzerland (4). Although *R. conorii* has always been considered to produce a less severe disease than *R. rickettsii*, severe forms of MSF have been reported in 6% of patients and the mortality rate may reach 2.5% (205). For this reason, we cannot consider it to be a mild form of spotted fever. Because of the frequent lack of several classical clinical features, a diagnosis score has been proposed to facilitate the diagnosis of this disease (204). The onset of signs is generally sudden, and in typical cases the patients have fever (>39°C), rash, and eschar (Fig. 6A). Headache, myalgia, and arthralgia are characteristic symptoms of boutonneuse fever. Patients with malignant forms have a petechial rash and neurological, renal, or cardiac problems, especially elderly people (206). Thrombosis of the deep venous vessels and acute pericarditis have also been described as complications of boutonneuse fever (70, 145). Variations in the severity of MSF have been encountered in different countries and even in different areas of the same country. For example, in the northeastern part of Catalonia in Spain, the disease is milder then elsewhere in Spain (86, 89, 225).

Siberian tick typhus. Siberian tick typhus was first described in Primorye in the spring-summer season of 1934 to 1935. It is well described in the former USSR, where literature relating to SFG and TG rickettsioses is abundant (80, 151, 211). The disease is also prevalent in Pakistan (216) and has recently been documented in northern China (87, 293). Its incubation period is usually 4 to 7 days after the tick bite. Thereafter, an ulcerated necrotic lesion appears at the inoculation site, often accompanied by regional lymphadenopathy. Fever (38 to 39°C), headache, myalgia, and digestive disturbances are concomitant symptoms and can last for 6 to 10 days without treatment. The rash, which may be purpuric, usually occurs 2 to 4 days after the onset of clinical symptoms. The central nervous system is often affected during infection. This disease is considered to be a mild form of spotted fever, and it is seldom associated with more profound complications (211).

Queensland tick typhus. Queensland tick typhus has been recognized as a disease since 1946, when the first cases were observed among Australian troops training in the bush of northern Queensland (193). By 1989, only a further 21 cases had been reported (234). More recently, the number of reported cases has increased to 62, showing that rickettsioses are widespread all along the eastern coast of Australia (75, 232, 234). More than one causative agent of rickettsiosis is found in Australia. *R. australis*, the etiologic agent of Queensland tick typhus, is prevalent in the northeastern part of the country, while *R. honei* has been isolated from rickettsiosis patients on Flinders Island, which lies close to Tasmania in the far south. This new disease will be discussed below. The areas of endemic infection by the two organisms have yet to be established. Although *R. australis* and *R. honei* show clear biological and genotypic differences (18), the clinical features of the diseases they cause are quite similar. After a sudden onset, characterized by fever, headache, and myalgia, patients usually develop a rash (maculopapular or vesicular) within the first 10 days. An eschar seems to be more prevalent in cases from the north (65%). Bites clearly associated with ticks are reported more often in the north than in the south, where lesions attributed to "insect" bites are frequently mentioned. Lymphadenopathy is

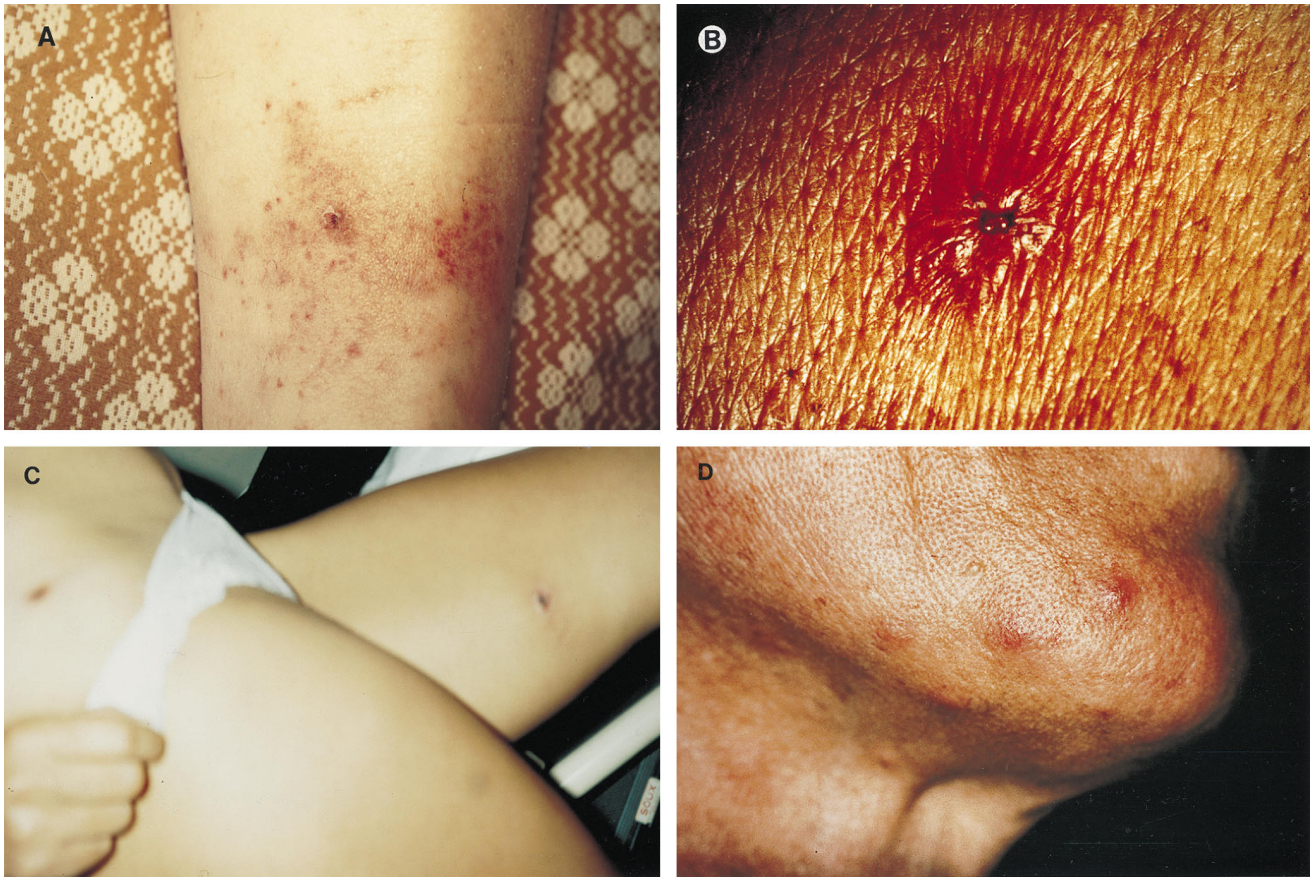

FIG. 6. Lesion of spotted fevers. (A) Tâche noire and purpuric fever eruption in a patient with MSF. (B) Tâche noire in a patient with Japanese spotted fever (courtesy of F. Mahara). (C) Multiple tâche noire in a patient with African tick bite fever. (D) Vesicular eruption on the face of a patient with African tick bite fever.

also a common feature. Only a single fatal case of Queensland tick typhus has been reported to date (235). The common tick species biting humans in Queensland is known to be *Ixodes holocyclus*. This and *I. tasmanii* have been confirmed to harbor *R. australis* in a study from Queensland (57). The latter tick seems to play a role in the maintenance of this rickettsia in small animals (57). Moreover, Cook and Campbell (64) detected antibodies in 54 of 307 bandicoots and rodents trapped in northern Queensland.

