23 The Family *Rickettsiaceae*

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Abstract
The *Rickettsiaceae* family is composed of two genera, *Rickettsia* and *Orientia*, which are obligate intracellular bacteria that belong to the order *Rickettsiales*. The species of these genera are divided into two groups based on antigenic, molecular, and ecological patterns: (1) the typhus group, composed of the species *Rickettsia prowazekii, Rickettsia typhi*, and *Orientia tsutsugamushi*, which are transmitted by lice, fleas, and mites, respectively; and (2) the spotted fever group (SFG), which is composed of more than 23 valid species. The transmission of great majority of species in SFG is associated with ticks, with the exception of *Rickettsia felis* and *Rickettsia akari*, which are associated with fleas and mites, respectively. Other *Rickettsia* species, such as *Rickettsia bellii* and *Rickettsia canadensis*, are not included in either of the two groups. In SFG, at least 12 species of *Rickettsia* cause infections in humans (*Rickettsia rickettsii, Rickettsia conorii, Rickettsia africae, Rickettsia parkeri, Rickettsia australis, Rickettsia honei, Rickettsia sibirica, Rickettsia japonica, Rickettsia massiliae, Rickettsia aeschlimannii, R. akari*, and *R. felis*). However, species of nonpathogenic rickettsiae or of unknown pathogenicity might have a key role in the natural history of the pathogenic species; ticks infected by a kind of rickettsia that is nonpathogenic to humans (e.g.: *Rickettsia montana, Rickettsia peacockii*) may become unable to maintain (via transovarial transmission) infection by other pathogenic species (e.g., *R. rickettsii*). This fact is of great practical importance because there are populations of ticks infected with nonpathogenic rickettsiae for which the infection rate is often higher compared to pathogenic rickettsiae.

Taxonomy, Historical and Current

Short Description of the Family and Their Genera

Rickettsiaceae (Pinkerton, 1936)

Members of this family are Gram-negative bacteria that grow only inside living cells and therefore are characterized as obligate intracellular parasites. They have high polymorphism and reside in the cytoplasm or nucleus in the host, where they divide by binary fission (Raoult and Roux 1997). They are classified as α-proteobacteria and belong to the order *Rickettsiales*. Within the *Rickettsiaceae* family, they currently have two genera: *Rickettsia* and *Orientia*, following Dumler et al. (2001). Based on their 16S rRNA and *groEL* genes, *Rickettsia* and *Orientia* represent very closely related evolutions of microorganisms in *Alphaproteobacteria* (Lee et al. 2003, Batra and Bakshi 2011).

The organisms identified as *Rickettsia* can be divided in two main groups by phylogenetic characteristics and clinical characteristics of disease: the typhus group (TG), composed of *Rickettsia typhi* and *Rickettsia prowazekii*, and the spotted fever group (SFG). SFG consists of more than 25 species, including *Rickettsia rickettsii* and *Rickettsia parkeri* in the American continent, *Rickettsia conorii* and *Rickettsia africae* in Europe and Africa, and *Rickettsia japonica* and *Rickettsia israeli* in Asia and the Middle East. However, new studies have shown that some species showed marked phenotypic differences and proposed two groups: the ancestral group (AG) composed of *Rickettsia bellii* and *Rickettsia canadensis* (of unknown pathogenicity in humans), and the transitional group (TRG), consisting of *Rickettsia akari*, *Rickettsia australis*, and *Rickettsia felis* (Gillespie et al. 2007). This newly proposed organization of rickettsial species was heavily based on a comparison of major conserved gene sequences (e.g. *ompB*, *gltA*) within the genus. In addition, because the best intrinsic aspects of bacterial growth temperature have a G + C content related to the genome of the bacterium, the ability to polymerize actin in the host cell (Heinzen et al. 1993; Teysseire et al. 1992), the cross-reaction of sera from a patient with rickettsial infection, the distribution of strains, and hemolytic activity are used to differentiate between different rickettsiae groups (Fournier and Raoult 2007).

The genus *Orientia* is classified in a single group, the scrub typhus group, represented by the species *O. tsutsugamuchi*.

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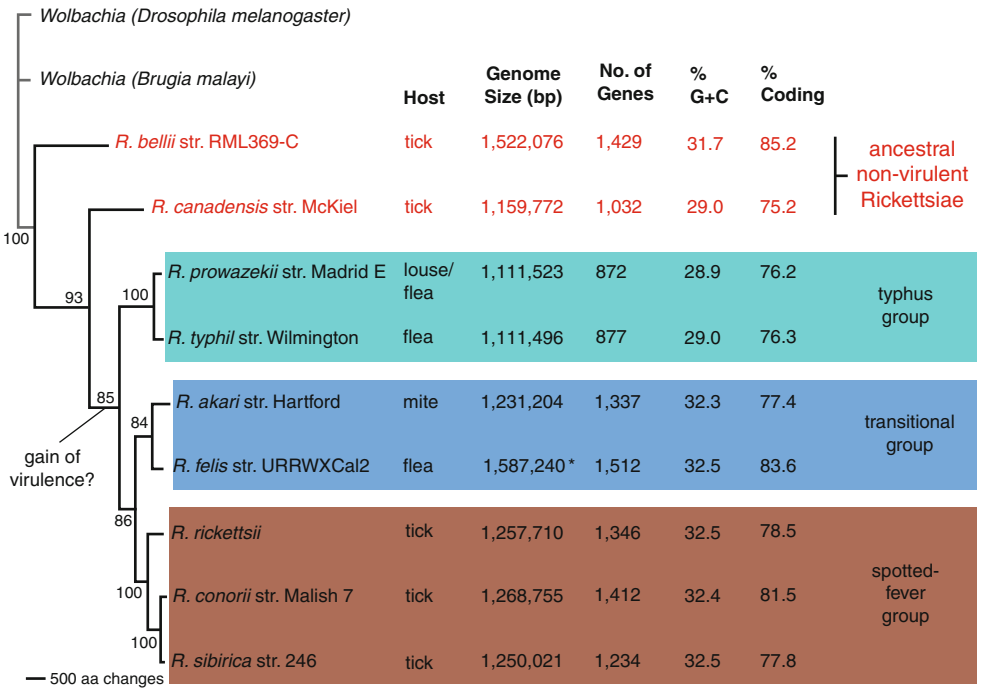

Fig. 23.1
Phylogeny estimation describing the four groups of the *Rickettsiaceae* family (Gillespie et al. 2007)

This organism was given its own genus designation because it is phylogenetically distinct from the other rickettsiae (Tamura et al. 1995).

This group of diseases affects humans through parasitism by blood-sucking arthropods, including ticks, fleas, lice, and mites (Hoogstraal 1967; Friedhoff 1990). These microorganisms thrive in nucleated cells from vertebrate and invertebrate, preferably a vertebrate host and endothelial cells (Weiss and Moulder 1984), and invertebrates, intestinal cells, salivary glands, and ovaries (Parola et al. 2005).

Regarding rickettsiosis, the genus *Rickettsia* is the most important due to the clinical and epidemiological aspects associated with human infections. The distribution of rickettsiae around the world is directly linked to their arthropod vectors, which includes a variety of species, described in regions of different countries from all continents (Parola et al. 2005).

Phylogenetic Structure of the Family and Its Genera

Previously, the original classification and phylogenic arrangement of the genus *Rickettsia* was determined by serological studies. The genus was subdivided into the conventionally well-defined TG and SFG, based mainly on phenotypic and serological features (Vitorino et al. 2007). Since the original organization, conserved genera-specific genes have been sequenced, compared among available *Rickettsia* species, and used to construct phylogenies based upon gene differences and their likely evolution (Reif 2009).

Many types of studies are realized throughout analysis of phylogenetic relation. According to the endosymbiont hypothesis, the mitochondria may be derived from an ancestral Alphaproteobacterium. Phylogenetic studies indicate that the mitochondrial ancestor is most closely related to the *Rickettsiales* (Brindefalk et al. 2011; Andersson et al. 1998). The most ancestral species of the genus appear to be *Rickettsia bellii* and *Rickettsia canadensis* (Stothard et al. 1994); because of their characteristics, these species have been recognized as belonging to ancestral group of *Rickettsiaceae*.