Israeli spotted fever. The first cases of rickettsial spotted fever in Israel were reported in the late 1940s and were diagnosed as RMSF (104). The number of cases increased following the development of new settlements in the rural areas of Israel. Although the disease presents with clinical features similar to those of MSF, the typical eschar at the inoculation site is usually lacking. In 1974, Goldwasser et al. (105) isolated and characterized the rickettsial agent of the disease, finding it to be slightly different from *R. conorii*. Antigenically, the causative organism is distinguishable from the reference strain of *R. conorii*, and recent comparison of rOmpA gene sequences has also demonstrated it to be distinct (157, 221). Several fatal cases and severe forms have been described, and the prevalence of the disease seems to be increasing (104, 105, 108). An epidemiological survey showed that among infected children, 714 patients were male and 213 were younger than 9 years. The incubation period was estimated to be about 7 to 8 days after the tick bite, and the symptoms observed in all the patients were fever and a rash which usually started on the hands and feet and extended centripetally. The prevalence of arthralgia, headache, vomiting, and myalgia varied from 13 to 33%. A primary lesion, resembling a small pinkish papule rather than a real eschar, was found less often (7%) than spleno- or hepatomegaly (35 to 30%) (108). Fatal cases have also been reported (291). Asymptomatic infections have been described and authenticated by seroconversion (230). However, the test used was not specific enough to ensure that the Israeli isolate was definitely the agent provoking seroconversion.

Rickettsialpox. Rickettsialpox was first described in New York City in 1946 (127). Most of the cases were associated with a single housing development. The disease was meticulously reported by the New York City Department of Health (106, 107, 126–128, 218). The disease is caused by *R. akari* and is transmitted by the bite of *Allodermanyssus sanguineus*, a mite ectoparasite of the domestic mouse (*M. muscularis*). The onset of this mild disease usually occurs 7 to 10 days after mite bite. At the inoculation site, a painless red papula appears and becomes vesicular over the following days. The scab that usually appears when the vesicular lesion bursts persists for about 3 weeks. Regional lymph nodes may be slightly enlarged. Fever appears suddenly, accompanied by chills, headache, myalgia, anorexia, and photophobia. A rash sometimes develops simultaneously with these signs but may instead develop several days later. Cutaneous lesions are maculopapular and develop into a vesicular form. When the vesicles dry out, they are usually replaced by crusts that do not produce scars. Because of its clinical resemblance to chickenpox, the disease was called rick-

ettsialpox. Even untreated, patients recover spontaneously in 1 to 3 weeks. Varicella and, in the early stages of rickettsialpox, other rickettsioses have to be considered in the differential diagnosis of this disease. The disease is still prevalent in New York City (40, 134) and has also been found in the Ukraine (78), in Korea (133), and recently in Slovenia (196). Despite such a wide geographical spread, *R. akari* strains are very homogeneous; macrorestriction analysis of the U.S. and Ukrainian strains shows them to be indistinguishable (78).

Newly Described Diseases

Japanese or Oriental spotted fever. Japanese spotted fever was described by Mahara et al. (154–156), a Japanese physician. He was alerted by the observation of two cases of highly febrile exanthema within 3 months of each other during the summer of 1984. Both patients lived in the countryside, one had a black eschar, and both had collected bamboo shoots on the same mountain (Fig. 6B). The patients' sera tested positive in the Weil-Felix test (154) and then by MIF with *R. montana* as the antigen (260). The causative organism has recently been isolated from patients (262) and characterized as a new SFG rickettsia. The disease is now known to be endemic in the southwestern part of Japan, where more than 100 cases have been described. The agent, *R. japonica* (292), and its tick vectors, *Haemaphysalis longicornis* (261) and *Dermacentor taiwanensis* (244), have now been characterized. The ticks *Ixodes ovatus* and *H. flava* have also been incriminated as vectors of this rickettsia. It is interesting that this disease is a "typical" spotted fever, as described in 1899 for RMSF and in 1909 for MSF. That it was not identified until 1986 in Japan, a country with an excellent medical infrastructure, is very surprising. Nothing, it seems, can replace the curiosity of a single physician. The first diagnosis was made by the Weil-Felix test, which is also used to diagnose scrub typhus, a disease endemic in parts of Japan although not in the area where Mahara observed his cases. He chose this test because of suspicion of an atypical scrub typhus with a novel clinical presentation and epidemiology. The Weil-Felix test is a very old test, having been introduced during the First World War, and therefore it would have been available for use whenever the syndrome was first observed. Thus, the recognition of this new clinical entities depended primarily on the physician's observations and suspicion. Once a preliminary diagnosis is established, a battery of modern methods makes it possible to confirm observations and to isolate and characterize the etiological agent. The clever part is simply being aware of a possible new disease.

Flinders Island spotted fever. Flinders Island spotted fever was described by Stewart, the only doctor of Flinders Island of Tasmania, south of Australia. He described 26 cases, observed over 12 years, of a summer febrile illness associated with a rash that was erythematous in the majority of patients and purpuric in two patients with severe cases associated with thrombocytopenia (242). The patients presented with a local lesion (eschar) in 25% of cases, and enlarged local nodes in 55% of cases. The patients' sera were initially assayed serologically by the Weil-Felix test and subsequently by MIF, and the results confirmed that the agent was a SFG rickettsia. At the time of Stewart's observations, three rickettsial diseases were known in Australia: (i) murine typhus, which is widespread in Australia, presents as a mild disease characterized by a transient erythematous rash, and is associated with rat fleas; (ii) scrub typhus, which is associated with generalized polyadenanopathy, and is found only in northern Australia; and (iii) Queensland tick typhus, as described above. The main clinical difference between Queensland tick typhus and Flinders Island spotted fever is the occurrence of a vesicular rash and of local lymph node enlargement in the former. It was the fact that the disease was observed far from its described area of endemicity that led to the suggestion that it was a distinct rickettsial infection. This suggestion was confirmed in 1992 (18), when a new isolate was obtained from patients. The isolate was characterized by sequencing of the 17-kDa antigen gene and proposed as a new species, *R. honei* (19). Two lessons can be drawn from this episode: first, again, the key role of a curious general practitioner, who patiently collected data for 12 years in a manner resembling that leading to the descriptions of RMSF (214) and MSF (63), and second, cell culture isolation systems and the power of DNA sequence analysis. Both allowed confirmation of a new etiological agent (18, 19).

Astrakhan fever. In Astrakhan on the Caspian Sea, an eruptive febrile summer disease had been observed since 1983. It was apparently unknown before this time (247) and was characteristic enough to be considered a new disease, which was defined as and named Astrakhan fever. The presence of tâche noire was reported in 20% of patients. In 1989, physicians from Astrakhan began sending sera to the laboratories of Balayeva (23) and Tarasevich (246, 247) at the Gamaleya Institute for Epidemiology and Microbiology in Moscow for testing for rickettsial antibodies. Sera tested in Moscow were found to be positive by the complement fixation test in 50 to 70% of patients (23, 246, 247) when *R. conorii* was used as the antigen. The clinical and epidemiological aspects have been described to include dog ticks as vectors (81, 247). The causative agent of the disease has recently been isolated from patients (69) and from *Rhipicephalus pumilo* ticks (83). The as yet unnamed bacterium was shown to be closely related to, but distinct from, *R. conorii*. In this instance, attention was drawn to the disease by an apparent outbreak. Patients were not treated and remained sick for 2 to 4 weeks. Locally, it was thought that the illness was related to environmental changes following the construction of a CO_2-producing petrochemical complex. Interestingly, it has been shown that CO_2 produced by human respiration attracts ticks. Ticks possess powerful CO_2 receptors, enabling them to detect potential animal hosts. The hypothesis of a rickettsiosis was proven with the aid of modern cell culture systems and molecular identification techniques (69) in the National Reference Center in Moscow rather than in Astrakhan, where physicians were reluctant to admit that they had encountered a rickettsial disease. The disease, however, is very similar to MSF in the preantibiotic era, and with this knowledge, it is difficult to mistake the illness for other infections.

African tick bite fever. African tick bite fever has been discovered twice. During the 1930s, Pijper described a very mild disease that was transmitted by tick bite in South Africa, and which often occurred without a rash. He reported that people were infected in the bush by the bite of ticks of the *Amblyomma* complex. He isolated the causative agent and determined that it was different from *R. conorii* by cross-protection studies in guinea pigs. The isolate, along with all Pijper's data, which was published in little known journals (190, 191), was lost. Pijper's successor, Gear (94, 95), isolated *R. conorii* from a *Rhipicephalus* tick, and therefore South African tick bite fever was considered to be a variant of MSF. However, a publication in 1990 by Kelly (139), a veterinary scientist, reported the isolation of rickettsial strains from *Amblyomma hebraeum* ticks in Zimbabwe. He demonstrated that the strains were distinct from *R. conorii* and were indistinguishable from an isolate obtained from *Amblyomma variegatum*, *A. gemma*, and *A. cohaerens* ticks collected between 1969 and 1971 in Ethiopia (53). This rickettsia was shown to be much more

prevalent in ticks in Zimbabwe than was *R. conorii* and to infect *Amblyomma* ticks, which bite a wide range of animals including humans, as opposed to *R. sanguineus* complex ticks, which rarely bite humans (100). A high seroprevalence of antibodies against this new isolate was also demonstrated among the population, with infection reaching 80% in some areas of endemicity (140). A review of Pijper's papers years later led to the hypothesis that this new rickettsia (*R. africae*) was responsible for the vast majority of the cases of rickettsiosis in Zimbabwe. However, since the disease is mild, patients are not hospitalized, making the acquisition of material to test this theory difficult. Blood was finally collected from suspected patients, and *R. africae* was isolated by the shell vial assay, thus confirming the presence of a second tick-transmitted rickettsiosis in Africa (137, 138, 141). Several cases have subsequently been reported among travellers returning from either Zimbabwe or South Africa. Examination of these cases has allowed the determination of a typical clinical picture for the disease, which is generally milder than MSF (42). Tâche noire is common and is usually accompanied by a tender large lymph node. Multiple eschars can also be observed, a critical point, because *R. sanguineus* complex ticks rarely bite humans, and thus the chance of being bitten more than once at the same time is virtually zero. Consequently, the occurrence of two eschars on a patient (Fig. 6C) all but excludes the possibility of a disease transmitted by *R. sanguineus* complex ticks and therefore rules out a *R. conorii* infection. If observed at all, some of the few eruptive lesions are vesicular (Fig. 6D); a vesicular rash is never observed in MSF. By indirect evidence such as seroepidemiology (254) or retrospective analysis of published imported cases, we believe that the prevalence of African tick-bite fever is high. The attack rate has been reported to be up to 30% in U.S. military citizens in Botswana (136). The key point in this episode is that the rickettsial species was isolated and characterized before the disease was recognized. We are currently developing monoclonal antibodies to *R. africae* for use in assays to distinguish between *R. conorii* and *R. africae* in culture and skin biopsy samples (290).

California flea rickettsiosis. Adams et al. reported that in 1967, while studying the changing ecology of murine typhus in southern California, they noticed a shift in the distribution of cases from central Los Angeles to eastern Los Angeles and Orange county (3). In this area, the usual reservoir (the Norway rat, *Rattus norvegicus*) and vector (the Oriental rat flea, *Xenopsylla cheopis*) were prevalent. However, the group also noted that opossums were present in places where cases were observed and that the seroprevalence of antibodies to *R. typhi* in opossums was high (282). These opossums were parasitized by cat fleas (*Ctenocephalides felis*) which were infected by a rickettsia (2). This rickettsia has since been grown successfully (197) and has been shown to be transmitted transovarially in cat fleas (14). The rickettsia was originally named the ELB agent and subsequently renamed *R. felis* (124). Although *R. felis* is distinct from *R. typhi*, antibodies specific for *R. felis* demonstrated a strong cross-reaction with *R. typhi*, and thus patients with *R. felis* were diagnosed as having murine typhus when serology was used. No clinical description of the disease is presently available, and it has not yet been encountered in other parts of the world, except for one occasion when DNA from *R. felis* was amplified from a patient from Texas with fever and headache (231); one can therefore suspect that some of the reported murine typhus cases in Texas may be due to *R. felis*. This single patient apparently had no rash, although the description of the clinical spectrum of the disease is incomplete. In this case, the disease does not appear to be new, yet even now virtually nothing is known of it except that cases without rash can be observed.