A reorganization of *Rickettsia* species within the genus *Rickettsia* has been proposed, based on phylogenetic analysis of a number of conserved genus-specific genes and the presence of a plasmid in *R. felis* (Gillespie et al. 2007). In this new arrangement, rickettsiae are organized into one of four groups: the ancestral group (AG; e.g. *R. bellii* and *R. canadensis*), the typhus group (TG; e.g. *Rickettsia typhi* and *Rickettsia prowazekii*), the transitional group (TRG; e.g. *Rickettsia felis* and *Rickettsia akari*), and the spotted-fever group (SFG; e.g. *Rickettsia rickettsii*, *Rickettsia sibirica*) (*Fig. 23.1*).

On account of phylogenetic studies based on the sequence of the 16S rRNA gene of members of the family *Rickettsiaceae*, it was initially observed that *Rickettsia tsutsugamushi*, the agent of scrub typhus, was found to be distinct enough by 16S rRNA gene sequence comparison to warrant transfer into the genus *Orientia*, which includes a single species, *Orientia tsutsugamushi* (Tamura et al. 1995) (*Fig. 23.2*). In addition, polymerase chain reaction (PCR) coupled with restriction fragment length polymorphism (RFLP) applied to the *gltA* and *ompA* genes showed

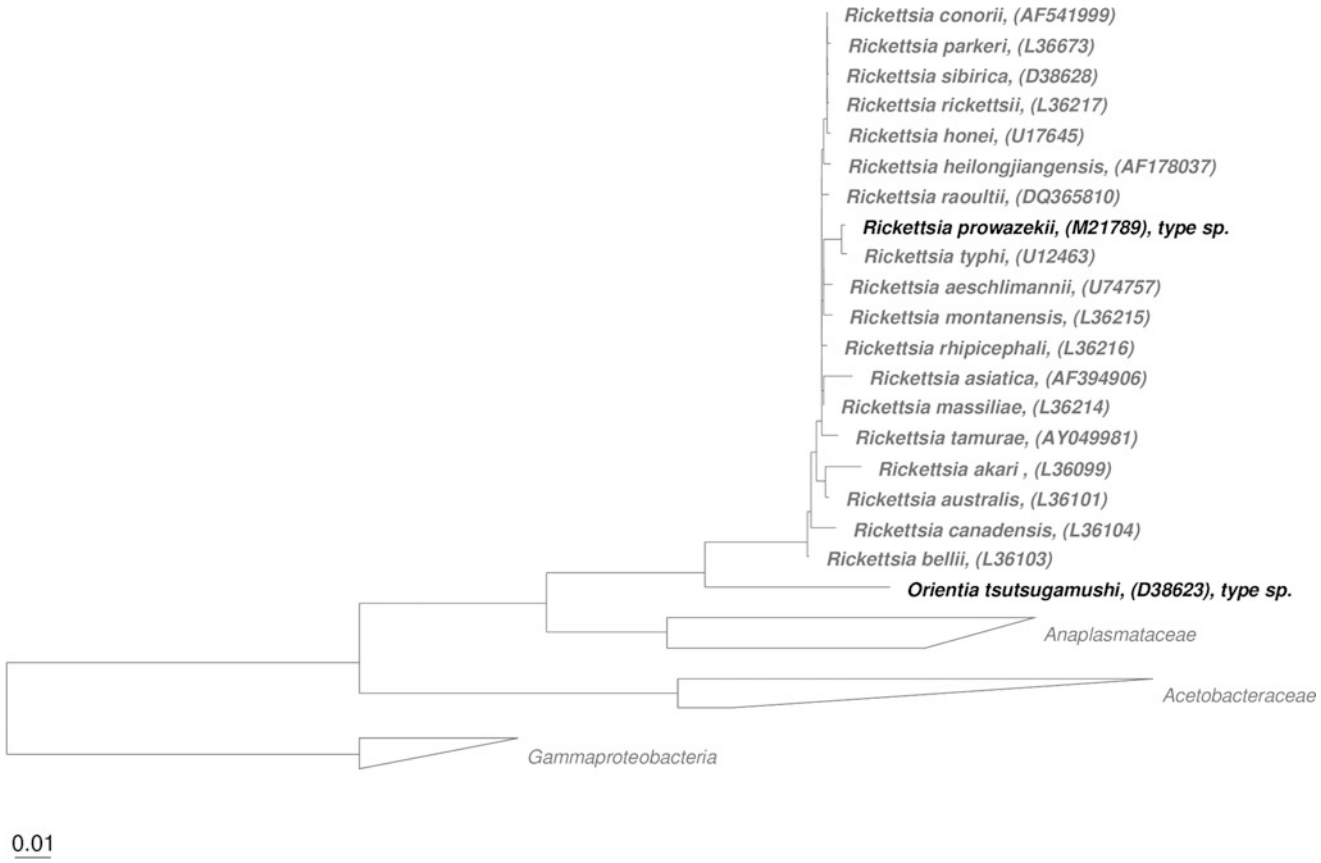

Fig. 23.2

Phylogenetic relationships of the organisms belonging to the *Rickettsiaceae* family in relation to the *Anaplasmataceae* family and others classes beyond *Alphaproteobacteria*, based on DNA sequences. The GenBank numbers are provided to the *right* of the species names

that *R. canadensis* and *R. bellii* occupied an intermediate position between the typhus and SFGs (*Table 23.1*).

- Phylogenetic reconstruction of the family *Rickettsiaceae* based on 16S rRNA and created using the maximum likelihood algorithm RAxML (Stamatakis 2006). The sequence dataset and alignment were used according to the All-Species Living Tree Project (LTP) database (Yarza et al. 2010; http://www.arb-silva.de/projects/living-tree). Representative sequences from closely related taxa were used as outgroups. In addition, a 40% maximum frequency filter was applied in order to remove hypervariable positions and potentially misplaced bases from the alignment. Scale bar indicates estimated sequence divergence.

In the *Rickettsiales* order are the *Anaplasmataceae* and *Rickettsiaceae* families, which are the most commonly studied. However, the *Holosporaceae* family was recently described by Görtz and Schmidt (2005); entered in that order were two new families, *Pelagibacteraceae* (Thrash et al. 2011) and *Midichloriaceae* (Montagna et al. 2013) (*Fig. 23.3*). According to Montagna et al. (2013), phylogenetic studies provided evidence for the deep branching of a lineage of the *Rickettsiaceae* that infects aquatic protista (Vaninni et al. 2005). Considering that current evidence, this places the family of ciliate-infecting bacteria *Holosporaceae* as the sister group of the lineage leading to *Rickettsiaceae*, *Anaplasmataceae*, and *Midichloriaceae*. There is evidence indicating that intracellular *Rickettsiales* were originally associated with aquatic/environmental protista that served (and potentially still serve) as an ecological and evolutionary reservoir for *Rickettsiales*-infecting animals (Montagna et al. 2013), based on phylogenetic studies.

Molecular Analyses

According to the genome project database of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/genomes/lproks.cgi), 21 genomes of *Rickettsia* and one of *Orientia* species have been sequenced completely: *Rickettsia prowazekii* str. Breinl, *R. typhi* str. B9991CWPP, *R. conori* str. Malish 7, *R. rickettsii* str. Brazil, *R. parkeri* str. Portsmouth, *R. felis* URRWXCal2, *R. akari* str. Hartford, *R. bellii* OSU 85-389, *R. sibirica* 246, *R. africae* ESF-5, *R. monacensis* IrR/Munich, *R. peacockii* str. Rustic, *R. australis* str. Cutlack, *R. rhipicephali* str. 3-7-female6-CWPP, *R. slovaca* 13-B, *R. canadensis* str. CA410, *R. massiliae* MTU5, *Orientia tsutsugamushi* str. Boryong, *R. montanensis* str. OSU 85-930, *R. philipii* str. 364D, *R. japonica* YH, *R. heilongjiangensis* 054 (*Table 23.2*). The availability of the complete sequences of

Table 23.1
Brief description of the set of commonly sequenced genes used for phylogenetic studies in the *Rickettsiaceae* family