Infection due to "*R. mongolotimonae*." In 1991, an SFG rickettsia was isolated from *Hyalomma asiaticum* collected in Inner Mongolia, a Chinese province, and subsequently characterized (293). It was antigenically and genotypically unique among SFG rickettsiae. Surprisingly, an indistinguishable isolate was obtained from the blood and the skin of a patient in 1996 by the shell vial assay. The patient was hospitalized in Marseille, France, in March (an atypical month for MSF) with a mild disease characterized by only a few spots on his body and an inoculation eschar. This patient was a resident of Marseille with no prior travel history or contact with individuals from Mongolia. The only possibly relevant history was that the patient had collected compost from a garden where migratory birds were resting (200). Birds carry ticks, and *Hyalomma* species can parasitize birds. We speculate that the patient was bitten by a migratory bird tick collected with the compost. Some birds could travel from the Arctic to Africa on a route passing through Mongolia and France (56). Dog ticks, prevalent in Marseille, are absent in March and are infected only with *R. massilliae*, *R. rhipicephali*, or *R. conorii*. The new species name has been proposed as "*R. mongolotimonae*" to acknowledge the names of the two places (Mongolia and La Timone Hospital, Marseille) where it has been encountered. The prevalence of the disease is unknown, but if the theory about migratory birds is correct, it could explain some sporadic cases of SFG rickettsioses occurring in areas that do not have endemic infection or during unusual seasons (257). Moreover, there is likely to be an as yet unrecognized rickettsiosis in Inner Mongolia waiting to be discovered.

Lessons from the recently described rickettsioses. None of the described diseases are new, only newly described or newly identified. What was critical in their discovery? The physician is frequently the first to be aware of something new, as in the case of Flinders Island spotted fever, Japanese spotted fever, and Astrakhan fever. In some cases, epidemiologic evidence plays a major role, as for California flea rickettsiosis or African tick bite fever. In one case, the realization of the prevalence of a *Rickettsia* species in ticks led to a prospective work to detect clinical cases (African tick bite fever), and such a work should be undertaken with California flea rickettsiosis. In some instances, technological advances have allowed easier diagnosis of infection, such as in the case in Texas when *R. felis* was identified in a blood sample not collected specifically for the isolation of rickettsiae, and in our case of "*R. mongolotimonae*." In these two cases, the identification of isolates by molecular methods allowed the incrimination of new species in human diseases.

How and where will we find other rickettsial diseases? Potential candidates are described in the section on rickettsiae of unknown pathogenicity. Additionally, the etiology of some human diseases may be rickettsial, such as acute cerebral vasculitis or the Israeli illness associated with antibodies to *R. typhi*. Moreover, we can expect new diseases in places where no spotted fevers have yet been described. It should be expected that in countries where spotted fevers are known, several may exist in the same place, making serological surveys difficult; this has been the case in Marseille, France, and in Southern Africa, and we suspect that it occurs elsewhere. Atypical cases of recognized spotted fevers are candidates for new etiologies, such as cases without rash, cases occurring in winter, and cases with multiple eschars. In these circumstances, the genomic identification of the involved agent may incriminate rickettsiae.

TABLE 3. Rickettsiae of unknown pathogenicity and their arthropod reservoirs

Rickettsia	Tick reservoir	Geographical locations	Reference(s)
"*R. slovaca*"	*Dermacentor marginatus*	Slovakia, Armenia, Russia, France, Switzerland, Portugal	26, 210
R. canada	*Haemophysalis leporispalustris*	Canada	170
R. massiliae	*Rhipiceephalus turanicus*, *R. sanguineus*, other *Rhipicephalus* sp.	France, Greece, Spain, Portugal, Central Africa	15, 24
Bar 29	*Rhipicephalus sanguineus*	Spain	31
R. rhipicephali	*Rhipicephalus sangguineus*, *Dermacentor andersoni*	United States, France, Portugal, Central AFrica	51
R. parkeri	*Amblyomma maculatum*	United States	177
R. montana	*Dermacentor variabilis*, *D. andersoni* Genera *Microtus*, *Peromiscus*	United States	32
R. bellii	*Dermacentor* species, *Ornithodoros concanensis*, *Argas cooleyi*, *Haemophysalis leporispalustris*	United States	188
"*R. aescchlimannii*"	*Hyalomma marginatum*	Morocco	29
Strain S	*Rhipicephalus sanguineus*	Armenia	79
"*R. amblyommii*"	*Amblyomma americanum*	United States	195
Unamed rickettsia from Pakistan (JC 880)	*Rhipicephalus sanguineus*	Pakistan	215, 216
HL-93	*Haemophysalis concinna*	China	294
Thai tick typhus rickettsia	*Ixodes* + *Rhipicephalus* pool	Thailand	216
R. helvetica	*Ixodes ricinus*	Switzerland, France	47
AB bacterium	*Adalia bipunctata* (Ladybird beetle)	England, Russia, United States	22, 280
R. peacockii	*Dermacentor andersoni*	United States	171
Pea aphid rickettsia	*Acrythosiphon pisum*	United States	61
R. texiana	*Amblyomma americanum*	United States	10
Unnamed rickettsia	*Dermacentor occidentalis*	United States	189
Unnamed rickettsia	*Dermacentor parumapertus*	United States	189
Unnamed rickettsia	*Ixodes pacificus*	United States	130
Unnamed rickettsia	*Amblyomma americanum*	United States	189

RICKETTSIAE OF UNKNOWN PATHOGENICITY

Rickettsiae of unknown pathogenicity constitute a reservoir of potential pathogens. Surprisingly, isolates of unknown pathogenicity have a tendency to be considered nonpathogenic. Members of this group of rickettsiae are described below according to their arthropod hosts, the factor which determines their ability to come into contact with human beings and consequently to infect them (Table 3). Species are discussed and compared to 14 species presently recognized as human pathogens.

Rickettsiae Isolated from *Rhipicephalus* Species

Rhipicephalus ticks rarely bite human beings but exist in such close proximity that bites do occur and thus infection is transmitted, as seems to be the case for MSF, Astrakhan fever, and Israeli spotted fever. Five rickettsiae of unknown pathogenicity have been isolated from *Rhipicephalus* ticks.

R. rhipicephali (51) was first reported from Mississippi in 1975 (54). It was isolated from *Rhipicephalus sanguineus* and has subsequently been detected in France (71) and Portugal (17) in the same tick species. It has been suspected as causing fever in a patient after a dog tick bite (51).

The Thai tick typhus agent was obtained from a pool of *Ixodes* and *Rhipicephalus* ticks in Chiang Mai, Thailand (216). Its pathogenicity is completely unknown, and it is distinct from other rickettsiae both antigenically and genetically (221, 223).

A unnamed rickettsia (JC880) was reported in Pakistan (215, 216) in 1970 and 1973; five isolates were available and have now been shown to be distinct from *R. conorii*, *R. sibirica*, Thai tick typhus rickettsia, *R. parkeri*, *R. rickettsii*, *R. akari*, and *R. australis.*

R. massiliae (30) is a recently described rickettsia that parasitizes *Rhipicephalus sanguineus* and *Rhipicephalus turanicus* in France (24) and Portugal (17), and similar rickettsiae have since been isolated in Greece (15). A closely related strain (Bar 29) has also been isolated in Barcelona from *Rhipicephalus sanguineus* (31). This bacterium is identical to an isolate that we previously described as MTU5 (24) and is slightly different from *R. massiliae* by antigenic and phenotypic criteria. *R. massiliae* has also been detected in various African *Rhipicephalus* species in central Africa (255). The geographic distribution of this rickettsia and its predominance in *Rhipicephalus* species in certain areas such as Catalonia, Spain, make it a pathogen of potential importance. This bacterium exhibits a natural resistance to rifampin in cell cultures (31).

Strain S (79) has been isolated from *Rhipicephalus sanguineus* in Armenia. Spotted fever is endemic in that country, and *R. sibirica*, a known pathogen, has also been isolated. Consequently, the pathogenic role of strain S is unknown and requires investigation.

Rickettsiae Isolated from *Dermacentor* Species

In the United States, *R. rhipicephali* has been isolated frequently from *D. occidentalis* and *D. andersoni* and occasionally from *D. variabilis* (91, 188).

R. bellii (188) is the species most usually isolated from ticks in the United States. It was first obtained from *D. variabilis* in 1966 by Bell and has subsequently been recovered from *D. andersoni*, *D. occidentalis*, and *D. albipictus* and from members of other tick genera such as *Ornithodoros concanensis*, *Argas cooleyi*, and *Haemophysalis leporispalustris* (188). The species is apparently confined to America, where its pathogenic role has yet to be discovered.

R. montana (32) was first isolated from *D. variabilis* and *D. andersoni* in Montana in 1953. It is nonpathogenic for guinea

pigs but has been isolated from rodents (genera *Microtus* and *Peromiscus*) (189), and its role in human disease is unknown.

"*R. slovaca*" was isolated in Czechoslovakia in 1968 (41) from *D. marginatus*. It has since been isolated in France (25), Armenia (80), Switzerland (26), and Portugal (17). It has been suspected of being the agent of meningoencephalitis in Slovakia, following detection of a seroconversion in two patients by complement fixation (80). This relationship has yet to be confirmed.

Unnamed species were isolated from *D. occidentalis* in California and *D. parumapertus* in Nevada and Utah (189). *R. peacockii*, the East-side agent, is a rickettsia strictly located in *D. andersoni* ovarial tissue in Montana (178). It is not found in hemocytes, and attempts to grow it have been unsuccessful. Sequencing of 16S rRNA- and rOmpA-encoding genes showed that it is close to but distinct from *R. rickettsii* (171).

Rickettsiae Isolated from *Amblyomma* Species

R. parkeri was isolated in 1937 (177) from *A. maculatum* collected from Texan cows. It has never been recovered outside the United States. It has recently been shown to share a close genotypic relationship with the newly described species *R. africae*, which also parasitizes *Amblyomma* species in subsaharan Africa. The pathogenic role of *R. parkeri* is unknown, but *Amblyomma* ticks are usually capable of biting humans.

A rickettsia isolated from blood and lymph nodes of patients and from *A. americanum* ticks collected in Texas has been described. This rickettsia, named *R. texiana*, was responsible for over 1,000 cases of Bullis fever among World War II troops in training at Camp Bullis (10).

Unnamed rickettsial species were isolated previously from *A. americanum* in 1971 in Alabama (189). Rickettsial strains were also isolated from *A. variegatum* in Guadeloupe (French West Indies) in 1966 (58), but unfortunately, these strains, which were distinct from *R. conorii*, were not characterized and were lost. "*R. amblyommii*" (195) has recently been isolated from the lone star tick *A. americanum*. Little is known about its prevalence or its pathogenicity.

Rickettsiae Isolated from Other Arthropods

R. canada was first isolated from *Haemaphysalis leporispalustris* ticks removed from rabbits in Ontario, Canada (167). It has been considered as a member of the typhus group rickettsia based on serologic cross-reactions (132). However, the genetic data obtained by both the sequences of the 16S rRNA and the citrate synthase gene showed that *R. canada* is outside the typhus group (223, 224). The role of *R. canada* as a human pathogen is not established. Serological evidence of human infection has been reported (36). In four patients presenting with an RMSF-like disease in California and Texas. A role for *R. canada* in acute cerebral vasculitis is also suspected (see above) (118, 149, 279). However, the pathogenic role in humans needs more investigation.

R. helvetica was first isolated from *Ixodes ricinus* in Switzerland in 1979 (47). It has also recently been isolated from the same tick species in central France (179). An SFG rickettsia, similar to *R. helvetica*, was recently described from *Ixodes* ticks in Sweden (172).

R. aeschlimanni has been obtained from the Moroccan tick *Hyalomma marginatum* (29). The AB bacterium has been detected in the ladybird beetle *Adalia bipunctata* by molecular methods. It has not been grown to date, and if it is restricted to ladybird beetles, it is difficult to consider it a potential human pathogen (22, 280). Pea aphid rickettsia was detected in hemolymph of the pea aphid (*Acyrthosiphon pisum*) a plant-feeding arthropod. It was impossible to culture this bacteria from pea aphid hemolymph (61). An unnamed species has been obtained from *I. pacificus* ticks in Oregon (130). A new rickettsia, HL-93, was isolated from *Haemophysalis concinna* ticks in China (294).

RICKETTSIOSES AROUND THE WORLD

The geographic distribution of pathogenic rickettsiae and of rickettsiae of unknown pathogenicity is summarized in Fig. 7 and 8.

America

RMSF occurs in the United States, Canada, Mexico, parts of Central America including Costa Rica and Panama, and parts of South America including Colombia and Brazil (270), where it represents a significant public health problem (93).