Gene	Characteristic of the gene	References
Citrate synthase gene (*gltA*)	The citrate synthase gene is one of the most common genes used to differentiate *Rickettsia* species from other bacterial species.	Bouyer et al. 2001, Regnery et al. 1991
16S rRNA gene	The 16S rRNA gene has stability as a housekeeping gene, which makes it less affected by host immune responses and other environmental stresses.	Reif 2009, Higgins et al. 1996
17-kDa protein gene (*htrA*)	The 17-kDa common antigen gene (*htrA*): The 17-kDa antigen found in all species of *Rickettsia* is an immunologically important surface protein and is one of the most characterized loci in any rickettsial genome. Only bacteria in the genus *Rickettsia* are known to possess the 17-kDa antigen gene; areas of divergence present in this gene between rickettsial species aided in the formation of the SFG and TG.	Anderson et al. 1987, 1988, Anderson and Tzianabos 1989, Tzianabos et al. 1989
OmpA protein gene (*ompA*)	The 190-kDa outer membrane antigen protein gene (*ompA*) is the major gene that defines the SFG. The immunodominant OmpA protein is believed to have a critical role in SFG rickettsial pathogenesis (e.g. cell adhesion and invasion) (Li and Walker 1998; Crocquet-Valdes et al. 2001). Antigenic variation in the conserved regions of this gene that flank tandemly repeated sequences are useful in differentiating *Rickettsia* species and strains within the SFG.	Regnery et al. 1991, Gilmore 1993, Zavala-Castro et al. 2005
OmpB protein gene (*OmpB)*	The 120-kDa protein outer-membrane protein B.	Eremeeva et al. 1994, Roux and Raoult 2000
groEL genes	The *groEL* genes, which encode the 60-kDa heat shock protein GroEL, are ubiquitous in both prokaryotes and eukaryotes and encode highly conserved housekeeping proteins that are essential for the survival of these cells. The *groEL* genes provide the defining evolutionary relationships among the members of the eubacterial lineage. *groEL* gene analysis is useful for the differentiation of STG strains and strains of the genus *Rickettsia.*	Lee et al. 2003
Gene D (*SCA4*)	'Gene D' is the PS120-protein-encoding gene, first described in *Rickettsia conorii* and *Rickettsia japonica.* 'Gene D' is considered as a complementary approach in phylogenetic studies of rickettsiae because it presents significant bootstrap values to the most of the nodes.	Sekeyova et al. 2001

different species belonging to a single genus enables comparative genomics to identify differences and commonalities among them.

The size, architecture, and composition of bacterial genomes vary incredibly. A common characteristic of bacteria of the family *Rickettsiaceae* is the small genome, ranging from 1.11 (*Rickettsia* sp.) to 2.13 Mb (*Orientia tsutsugamuschi*) (*Table 23.1*). One factor that clearly contributes to the genome reduction is the habitat. The genome reduction is probably related to adaptations to a parasitic and pathogenic lifestyle, in which certain functions are no longer required (Moran 1996), resulting in total dependence on host cells. In a study on the evolution of the *Rickettsiales*, it was demonstrated that evolution from a free-living lifestyle to an obligate intracellular one was associated with the loss of 2,135 genes (Georgiades et al. 2011a). Furthermore, the 12 deadliest epidemic species for humankind have significantly smaller genomes, with fewer open reading frames (ORFs), than less dangerous species (Georgiades et al. 2011b). One of the best examples of genomic reduction of epidemic bacteria is *R. prowazekii*, the agent of epidemic typhus. No virulence genes have been identified in its genome, and 24 % of its small genome is composed of pseudogenes and non-coding DNA (Andersson et al. 1998; Bechah et al. 2010). In fact, the genome of *R. prowazekii* (834 ORFs) represents a subset of *R. conorii* (1374 ORFs); it possesses almost no genes that are not present in *R. conorii*, with the exception of four TG-specific genes that also are present in *R. typhi* (RP624, RP338, RP164 and RP174) and absent from, or split in, other rickettsiae (Gillespie et al. 2008; Ammerman et al. 2009).

In *Listeria monocytogenes* and *Shigella* (Goldberg and Theriot 1995; Moliner et al. 2010), intracellular motility is considered a virulence factor. In *R. prowazekii*, this characteristic was not observed because the bacteria have no motility within cytoplasm (Andersson et al. 1998). In a study of comparative genomics between *R. rickettsii* and *R. africae*, pathogenicity development was associated with the loss of essential genes (Fournier et al. 2009). Studies of rickettsial genomics challenge traditional concepts of pathogenesis, which were based principally on the acquisition of virulence factors.

Another intriguing phenomenon about the reduced rickettsial genomes concerns the large fraction of non-coding DNA and

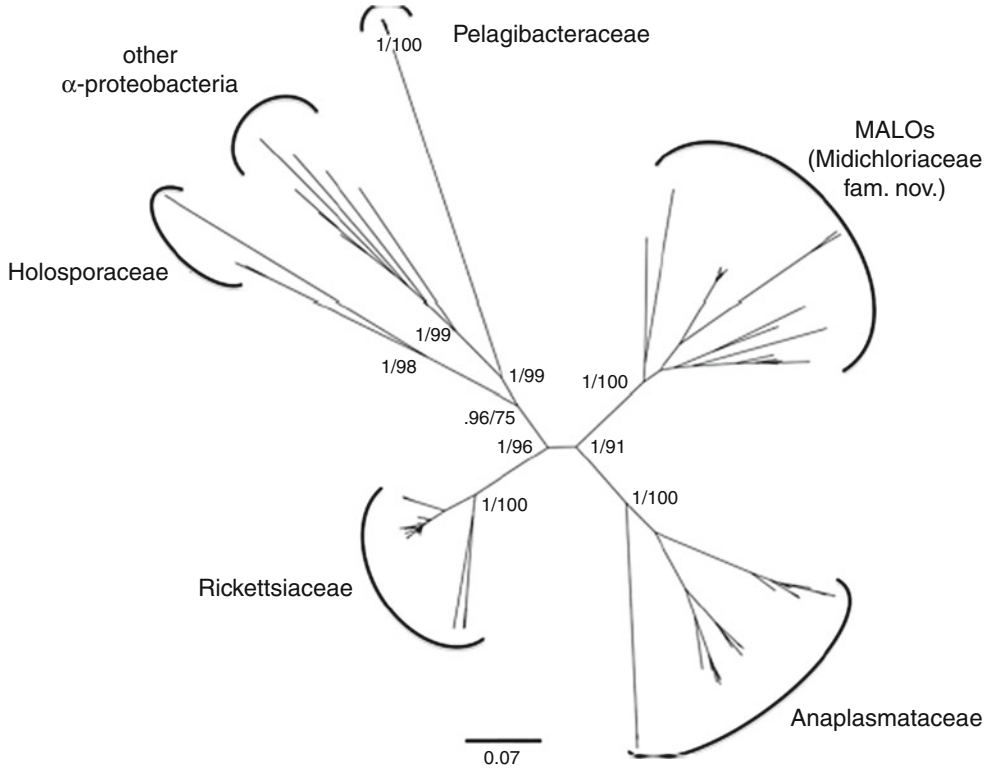

Fig. 23.3

An unrooted Bayesian phylogram of the *Rickettsiales* based on the 16S rRNA gene sequence. Bayesian posterior probability and ML (Maximum Likelihood) bootstrap values are reported for the main lineages (Montagna et al. 2013)

possible functionality of these "non-coding" sequences because of the high conservation of these regions. "Pathogenicity islands" (a set of genes coding for virulence traits) are present in many bacteria; however, they are not present in species of *Rickettsia* spp. vertebrate-pathogenic (Hacker and Kaper 2000). In many studies, the presence of plasmids in *Rickettsia* species does not appear to be directly associated with pathogenicity (Fournier et al. 2009; Ogata et al. 2005). However, it has been suggested that the genes encoding the proteins responsible for recognition, invasion, and pathogenicity are located in plasmids of some *Rickettsia* spp. species. The acquisition of new genes does not necessarily imply pathogenicity change, as has been shown in several studies on comparative genomics of *Rickettsia* sp. Furthermore, the emergence of virulent traits has occurred with the loss of gene function. In *Shigella* spp. and *Yersinia* spp., such events may provide a selective advantage, as noted by a series of genetic events that contribute to the emergence of virulence (Maurelli et al. 1998; Sun et al. 2008).

Compared with other bacteria species with sequenced genomes, the percentage of non-coding DNA in *Rickettsia* species not decreased, suggesting that the reduction of the genome is associated with the loss of genes (Rogozin et al. 2002). Genome analysis of various rickettsial genomes shows that the genome reduction process described for alpha-proteobacteria (Boussau et al. 2004) has occurred independently in different rickettsial lineages, leading to the existing species assemblage (Blanc et al. 2007b).