Recent studies reveal that only a small proportion of ticks are infected by rickettsiae in the United States, and of these many are *R. bellii*, with only a small percentage containing *R. rickettsii* (46, 77, 150, 153, 165, 187, 194). In North Carolina, the state with the highest incidence of RMSF, only 3.4% of *D. variabilis* ticks contained SFG rickettsiae. Among 72 SFG rickettsiae isolated, only one was *R. rickettsii*, the remainder being *R. montana*. In western Montana, 8.3% of ticks contained rickettsia-like organisms in hemolymph. Of the rickettsiae isolated, 42.6% were *R. rhipicephali*, 38.7% were *R. bellii*, 9.4% were *R. rickettsii*, and 7.5% were *R. montana. R. rickettsii* is still prevalent in the southern and southeastern regions of the United States (267, 270). Cases have also been reported recently in New York City (229), where infected *D. variabilis* ticks were found in local parks.

R. akari, the agent of rickettsialpox, is still prevalent in the United States, and cases are sporadically described, in particular in New York City (40). *R. texania* was described in Texas (10). Murine typhus is prevalent in Texas. It peaks in June and affects more Hispanics than other ethnic groups (270). Epidemic typhus is sporadically observed in Peru and in Guatemala (217). *R. felis* is an emerging pathogen that needs further investigation. Currently, the disease it produces is known in Texas, and epidemiologic evidence suggests its presence in California (3, 282).

Rickettsiae not linked to human disease. Six *Rickettsia* spp. have not been associated with disease: *R. rhipicephali*, *R. canada*, *R. montana*, *R. parkeri*, "*R. amblyommii*," and *R. bellii*, which all live in ticks capable of biting humans. Several unnamed strains have been described. *R. bellii* has been demonstrated to be the predominant species of rickettsia in 23 countries in seven states, including 39% of isolates from *D. andersoni* in Montana, 82% of isolates in North Carolina, and 83% of isolates in Ohio. On Long Island, N.Y., all isolates of SFG rickettsiae from adult *D. variabilitis* were *R. montana*. In western Montana, *R. montana* and *R. rhipicephali* are also present. The East-side agent, *R. peacockii*, which parasitizes 66% of the Montana *D. andersoni* ticks, is not circulating in the tick and probably is not able to be transmitted.

Diseases possibly due to rickettsiae. Rickettsioses that do not produce a rash, as reported recently by Sexton and Corey, are obvious candidates for discovery as new rickettsioses (233). The example of infection due to *R. felis* is demonstrative. Acute cerebral vasculitis is another disease of uncertain etiology and in which a rickettsial agent has been serologically implicated. In 1986 in Virginia (160, 279), four males and one female aged between 16 and 59 years were afflicted by an unknown disease. All had severe illness characterized by fever, headache, and

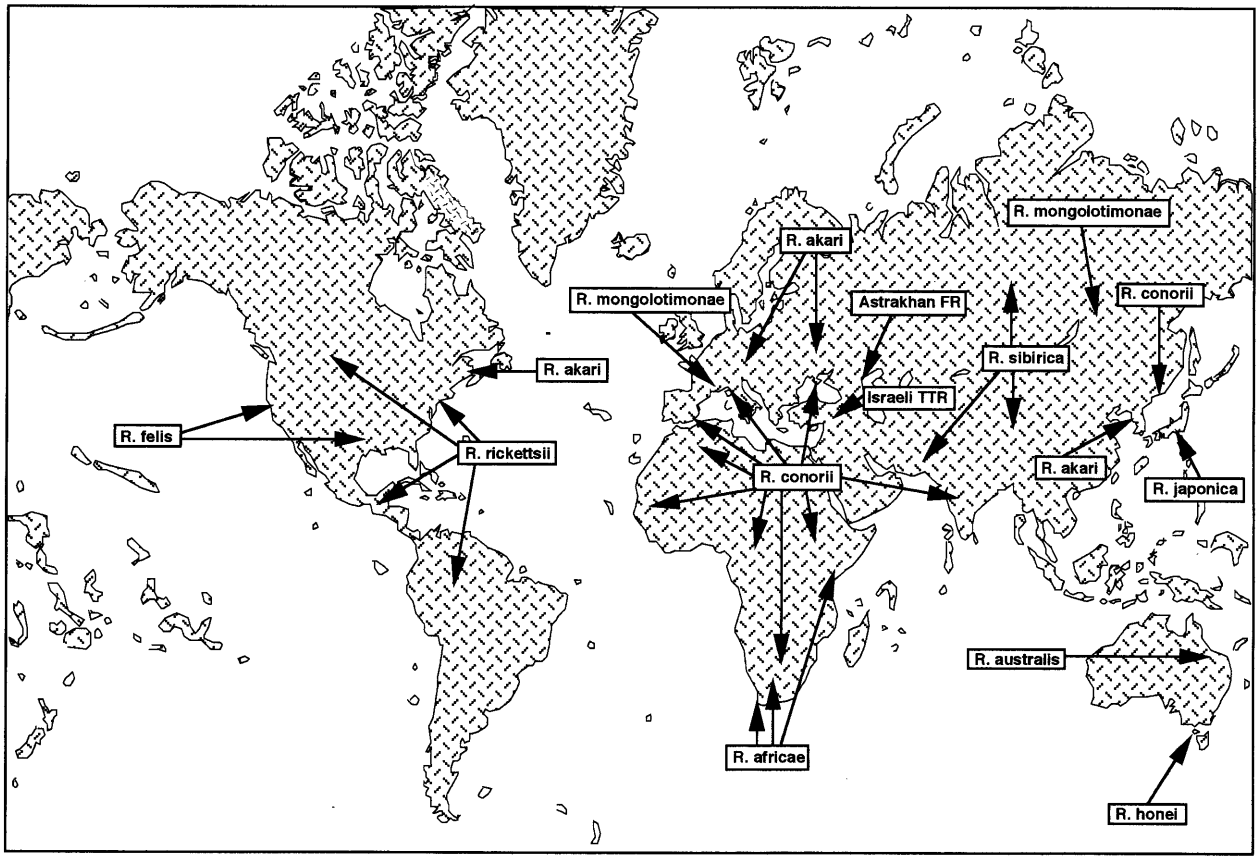

FIG. 7. Geographical distribution of pathogenic rickettsiae.

focal neurologic involvement. Brain biopsy in two patients and autopsy findings in another showed vasculitis and perivasculitis. All the patients were treated with antibiotics, and three were treated with antiviral therapy. Extensive microbiologic investigations revealed only cross-reacting antibodies to *R. typhi*. Sera were also examined by Western blot analysis (117), and the results indicated that the patients had specific antibodies to a typhus group antigen (117). One other case has also been reported (149) in a 37-year-old woman from Ohio, who was hospitalized in December 1985 with fever and headache. She developed tremor of the upper extremities, head, and neck and generalized myoclonus. The cerebrospinal fluid showed a lymphocytic pleocytosis. One month prior to the onset of her illness, she had trapped field mice in her house. Serologic studies showed antibodies to TG rickettsiae. Western blot analysis revealed antibodies unique to *R. canada*. Whether acute febrile cerebrovasculitis will emerge as a real entity remains to be demonstrated. PCR amplification of the 16S rRNA gene, coupled with DNA sequencing, should now be applied to the study of such patients (149).