The changes in genome size may occur by gene duplication and the emergence of new sequences. In rickettsia, genomes have been found with repeating elements without defined cellular function (Ogata et al. 2000). In bacterial genomes, repeated elements are located in intergenic regions (Van Belkum et al. 1998). In some of these, variable numbers of tandem repeats (VNTR) represent an interindividual variability in the sequence length; some have been used for genetic typing (Eremeeva et al. 2006; Fournier et al. 2004).

In *Rickettsia* spp., elements palindromic repeat (EPR) of approximately 100–150 bp were found. These repetitions can invade coding and non-coding regions of the genome (Amiri et al. 2002; Claverie and Ogata 2003; Ogata et al. 2000). When inserted within genes encoding proteins, these repeats generated a new reading frame as a part of a preexisting gene. Therefore, the final gene product has an additional peptide segment (30–50 amino acids). Over evolutionary time, multiple random insertions of such elements within genes, followed by selection on the resulting peptide sequences within the context of different host proteins, may have contributed to the emergence of new protein sequences, domains, and functions. Thus, EPR enhance sequence diversity in coding regions of *Rickettsia* spp.

Mobile elements have been identified in the rickettsia genome by genome comparative studies. These elements mediate the DNA movement within and between genomes, by means of transposable elements, plasmids, bacteriophages, and genes associated with horizontal mobility. These mobile elements

Table 23.2
Sequenced bacterial genomes of *Rickettsiaceae*

Bacteria	Genome	G+C %	Proteins	Genes	Reference/GenBank
Rickettsia prowazekii str. Breinl	One chromosome (1.11 Mb)	29	920	956	CP004889.1
R. typhi str. B9991CWPP	One chromosome (1.11 Mb)	28.9	839	875	CP003398.1
R. conori str. Malish 7	One chromosome (1.27 Mb)	32.4	1,374	1,414	NC_003103.1
R. rickettsii str. Brazil	One chromosome (1.26 Mb)	32.5	1,332	1,369	NC_016913.1
R. parkeri str. Portsmouth	One chromosome (1.30 Mb)	32.4	1,318	1,355	NC_017044.1
R. felis URRWXCal2	One chromosome (1.59 Mb) and two plasmids (pRF-62.9 Kb and pRF delta-39,8 Kb)	32.6	1,512	1,551	NC_007109.1 (chromosome), NC_007110.1 (pRF), NC_007111.1 (pRF delta)
R. akari str. Hartford	One chromosome (1.23 Mb)	32.3	1,257	1,292	NC_009881.1
R. bellii OSU 85-389	One chromosome (1.53 Mb)	31.6	1,429	1,511	NC_009883.1
R. sibirica 246	One chromosome (1.25 Mb)	32.4	–	–	NZ_AABW00000000.1
R. africae ESF-5	One chromosome (1.29 Mb) and one plasmid (pRAF-12.36 Kb)	32.4	1,041	1,167	NC_012633.1 (chromosome), NC_012634.1 (pRAF)
R. monacensis IrR/ Munich	One chromosome (1.27 Mb)	32.3	1,460	1,503	NZ_CBUA000000000.1
R. peacockii str. Rustic	One chromosome (1.31 Mb) and one plasmid pRPR-26,42 Kb)	32.6	947	984	NC_012730.1 (chromosome), NC_012732.1 (pRPR)
R. australis str. Cutlack	One chromosome (1.32 Mb) and one plasmid (p-MC5-1 26.66 Kb)	32.3	1,261	1,297	NC_017058.1 (chromosome), NC_017041.1 (pMC5-1)
R. rhipicephali str. 3-7-female6-CWPP	One chromosome (1.31 Mb) and one plasmid (pMCC-1 15. 09 Kb)	32.4	1,266	1,302	NC_017042.1 (chromosome), NC_017055.1 (pMCC-1)
R. slovaca 13-B	One chromosome (1.28 Mb)	32.5	1,112	1,323	NC_016639.1
R. canadensis str. CA410	One chromosome (1.15 Mb)	31	1,016	1,052	NC_016929.1
R. massiliae MTU5	One chromosome (1.38 Mb) and one Plasmid (pRMA-15.28 Kb)	32.5	980	1,436	NC_009900.1 (chromosome), NC_009897.1 (pRMA)
Orientia tsutsugamushi str. Boryong	One chromosome (2.13 Mb)	30.5	1,182	2,216	NC_009488.1
R. montanensis str. OSU 85-930	One chromosome (1.28 Mb)	32.6	1,217	1,254	NC_017043.1
R. philipii str. 364D	One chromosome (1.29 Mb)	32.5	1,344	1,380	NC_016930.1
R. japonica YH	One chromosome (1.28 Mb)	32.4	971	1,010	NC_016050.1
R. heilongjiangensis 054	One chromosome (1.28 Mb)	32.3	1,297	1,338	NC_015866.1

Source: http://www.ncbi.nlm.nih.gov/genome/?term=Rickettsia

constitute "the mobilome" (Frost et al. 2005; Koonin and Wolf 2008). Some *Rickettsia* spp. contain transposases (Blanc et al. 2007a; Ogata et al. 2005), phage-related genes (Andersson et al. 1998; McLeod et al. 2004; Ogata et al. 2001, 2005, 2006), plasmids (Baldridge et al. 2007, 2008; Fournier et al. 2008; Ogata et al. 2005), and an apparatus for conjugation (*tra* genes) (Blanc et al. 2007a; Fournier et al. 2008; Ogata et al. 2005, 2006). In intracellular bacteria, the lateral gene transfer (LGT) phenomenon has long been considered rare (Audic et al. 2007); in rickettsiae, the discovery of the mobilome is possible. Ogata et al. (2006) described various mechanisms in rickettsial genomes for the influx of foreign DNA sequences. Further analysis of the rickettsial genome can identify candidates for LGT between *Rickettsiae* species.

The transfer of genes between chromosomes and plasmids was observed after analysis of *R. felis* genome. Eleven genes

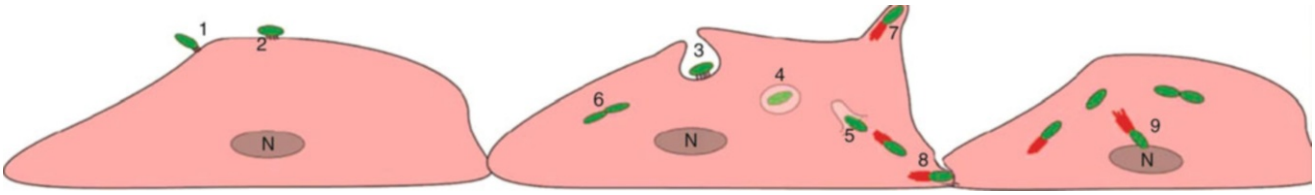

Fig. 23.4

Spotted fever group rickettsia–endothelial cell interaction. (*1*) Adhesion of bacteria to the cell membrane via adhesins; (*2*) Recruitment of more receptors; (*3*) Engulfment of the bacteria by cell extensions; (*4*) Inclusion of bacteria in phagocytic vacuoles; (*5*) Rickettsial enzymes lyse vacuolar membrane; (*6*) Replication by binary fission; (*7*) Host actin-based mobility; (*8*) Cell-to-cell spread; and (*9*) Invagination into the endothelial cell nucleus (*N*) (Walker 2007)

located on plasmid PRF exhibiting homologous chromosome of *R. felis* were observed. These two genes encoded heat shock proteins, thymidylate kinase, and patatin-like phospholipase transposase seven. All sequenced *Rickettsia* genomes exhibit patatin-like phospholipase-coded chromosome (pat1). In plasmid PRF, *R. felis* has a paralog additional pat2. Phylogenetic reconstruction among species of *Rickettsia* shows a close relationship for pat1 of *R. felis* and *R. Akari* pat2 with *R. felis*. This provides a clear case of transfer of genes from plasmid to chromosome; the chromosome was replaced by pat1 pat2 plasmid-encoded in the lineage, leading to the ancestor of *R. felis* and *R. akari* (Ogata et al. 2005).

The development of technologies for large-scale genome sequencing may modify the way we view microbiology, thus starting a postgenomic era in which the development of computational tools for the analysis of biological data is a priority. The genome comparative analysis of the family *Rickettsiaceae* with other bacteria has provided information about the bacteria-host relationship, pathogenicity, and evolutionary history. The ultimate understanding of the molecular mechanisms related to adaptation to different conditions requires the application of global approaches, including differential transcriptome analysis using platforms such as HiSeq 2000 (Illumina). Such a methodology has not yet been extensively applied for *Rickettsiaceae*, but we believe that experimental progress will make the technology more accessible in a few years. A better knowledge of a microorganism can also be gained by proteomic analysis.

Phenotypic Analyses

All the microorganisms of *Riackettsiaceae* are similar in the following aspects: they are obligate intracellular parasites, morphologically similar to gram-negative bacteria, and survive in vertebrates and arthropod hosts. When human infection occurs, this is mediated by arthropod vectors.

Bacteria of the genus *Rickettsia* and *Orientia* are small rods. *Rickettsia* measure 0.3–0.5 μm^2 in length and 0.8–2.0 μm^2 in width, whereas *Orientia* measure 0.5–0.8 μm^2 in length and 1.2–3 μm^2 in width, respectively. Generally, SFG members have an optimal growth temperature of 32 °C, whereas TG members have an optimum growth temperature of 35 °C. SFG members have 32–33 % GC content and TG members have nearly 29 % GC. In addition, only SFG members can enter the nucleus of parasitized cells (Heinzen et al. 1993; Teysseire et al. 1992).

Pathogenic *Rickettsiaceae* do not have flagella, although *Rickettsiae* of the SFG members move by a peculiar mechanism. They use the host cell actin to form a tail and move freely in the cytoplasm of the cell; however, this mechanism only occurs after escaping the phagocytic vacuole. The scientists suppose that the polymerization of actin and the tail formation play a fundamental role in the invasion movement from cell to cell in host infection; this could explain why *Rickettsiae* spreads rapidly in host organisms (Heinzen et al. 1993, 1999; Gouin et al. 1999) (*Fig. 23.4*). Concerning the polymerization of actin in the host cell, SFG members are capable and TG members are not capable.

The membrane structure of *Rickettsiaceae* is similar to those observed in gram-negative bacteria, in general. It has a trilamellar structure, as follows: a lipopolysaccharide outer membrane and inner membrane composed of a phospholipid bilayer, separated by a peptidoglycan layer in a periplasmic space. Furthermore, SFG members are surrounded by an electron-lucent slime layer when they are infecting a host cell (Raoult and Parola 2007; Quinn et al. 2011). One exception is *O. tsutsugamushi*, which lacks muramic acid, glucosamine, heptose, 3-deoxy D-mannooctulosonic acid (KDO), and hydroxy fatty acids—the basic components of peptidoglycan and LPS, so it is deficient of both in the cell wall (Amano et al. 1987). This fact explains why *O. tsutsugamushi* is resistant to beta-lactam antibiotics (Miyamura et al. 1989).

The major antigens of *Rickettsiae* are lipopolysaccharides, lipoproteins, outer membrane proteins of the surface cell antigen (SCA) family, and heat shock proteins. Other antigens have been characterized in *Rickettsiae* species, including a 17-kDa lipoprotein and members of the autotransporter protein family SCA, which includes the 120 kDa protein S-layer (OmpB or SCA5), OmpA (present only in SFG *Rickettsiae*), and Sca4 (Anderson et al. 1990; Blanc et al. 2005; Fournier et al. 1998b).

With light microscopy, the majority of *Rickettsiaceae* stains well with Giménez, Macchiavello, and Giemsa stains. *Rickettsia akari* does not stain with Giemsa or eosin-azure-based techniques, just with Macchiavelo, Giménez, and carbol basic fuchsin (Giménez 1964). In addition, *O. tsutsugamushi* does not stain with Macchiavello, and a modification of the Gimenez stain is

required (Timoney et al. 1992). Concerning microscopy fluorescence, it is possible to use acridine orange stain in cell culture or smears. Also, materials such as host tissue stains could be evaluated by immunohistochemistry or fluorescein-conjugated polyclonal antibodies (Raoult and Parola 2007).

Due to the strictly intracellular nature of *Rickettsiaceae*, traditional identification methods used in bacteriology cannot be applied. It must be cultivated in tissue culture or the yolk sac of developing chicken embryos (Teysseire et al. 1992). *Rickettsiaceae* can be isolated from decanted plasma, blood collected on heparin or citrate anticoagulant (because EDTA anticoagulant is harmful to the cell monolayers used for recovery of rickettsiae), skin biopsy, necropsy tissue, and arthropod samples (Brouqui et al. 2004). The material can be inoculated into experimental animals, in primary cultures of chicken embryo, HEL, MRC5, WI-38, LLC-MK2, BSC-1 or Hep-2 cells, further VERO cells, and L929 cells, which are used more often (Cox 1941; Cory et al. 1974; Brouqui et al. 2004). Today, cell culture systems have replaced the yolk sacs of developing chicken embryos and experimental animal inoculation, which were widely used in the past (La Scola and Raoult 1997). All of these procedures must be performed only in Biosafety Level 3 laboratories. The cultivation of *Rickettsiae* in cell monolayers is observed by the disruption of massively infected cells. SFG members form a plaque with a diameter of 2–3 mm in 5–8 days, while TG members form a plaque smaller than 1 mm in 8–10 days (Raoult and Parola 2007).

Concerning the metabolism of *Rickettsiaceae*, they do not use glucose as an energy source; rather, they use glutamate. Likewise, they do not synthesize or degrade nucleoside monophosphates (Raoult and Parola 2007).

Isolation, Enrichment and Maintenance Procedures

Isolation and Enrichment

Because of their intracellular binding, bacterial culture is one of the necessary procedures for cells' isolation. Another method for their isolation is the inoculation of biological material containing the bacteria in guinea pigs and embryonated eggs. However, cell culture is currently the most widely used system for primary isolation. Tissue culture requires prior disaggregation of the original tissue where the cells are grown and an adhesive layer on a solid substrate or suspended in culture medium.

For the isolation and cultivation of the parasite, samples used can be obtained from minced clot plasma, skin biopsy, and autopsy tissue samples (La Scola and Raoult 1997); arthropod material is collected and inoculated into cell lines (Paddock et al. 2010). It is important to note that performing routine laboratory cell isolation and cultivation of many of these rickettsial agents requires biosafety level 3 (BSL3) (Champman et al. 2006), which becomes impractical for implementation in many laboratories.

The cell cultivation may be performed in different lines, including Vero cells, MRC5 cells, L-929 cells, HEL cells, LLC-MK2, BSC-1, or Hep-2 (Cox 1941; Cory et al. 1974; Jonhson and Pedersen 1978; Dumler and Walker 2005). Vero or L929 cells have been shown to allow better and faster isolation of rickettsiae, especially from heavily infected samples, than HEL or MRC5 cells (Kelly et al. 1991; La Scola and Raoult 1997). However, these types of cell lines are established when an inhibition of cell division due to close contact occurs in the monolayer and may subsequently be used in a prolonged incubation.

The adaptation of the system shell vial employed for the detection of cytomegalovirus (Paya et al. 1987) has been successful for growing rickettsiae in VERO cells (La Scola and Raoult 1997), which may show multiplication of rickettsiae by the cytopathic effect (CPE). However, the cultivation of this technique has limitations because getting material from symptomatic patients is not always possible. Another important aspect of the isolation and cultivation of rickettsiae is the use of embryonic cell lineages of the tick, particularly for species struggling for multiplication in mammalian cell lines (Bell-Sakyi et al. 2007). Thus, using different strains of ticks, it is possible cultivation of different species of rickettsiae that have medical and veterinary importance (*Table 23.3*) (Bell-Sakyi et al. 2007).

Ecology

Rocky Mountain Spotted Fever (RMSF) is endemic to regions of North, Central, and South America. Cases have been reported from Canada to Argentina; however, some countries in these areas have not yet reported any cases. The greatest numbers of fatalities have been reported in the United States and Brazil. The distribution of RMSF reflects the distribution and abundance of the tick vectors *D. variabilis* (the American dog tick) and *D. andersoni* (*Table 23.4*).