Europe

In the Mediterranean area, *R. conorii* is the primary spotted fever agent and is transmitted through the bite of the brown dog tick, *Rhipicephalus sanguineus*. A review published in 1957 reported all data dealing with the geographical distribution of boutonneuse fever (or MSF) in the Mediterranean area and southern Europe, with cases reported in Italy, Spain, Portugal, Greece, Turkey, Cyprus, Palestine, Romania, and Bulgaria (174). In fact, *R. conorii* is prevalent around the Mediterranean Sea, south of the 45th parallel, and some foci have been detected in central and northern France, Belgium, and Switzerland and around the Black Sea in Russia. In northern parts of its zone of endemicity indoor heating allows *R. sanguineus* to survive indoors during the winter. Astrakhan fever rickettsia and *R. sibirica* were found in Russia (West of the Ural Mountains including Caucasus). *R. akari* has been described in the Ukraine (80) and recently in Slovenia (196).

Murine typhus is prevalent in Greece (258) and in Spain (226). In France, only imported cases have been observed (76).

Rickettsiae not linked to human disease. *R. massiliae* is a widely distributed rickettsia (France, Spain, and Portugal). *R. rhipicephali* and *R. helvetica* have not been implicated in disease to date. *R. helvetica*-infected *I. ricinus* ticks frequently bite humans. "*R. slovaca*" is prevalent in western Europe; it was considered in Russia to be avirulent form of *R. sibirica* (80). "*R. slovaca*" is found from France to the Crimea. Its role as a human pathogen has been debated; two cases of meningoencephalitis have been suggested (80).

Diseases possibly due to rickettsiae. Atypical cases of MSF are candidates for infections caused by a new rickettsia. This include "spotless" fever (43), winter cases (257), and cases with multiple eschars. Cases in patients infected outside of the recognized area of endemic infection are also of interest. In central France, a seroepidemiological study (199) showed that people living in the countryside had a very high prevalence of antibodies. In one area, Western blot analysis confirmed a high prevalence of antibodies specific to *R. conorii*. In this area, a prospective investigation identified patients from whom we

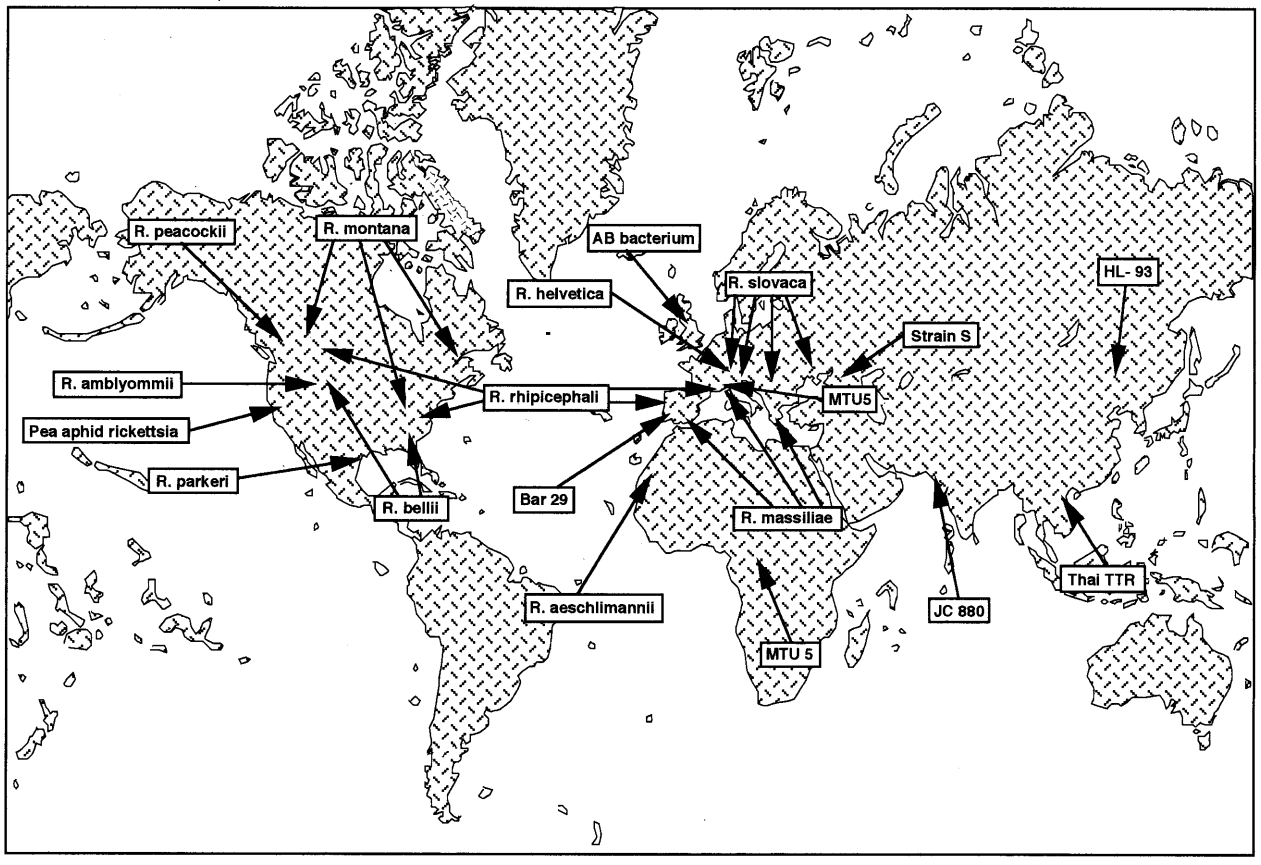

FIG. 8. Geographical distribution of rickettsiae of unknown pathogenicity.

isolated *R. conorii* and *Rhipicephalus* ticks, from which isolates of *R. conorii* were also obtained (34). However, in nearby areas we observed patients with a high prevalence of nonspecific antibodies directed against SFG lipopolysaccharides. We are now investigating the ticks of this area and hope to find a new *Rickettsia* species. Currently, only *R. helvetica* has been found in this region (179).