The transmission of RMSF almost always results from rickettsiae infection in a human host by a tick as it obtains a blood meal from that host (McCalla 1908). Tick vectors of *R. rickettsii* include at least four species: *D. andersoni, D. variabilis, R. sanguineus*, and *Amblyomma cajenennse* (Parker 1933; Burgdorfer 1975; Demma et al. 2005). Because *R. rickettsii* is passaged transovarially and transstadially, all hematophagous stages of these ticks are potentially capable of transmitting rickettsiae to a susceptible host. Rare routes of transmission of *R. rickettsii* to human hosts include blood transfusion (Wells et al. 1978) and inoculation of rickettsiae through mucous membranes following contact with fingers contaminated during the crushing of infected ticks removed manually from a human or animal (Price 1954; Spencer and Parker 1930; Gordon et al. 1984).

Most of reported cases of RMSF occur during the months of April through September in the temperate United States (Kirkland et al. 1995; Gordon et al. 1984; Treadwell et al. 2000), because this period coincides with the greatest host-seeking activity of the *Dermacentor* spp. ticks (Clark et al. 1998;

Table 23.3
***Rickettsia* species propagated in tick cell lines since 1995 (Adapted by Bell-Sakyi et al. 2007)**

Rickettsia species	Mammalian host (disease caused)	Tick cell line (s) used
Rickettsia rickettsii	Human (Rocky mountain spotted fever)	IDE2, DALBE3, ISE6, IDE8,
Rickettsia peacockii	–	DAE 100, ISE6, BME26, DVE1, DAE3, DAE15, IDE12, IDE2, IDE8, IRE11, CCE3
Rickettsia monacensis	?	ISE6, IRE11, DAE100, IDE8
Rickettsia helvetica	Humans (fever, perimyocarditis?)	IRE11
Rickettsia montanensis	Various small mammals	IDE2, DALBE3
Rickettsia sp. (spotted fever group)	?	RAE25, IDE2, IDE8
Rickettsia felis	Humans (flea-associated spotted fever)	ISE6

Eads and Smith 1983). In Brazil, tick surveys in the county of Pedreira documented peak numbers of larvae and nymphs of *A. cajennense* between June and October, the months coinciding with the most reports of RMSF in Brazil (de Lemos et al. 1997).

African Tick-Bite Fever is transmitted by ixodid ticks of the genus *Amblyomma*: *Amblyomma variegatum* in West, Central, and East Africa and the eastern Caribbean, and *Amblyomma hebraeum* in southern Africa (Kelly and Mason 1991; Kelly et al. 1994; Parola et al. 2001). In contrast to most other ticks of human importance, *Amblyomma* ticks are hunter ticks and exhibit a notoriously aggressive behavior. Both tick species prefer semihumid habitats with tall grass or bush. Cattle, wild game, and other ungulates are the principal hosts, although young stages may also parasitize birds and rodents. *A. hebraeum* and *A. variegatum* are active all year, but their number peaks during and after the rainy season, from January to May (Norval 1983). Both species act as vectors of *R. africae* and are also reservoirs in which the infection is maintained through transstadial transmissions (Kelly and Mason 1991).

Mediterranean Spotted Fever (MSF) is caused by *R. conorrii* and transmitted by *R. sanguineus*, which probably has the most widespread distribution of all Ixodid ticks. Although *R. sanguineus* has a worldwide distribution, *R. conorii* is confined to particular regions of the world. *R. sanguineus* lives in peridomestic environments shared with dogs but has relatively low affinity for humans. Because of these circumstances, cases of MSF are sporadic and typically encountered in urban areas.

MSF is endemic in northern Africa, southern Europe, and the Mediterranean area. It is a reportable disease in Portugal (Bacellar et al. 2003), where there is an annual incidence rate of 9.8 cases per 100,000 persons (De Sousa et al. 2003). Overall, in endemic countries, fluctuation in incidence has been variable; this may be due to variations in climatic conditions, such as an increase in temperature and the lack of rainfall (Espejo-Arenas et al. 1986; Segura-Porta et al. 1989; Raoult et al. 1992). Sporadic cases in nonendemic countries are also frequently observed as a consequence of tourism (Rolain et al. 2004; Jensenius et al. 2004). Notably, although *R. sanguineus* is prevalent in North America, no cases of MSF were described until recently on that continent. In Europe, cases are encountered in late summer; this period is associated with the peak of activity of immature ticks, which are difficult to observe even when attached to the body (Raoult and Roux 1997).

Louse-Borne Epidemic Typhus has the most serious epidemic potential of all rickettsiae. It should be considered a serious threat, even in developed countries (Drancourt et al. 1995; Brouqui et al. 1996; Van Der Laan and Smit 1996; Brouqui et al. 1999). Epidemic typhus is currently considered as a potential bioterrorism agent (category B, Centers for Disease Control and Prevention). The human body louse is the only established vector for *R. prowazekii.* Lice die of their infection and do not transmit their infection to their progeny; because of this, the main reservoir appears to be humans. Bacteremia may occur and be prolonged to contaminate lice and allow transmission. Humans who contract typhus retain some rickettsiae for the rest of their lives, leading immunocompromised patients to relapse with Brill-Zinsser disease, a milder but bacteremic form of typhus (Green et al. 1990).

In the louse, rickettsiae only infect the epithelial cells of the first part of the louse's digestive tract (Weigl 1924; Houhamdi et al. 2002), where they multiply. As a result of its excessive growth, infected epithelial cells release the rickettsiae into the gut lumen (Houhamdi et al. 2002). Massive quantities of rickettsiae are discharged in the feces (Houhamdi et al. 2002). The rupture of digestive epithelium allows the ingested blood to diffuse through the intestine to the hemolymph and the louse becomes red (Burgess 1995); because of this, typhus has also been named "red louse disease". Because the ruptured epithelial cells are not replaced, infection with *R. prowazekii* leads to the death of the louse shortly thereafter (Houhamdi et al. 2002). *R. prowazekii* is the only *Rickettsia* species unable to be transmitted transovarially to its progeny in its vector.

Lice live in clothing, and their prevalence is determined by the weather, humidity, poverty, and lack of hygiene. They are more prevalent during the colder months; therefore, epidemic typhus is more frequently reported during winter and the early

Table 23.4

Species classified in SFG rickettsiae pathogenic for humans, SFG rickettsia never isolated from humans, the typhus group, and the genus *Orientia* (La Scola and Raoult 1997)

Group	Species	Disease	Associated arthropod	Distribution
SFG rickettsiae (human pathogens)	*Rickettsia conorii sensu stricto*	Mediterranean spotted fever	*Rhipicephalus sanguineus*	Mediterranean countries, Europe, Africa, Asia
	Rickettsia conorii complex	Israeli spotted fever	*Rhipicephalus sanguineus*	Israel
	Rickettsia conorii complex	Astrakhan spotted fever	*Rhipicephalus pumilo*	Russia
	Rickettsia rickettsii	Rocky Mountain spotted fever	*Dermacentor variabilis, D. andersoni, Rhipicephalus sanguineus, Amblyomma cajennense*	North and South America
	Rickettsia sibirica	Siberian tick typhus	*Dermacentor nuttalli, Dermacentor marginatus, Haemophysalis concinna*	Northern China, Pakistan (Asia Siberia)
	Rickettsia akari	Rickettsial pox	*Allodermanyssus sanguineus*	USA, Ukraine, Croatia, Korea
	Rickettsia africae	African tick bite fever	*Amblyomma hebraeum*	Southern Africa
	Rickettsia australis	Queensland tick typhus	*Ixodes hlocyclus*	Australia
	Rickettsia japônica	Japanese tick typhus	*Haemophysalis longicornis*	Japan
	Rickettsia honei	Finders Island tick typhus	*Aponomma hydrosauri*	Finders Islands
SFG rickettsiae (never isolated from humans)	*Rickettsia massiliae*		*Rhipicephalus turanicus, Rhipicephalus sanguineus, other Rhipicephalus spp.*	France, Greece, Spain, Portugal, central Africa.
	Rickettsia rhipicephali		*Rhipicephalus sanguineus*	USA, France, Portugal, central Africa
	Rickettsia parkeri		*Amblyomma maculatum*	USA
	Rickettsia Montana		*Dermacentor variabilis*	USA
	Rickettsia belli		*Dermacentor spp.*	USA
Typhus group	*Rickettsia prowazekkii*	Epidemic typhus	*Pediculus humanus corporis*	Worldwide (most in highlands areas of South America, Asia, Africa)
	Rickettsia typhi	Murine typhus	*Xenopsylla cheopis*	Worldwide
Scrub typhus	*Orientia tsutsugamushi*	Scrub typhus	*Leptotrombidium deliense*	Eastern Asia, northern Australia, western Pacific Islands

spring (Patterson 1993). Infestation with body lice and louse-transmitted diseases is also being increasingly reported in the inner cities of developed countries (Koehler et al. 1992; Van Der Laan and Smit 1996; Jackson and Spach 1996; Rydkina et al. 1999).