The reasons behind the large difference in mortality observed in the series of Spanish patients in the western part of Salamanca (225) and the patients of northeastern Barcelona (89) are unknown but could be explained by strain or species differences. In the vast majority of the *Rhipicephalus sanguineus* ticks tested, a variant of *R. massiliae* (Bar 29) was found (31). This strain was rifampin resistant, perhaps explaining the failure of this antibiotic among patients in this area.

Asia

In the middle East and the Far East, *R. conorii* was described. In Israel, a strain of SFG rickettsiae causes a spotted fever without eschar. The bacterium is different from *R. conorii*, and its name has been proposed as "*R. israeli*." Murine typhus is also prevalent. In 1986, a series of 24 patients with a febrile disease of unknown etiology but with antibodies to *Proteus* OX19 was described in Israel (238). It was suggested that *R. typhi* was the agent of the disease, but since the bacterium had not been isolated, this was largely speculative, especially as the patients had atypical presentations. Their sera reacted against *R. typhi*, *Legionella bozemanii*, and OX19, but cross-reacting antibodies were removed by absorption with *R. typhi* (202). However, this outbreak may involve a new rickettsial agent.

In Armenia, one pathogenic rickettsia, *R. sibirica*, is found at its western limit of distribution, and two rickettsiae not currently associated with disease, strain S and "*R. slovaca*," are also present (21). In Crimea, "*R. slovaca*" was also characterized (20). In Russia, *R. akari* was found in the eastern region of the country. *R. conorii* may exist as far as Vladivostock (248). Epidemic typhus has long been a major problem, killing probably millions of people from World War I to World War II. Only Brill-Zinsser disease cases have been reported for 30 years (82), although it represents a major threat in this country.

In China epidemic typhus was a real problem until 1960. The last foci were observed in mountainous regions. However, the seroprevalence continues to be high, and consequently a large reservoir still exists. A large outbreak of murine typhus was observed from 1980 to 1982 along the Yellow River (168). *R. sibirica* and "*R. mongolotimonae*" are found in China; the latter has been isolated in Inner Mongolia and has recently been shown to be pathogenic for humans. HL-93 has also recently been isolated (294) but as yet has no demonstrated pathologic role in humans.

In India, an Indian tick typhus is described and the associated rickettsia has been isolated. It is closely related or identical to *R. conorii*. However, an eschar is rarely formed. Epidemic typhus occurred until recently in Kashmir (176), and murine typhus is prevalent throughout the country. In Pakistan, *R. conorii* and *R. sibirica* are found, as is an unnamed species of unknown pathogenicity (JC 880). In Korea, *R. akari* isolates were described.

In Thailand, spotted fever and murine typhus are prevalent (237, 281). Thai tick typhus rickettsia has been isolated from ticks. Its pathogenic role, despite its name, is unknown (215, 216).

In Japan, Japanese or Oriental spotted fever is prevalent, but another rickettsia has been suspected to be present in *I. ovatus* and *Haemophysalis flava* ticks in Kanagawa prefecture (135).

Africa

Epidemic typhus remains a major threat in Africa. It is endemic in Ethiopia, and sporadic cases have been reported in Uganda, Rwanda, and Burundi (131, 182, 286–288). Since 1996, we have been investigating a large outbreak in refuge camps, associated with the civil war in Burundi (289). Murine typhus is also prevalent in many African countries (254).

R. conorii is prevalent on the Mediterranean coast (Tunisia, Algeria, Morocco, Libya, and Egypt) but has also been isolated in Somalia (283), Kenya (where it was referred as to Kenya tick typhus), Central Africa (255), Zimbabwe, and South Africa (94). *R. africae*, which causes African tick bite fever, is prevalent in South Africa (42) and Zimbabwe and has been recovered from ticks in Ethiopia (53) and Central Africa (255).

Rickettsiae not linked to human disease. *R. rhipicephali*, *R. massiliae*, and MTU5 have all been isolated or genomically identified in Africa. Two new strains, "*R. aeschlimannii*" (MC16) (29) from Morocco and Hmr from Zimbabwe (27), have also recently been isolated in our laboratory.

Diseases possibly due to rickettsiae. We recently performed a study of human sera from Africans and observed a very high prevalence of antibodies to rickettsiae (254). Sub-Saharan Africa is probably the region where rickettsioses are most prevalent. In this study, we found antibodies directed against both SFG rickettsiae and *R. typhi*. This fact, together with the number of *Rickettsia* species found in African ticks (255), suggests that this continent is probably home to a number of undiscovered rickettsioses, the study of which should be facilitated through the extended use of PCR-based methods. For example, we have recently amplified and sequenced DNA derived from *R. prowazekii* from dry lice sent from Burundi on suspicion of an epidemic typhus outbreak (202a). This method of identification makes collaboration with Africa easier, since the problems associated with sample transportation and preservation have previously hindered such study.

Oceania

In Australia, two diseases are prevalent: Queensland tick typhus due to *R. australis* on the continent and Flinders Island spotted fever due to *R. honei* in the south. Little is known about other strains. However, even if the occurrence of the disease caused by *R. honei* on the southern coast of Australia is suspected (234), it has not yet been demonstrated, and no *R. honei* strain has been isolated outside of Flinders Island (19). A clear demonstration that the two diseases (Flinders Island spotted fever and Queensland tick typhus) are clinically and epidemiologically different has not yet been provided.

CONCLUSIONS

Rickettsioses are good examples of newly recognized or emerging infectious diseases. Traditionally, *Rickettsia* species have proven difficult to isolate and maintain, while strong serological cross-reactions between species have made specific identification and strain differentiation difficult. More recently, the advent of molecular biological methods, based predominantly on PCR, and the introduction of improved cell culture techniques have helped overcome these difficulties and have facilitated the identification of numerous new clinical syndromes associated with *Rickettsia* species. Investigations leading to the recognition of a new rickettsiosis can be triggered by a physician's curiosity, the observation of an epidemiological peculiarity, or the identification of the rickettsial nature of a disease. Two specific circumstances are perhaps most likely to be involved with an emerging rickettsiosis: first, the presence of a disease in a country where it was previously unknown and consequently unrecognized, such as Japan, and second, the presence of two SFG rickettsioses in the same area, such as in Zimbabwe. Atypical cases of rickettsiosis in regions of endemic infection could also, in reality, be due to a new agent and could have been misdiagnosed by a nonspecific serological test. The combination of PCR-based amplification of DNA and methods for the analysis of PCR products, such as RFLP or base sequencing, is the key to determining the true identity of infecting rickettsiae. The use of this approach will facilitate a better understanding of emerging rickettsioses and their agents.

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