Murine Typhus is mainly transmitted by rat flea *Xenopsylla cheopis* (Azad 1990; Chaniotis et al. 1994); other flea species and arthropod vectors also have been reported to transmit *R. typhi*, including the cat flea *Ctenocephalides felis*, lice, mites, and ticks (Azad 1990; Sexton 2005; Raoult and Roux 1997). The flea remains infected for life after a blood meal from an infected rat (Azad 1990). The primary reservoirs are rats belonging to the subgenus *Rattus*, mainly *Rattus norvegicus* and *Rattus rattus* (Azad 1990). However, various rodents and other wild and domestic animals have also been occasionally seen to act as hosts. Rats serve not only as simple hosts but also as amplifying hosts (Azad 1990).

Rickettsia enters the midgut epithelial cells of the flea, where it multiplies without causing any damage and is excreted with the feces. The bacterium is additionally maintained in fleas by

transovarial and transstadial transmission (Raoult and Roux 1997) and is then transmitted back to a susceptible vertebrate host upon subsequent feeding (Azad 1990).

The role of humans in the natural cycle of *R. typhi* is secondary, as they are only accidental hosts. While feeding on a human host, the flea defecates; the irritation caused by the bite causes the host to scratch and thus inoculate the rickettsiae into the flea-bite site or skin abrasions. *Rickettsiae* are also thought to infect humans via inhalation or contamination of the conjunctiva (Azad 1990; Raoult and Roux 1997).

Many reports illustrate that murine typhus is an emerging disease with worldwide distribution, but it is most prevalent in warmer countries (Raoult and Roux 1997). Most cases occur in late summer or early autumn; this seasonal variation directly correlates with the abundance of the vector fleas (Azad 1990). A decline in the incidence of murine typhus occurs when rat and flea control programs are initiated by public health services (Azad 1990; Sexton 2005).

Scrub typhus Transmission occurs within an area bounded by the Asiatic and Australian continents, to the north by Siberia and the Kamchatka Peninsula, to the south by Queensland, and to the west by Afghanistan. The public health impact of *O. tsutsugamushi* infection is greatest for agricultural laborers in rural areas (Duffy et al. 1990), although disease transmission also has been reported in suburban areas (Fan et al. 1987). Travelers to endemic areas can become infected (Woodruff et al. 1988), but it is not common. The majority of scrub typhus cases go undiagnosed because commercial diagnostic tests are generally unavailable in the rural tropics. *O. tsutsugamushi* was documented to be the most common cause of acute fever in Malaysia and Thailand (Lorandos 1934; Binford and Ecker 1947).

Transmission occurs by mites in zones where the primary forest has been cleared and replaced. Humans are accidental hosts, acquiring *O. tsutsugamushi* during feeding of a larval trombiculid mite of the genus *Leptotrombidium*. These chiggers only feed on mammalian tissue fluid once in their lifetime (McLeod et al. 2004); the reservoir of infection is through transovarial transmission. Chigger activity is determined by temperature and humidity. Mites are normally maintained in nature by feeding on a variety of wild rodents, but rodents are not a reservoir of *O. tsutsugamushi*.

Leptotrombidium deliense is the most important vector species in Southeast Asia and southern China, whereas *L. akamushi, L. scutellare*, and *L. pallidum* are the main vectors in Korea and Japan (Duffy et al. 1990; McLeod et al. 2004). *L. chiangraiensis* is a newly described vector found in cultivated rice fields in Thailand (Schmiela and Millera 1999).

Pathogenicity and Clinical Significance

Rickettsiae are obligately intracellular organisms that do not replicate extracellularly. Organisms in this family generally target macrophages, leukocytes, and endothelial cells. The main target cells of rickettsiosis are endothelial cells, which are most likely the result of vascular location and hematogenous dissemination. The lungs and brain are the critical target organs determining the lethality of rickettsioses. The events are visible in the skin: vasodilatation, perivascular edema, and disruption of vascular integrity (Raoult and Parola 2007). The pathogenesis of diseases caused by *Rickettsia* and *Orientia* genera differ greatly, according to the species of bacteria:

Rocky Mountain Spotted Fever (RMSF), the most severe of all the spotted fever group (SFG), is caused by *Rickettsia rickettsii*. It is a disease of the capillary circulation, being the only organism in the genus that invades beyond the blood vessel lining of the endothelium; it also invades adjacent vascular smooth muscle cells, particularly in arterioles. This bacteria is transmissible from the blood of infected patients to healthy hosts. These bacteria have the capacity to infect and replicate in the cytosol, and occasionally in the nucleus of vertebrate cells (e.g., endothelium, vascular smooth muscle, and macrophages) and invertebrate cells (e.g., hemocytes and salivary gland epithelium) (Raoult and Parola 2007).

In vertebrate hosts, these organisms are found in apparently uninjured endothelium of normal vessels, in areas of proliferated endothelium of the intima of vessels, in hyaline necrosed intima of more advanced lesions, in apparently normal and necrosed smooth muscle fibers of vessels with lesions, and in endothelial cells in the perivascular zones of proliferation. The largest masses are seen in smooth muscle cells of affected arteries and veins (Wolbach 1916).

Infection by *R. rickettsii* causes a significant reduction in key enzymes involved in the protection of endothelial cells from oxidative injury (Devamanoharan et al. 1994), resulting in increased levels of intracellular peroxides accompanied by ultrastructural indications of cell injury (Hong et al. 1998). Endothelial cell infection by *R. rickettsii* activates the nuclear transcription factor, which exerts an antiapoptotic effect (Joshi et al. 2003) by inhibiting proteins in the caspase family, which mediate apoptosis (Joshi et al. 2004). Inhibition of apoptosis is essential for host cell survival and site persistence of active infection (Clifton et al. 1998).

RMSF is a systemic illness that can involve endothelial cells of capillaries of all tissues and organs; however, the first signs and symptoms of disease resemble many other infectious syndromes. Following the bite of an infected tick, the disease begins with abrupt onset of fever accompanied by headache, nausea, vomiting, anorexia, and generalized myalgia. Other findings were recorded but with low frequencies. The rash begins small, typically on the wrists, ankles, and forearms, then evolves. It spreads centrally, and the entire body may be involved in few hours (Ong and Raffeto 1940; Harrell 1949). Characteristics of the rash considered to be classic for RMSF—that is, petechial lesions and a distribution that includes the palms and soles—occur in most of patients (Hazard et al. 1969; Kaplowitz et al. 1981; Ong and Raffeto 1940; Harrell 1949). Children are less likely than adults to present without a rash (Helmick et al. 1984). The life-threatening pathophysiologic consequences of infection and inflammation are microvascular damage and increased vascular permeability, which results in edema, localized hemorrhage, and hypoperfusion of one or more organ systems.

The invertebrate hosts for *R. rickettsii* include several species of ticks from several genera, including *Dermacentor*, *Rhipicephalus*, and *Amblyomma* (Burgdorfer 1988; Guedes et al. 2005). In these hosts, rickettsiae infect and replicate in several cell types, such as ovaries, salivary glands, midgut epithelium, and hemocytes (Sonenshine 1993). Vertebrates host that these bacteria has been isolated include domestic dogs, field voles (*Microtus pennsylvanicus*) (Badger 1933; Price 1954), pine voles (*M. pinetorum*), white-footed mice (*Peromyscus leucopus*), cotton rats (*Sigmodon hispidus*), cottontail rabbits (*Sylvilagus floridanus*), Rocky Mountain cottontail rabbits (*S. nuttallii*), snowshoe hares (*Lepus americanus*), opossums (*Didelphis virginiana*), chipmunks (*Tamias amoenus*), and golden-mantled ground squirrels (*Spermophilus lateralis*) (Burgdorfer 1988; McDade and Newhouse 1986; Schriefer and Azad 1994). The importance of birds as reservoir hosts for *R. rickettsii* remains unproven (Burgdorfer 1975).

Most cases of severe rickettsial disease are confirmed by serologic testing and on this basis are attributed to RMSF caused by *R. rickettsii*. However, the recent description of *R. parkeri* as a cause of rash-associated febrile illness (Paddock et al. 2004) in the United States highlights the low reliability of the results of serologic tests.

Despite the current availability of effective treatment and advances in medical care, many patients still die from rickettsial diseases. The majority of deaths are attributable to delayed diagnosis and failure to initiate specific antibiotic treatment within the first several days of the illness (Dalton et al. 1995; Kirkland et al. 1995). The recommended therapy for RMSF is doxycycline (CDC 2006).

African Tick-Bite Fever is probably an ancient disease in sub-Saharan Africa caused by *Rckettsia africae*. This disease may be one of the most common causes of acute febrile disease in rural sub-Saharan Africa, particularly in visitors from abroad. *R. africae* is a spotted-fever group rickettsia closely related to *R. parkeri* in North America and *R. sibirica* in Northeast Asia. These bacteria are mainly transmitted by *Amblyomma variegatum* and *Amblyomma hebraeum* ticks (Kelly and Mason 1991; Kelly et al. 1994; Parola et al. 2001). Humans are usually attacked by ticks on the legs, typically on moist skin behind the knee, in the groin, the perineum, or the axilla.

The pathophysiological hallmark of African tick-bite fever is the formation of focal or disseminated vasculitis (Toutous-Trellu et al. 2003). *R. africae* primarily invades the endothelial cells of smaller blood vessels (Jensenius et al. 2003a) and results in intramural and perivascular inflammation composed mainly of polymorphonuclear leukocytes, T-cells, and macrophages (Lepidi et al. 2006). The clearance of *R. africae* from endothelial cells is characterized by increased circulating levels of cytokines and chemokines (Jensenius et al. 2003b).

Rickettsia africae infection is symptomatic in few cases (Jensenius et al. 2002). The clinical course typically includes an abrupt onset of fever, nausea, headache, and neck myalgia commencing days after a tick bite (Raoult et al. 2001; Jensenius et al. 2003b). Most patients develop a painless large black crust surrounded by a red halo at the site of the tick bite. A painful regional lymphangitis (Brouqui et al. 1997) is detected in half of the cases. The majority of patients with African tick-bite fever develop mild to moderately severe illness that either resolves spontaneously within 10 days or responds promptly to anti-rickettsial treatment (Raoult et al. 2001; Jensenius et al. 2003b; Fournier et al. 1998a; Smoak et al. 1996). Complications are rare and no fatalities have ever been reported.

R. africae is susceptible in vitro to tetracycline, chloramphenicol, rifampin, fluoroquinolones, newer macrolides, and ketolides (Rolain et al. 1998, 2000). Most patients respond quickly to doxycycline. Patients with mild symptoms may not require any treatment at all (Jensenius et al. 2003a).

Mediterranean Spotted Fever (MSF) was described in Tunisia and was soon reported in the Black Sea littoral, India, the Middle East, and southern Africa. The causative agent was named as *Rickettsia conorii*. It was thereafter also known as "boutonneuse fever" because of a papular rather than macular rash. The brown dog tick *Rhipicephalus sanguineus* was recognized as a vector in Europe (Brumpt 1932); this vector is found throughout the world, but *R. conorii* is found only in some regions.

This bacterium does not normally infect humans during its natural cycle between its arthropod and vertebrate hosts, dogs. After an asymptomatic incubation of 6 days (Raoult et al. 1986; Martin Farfan et al. 1985), the onset of MSF is abrupt. Typical cases present with high fever and flu-like symptoms such as headache, chills, arthromyalgia, and a black eschar at the tick-bite site (Raoult and Roux 1997; Anton et al. 2003). The eschar is usually localized on the trunk, legs, and arms and rarely occurs in multiples. The rash often involves the palms and soles, but not the face. Generally, patients will recover within 10 days without any sequelae.

R. conorii multiply in the endothelial cells of small to medium vessels in human hosts, causing a vasculitis that is responsible for the clinical and laboratory abnormalities occurring in MSF (Parola and Raoult 2001). After phagocytosis and internalization, the phagocytic vacuole is lysed and rickettsiae escape the phagocytic digestion to multiply freely in the host cell cytoplasm and nucleus (Raoult and Roux 1997).

Before confirmation of the diagnosis, early empirical antibiotic therapy should be prescribed in any suspected cases. Doxycycline remains the treatment of choice for MSF (Raoult and Roux 1997). In patients with severe hypersensitivity to tetracyclines, chloramphenicol can be considered as an alternate therapy (Shaked et al. 1989).

Murine typhus, also known as endemic typhus, is a flea-borne infectious disease caused by *Rickettsia typhi*. The disease occurs in environments where rats and humans live in close proximity and typically in temperate and subtropical seaboard regions. The illness is less commonly diagnosed in developed countries than in the developing part of the world due to improved hygiene and rat control efforts. It is difficult to establish the true incidence because of the difficulty in distinguishing murine typhus from other causes of rash and fever.

R. typhi is a member of the typhus group of rickettsiae that also includes the agent responsible for epidemic typhus, *R. prowazekii*. *R. typhi* (or *R. mooseri*) is carried by the rat flea *Xenopsylla cheopis* and typically infects humans in markets, grain stores, breweries, and garbage depots. It usually causes a mild form of illness, but severe forms of the disease can also occur.

Scrub typhus is a chigger-borne zoonosis that is of greatest public health importance in tropical Asia; the causative agent is *Orientia tsutsugamushi*. The chigger bite can occur on any part of the body, is painless, and is not usually remembered by the patient (Sayen et al. 1946). Humans are accidental hosts. It is not known whether organisms deposited in the skin spread to internal organs via the bloodstream, the lymphatics, or by another mechanism. The disease begins as a small papule, enlarges, undergoes central necrosis, and acquires a blackened crust to form a lesion resembling a cigarette burn. Atypical eschar is pathognomonic when viewed by a clinician experienced in scrub typhus diagnosis. The rash appears on the trunk and spreads peripherally; however, it is often difficult to detect on dark-skinned persons.

O. tsutsugamushi infection appears to be a vasculitis, but the host cell in human scrub typhus is not known with certainty. The basic histopathological lesions suggest that macrophages are a more important target cell than the endothelium (Park and Hart 1946). Scrub typhus bacteria have been demonstrated in a variety of cells in humans, including monocytes, macrophages, Kuppfer cells, cardiac myocytes, hepatocytes, and endothelial cells (Settle et al. 1945; Allen and Spitz 1945; Walsh et al. 2001; Moron et al. 2001). In fatal cases, histopathology showed chiefly disseminated focal vasculitis and perivasculitis in vessels of the skin, lungs, heart, and brain. Endovasculitis and focal hemorrhage may be present but are less prominent than in Rocky Mountain spotted fever and epidemic typhus (Settle et al. 1945). The most important lesions are interstitial pneumonia with alveolar edema, hemorrhage, and meningoencephalitis (Settle et al. 1945; Allen and Spitz 1945; Park and Hart 1946).

Fever and headache begin abruptly and are frequently accompanied by myalgia and malaise. Cough, sometimes accompanied by infiltrates on the chest radiograph, is one of the most common presentations of scrub typhus infection (Chaykul et al. 1988). In severe cases, tachypnea progresses to dyspnea, the patient becomes cyanotic, and full-blown acute respiratory distress syndrome (ARDS) may develop. ARDS is associated with older age and preceding infiltrates on chest radiographs (Tsay and Chang 2002). Respiratory failure is the most common cause of death in severe scrub typhus infection, but survivors recover without sequelae.

O. tsutsugamushi infection can be cured and prevented by chloramphenicol and safer tetracyclines (Smadel et al. 1950). The response of mild scrub typhus to treatment with doxycycline or chloramphenicol is typically so rapid that resolution of fever is used as a diagnostic test; if the temperature has not returned to normal within 48 h after beginning doxycycline treatment, then the infection is not due to *O. tsutsugamushi* (Watt et al. 1996).

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