Update on Rickettsioses in Slovakia

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Summary. – The reported incidence of vector-borne diseases including various cases of Rickettsioses in humans is increasing due to a combination of climatic and social factors, escalating the opportunities for contact between people and ticks, fleas or lice. Many of the emerging infectious diseases currently challenging human health in Europe are transmitted by ticks which normally feed on domestic or wild animals. Each *Rickettsia* spp. has one or several tick vectors, and their geographical distribution varies according to geographical conditions; e.g.; altitude or temperature, which is gradually changing due to a global warming. Evidence of *Rickettsia* spp. particularly of a newly discovered species is a strong indication that a great number of diseases may be caused by so far undetected or unrecognized organisms. Their diagnosis relies mostly on rare "spot like" cooperation of clinicians with scientists, the members of the working groups that are devoted to the scientific studies of the corresponding research areas. The clinical picture of the disease caused by rickettsiae varies significantly from flu like symptoms to severe fatal outcomes, reflecting the various factors, e.g. a variability of virulence of rickettsial species due to cell invasion, dissemination of rickettsiae, genomics, immune response of an infected organism, or a tricky impact of a treatment. Several major reviews on rickettsioses have been previously published, e.g. in 1997 (Raoult and Roux, 1997a), in 2005 (Parola *et al.*, 2005), and in 2011 (Botelho-Nevers and Raoult, 2011). In this work we intend to present a short historical overview and to describe new trends in research studies of rickettsiology. The main focus will be on rickettsioses affecting Europe´s population.

Keywords: rickettsiae; tick; lice; fleas; vector; Slovakia

Contents:

1. Inauguration of Rickettsiology

Members of the family *Rickettsiaceae* have accompanied humans throughout the history of mankind. The founda-

tion stone of rickettsiology was laid in ancient times. Currently this field of study has entered into incredibly large dimensions. Rickettsiology began with a concentrated effort of scientists to assign possible vectors (ticks, lice, flea, and mite) and reservoirs (mammals, humans, dogs, rabbits, birds, reptiles) of these microbial agents. They are an inevitable prerequisite for the spread and evolution of the variety of diseases they cause (Merhej and Raoult, 2011a; Renvoise *et al.*, 2011). *Rickettsia prowazekii* (*R. prowazekii*), the agent of epidemic typhus, also known as louse-borne typhus is the oldest rickettsiosis and has caused more deaths than weapons during wartime. It was named in honour of Howard Taylor Ricketts and Stanislaus von Prowazek, who both died of typhus (da Rocha Lima, 1917). This microbe requires vertebrate hosts, human beings to maintain its life cycle and is transmitted by human body lice (*Pediculus humanus humanus*). Determination of the lice being a vector gained a Nobel Price for the man who discovered this, Dr. Charles Nicolle. Recently, an extra facet of its persistence in nature has been revealed as a natural reservoir of *R. prowazekii*: a North American squirrel (*Glaucomys volans*) in Virginia, USA, and thereafter in Florida and Maryland (Bozeman *et al.*, 1975) as well as in ticks in Ethiopia and Mexico.

A second agent, *Rickettsia typhi* (*R. typhi*) causes murine or endemic typhus. This bacteria was proved to be transmitted mainly by rats flea *Xenopsylla cheopis* (Azad, 1990), but occasionally by other flea species or arthropod vectors, including cat fleas *Ctenocephalides felis* (Irons *et al.*, 1944; Le Chuiton *et al.*, 1935; Lepine and Lorando, 1935), mouse fleas *Leptopsyllia segnis* or rat lice *Polyplax spinulosa* (Mouffok *et al.*, 2011;Schriefer *et al.*, 1994). The main reservoirs are rats (*Rattus rattus*, *R. norvegicus*,), but various rodents and other wild and domestic animals, such as house mice, cats, opossums (*Didelphis virginiana*) and skunks have also been seen to act occasionally as hosts.

The third oldest known representative or rickettsiae is *Rickettsia rickettsii* (*R. rickettsii*), discovered in 1904 (Wilson and Chowning, 1904) and the agent of Rocky Mountain spotted fever. The main role of ticks and tick bites as a mode of transmission was described and a first isolate obtained in 1910 (Ricketts and Wilder, 1910). The list of tick borne transmitted rickettsiae to the present day has grown enormously. Reservoirs of ricketttsiae are numerous mammals, dogs, rabbits, birds, *etc*. Inspection and discovery of the quantity of new, emerging, *candidate* rickettsiae has increased significantly. This has led to the creation of ever more modern investigative procedures and moved rickettsiology to being an elite science. An overwhelmingly large amount of scientific books and articles, describing the essence of the life and behaviour of these particular organisms have been written. There are currently 174 results accessible under the password "rickettsiae review" in PubMed.

2. Taxonomy

Taxonomic classification within the order Rickettsiales was originally based on relatively few phenotypic criteria (Raoult *et al.*, 1997a). Recent decades have brought extensive reorganization with the development of molecular tools. The family *Bartonellaceae* (Birtles *et al.*, 1995) and *Coxiella burnetii*, the agent of Q fever (Weisburg *et al.*, 1989) and *Rickettsiella grylli* (Roux *et al.*, 1997a) were removed from the order *Rickettsiales*, which nowadays includes three families, the *Anaplasmataceae*, to which belong *Anaplasma*, *Ehrlichia*, *Neorickettsia*, *Wolbachia* genera, *Rickettsiaceae* with *Rickettsia* and *Orientia* genera, and the Holosporaceae, which are mostly protest symbionts (Fig. 1). The bacteria from the first two families cause diseases of varying severity and are transmitted usually by arthropod vectors.

Based on the system of classification of rickettsiae, they are closely related phylogenetically and have a high degree of 16S rDNA nucleotide sequence similarity (Roux and Raoult, 1995). Recently, an international committee of expert rickettsiologists proposed guidelines to classify rickettsial isolates at various taxonomic levels and to clarify the nomenclature within the genus *Rickettsia* (Raoult *et al.*, 2005). The guidelines recommended the use of multiple approaches including phenotypic, genotypic and phylogenic criteria. Five rickettsial genes have been proposed to define the genus, the group and the species: 16S rRNA (rrs), *gltA, ompA, ompB* and sca4 (gene D) (Fournier *et al.*, 2003). Bacteria from *Rickettsia* genus are divided into spotted fever groups (SFG) with causative microorganisms which are mainly spread by ticks, currently including 26 validated species; typhus group (TG) with two members, transmitted by lice and/or fleas, namely *R. prowazekii* and *R. typhi;* and *R. bellii* and *R. canadensis*, the latter two groups lie outside the spotted fever and typhus groups (Merhej and Raoult, 2011b).

In addition, official criteria based on epidemiological, clinical, serotypical and genotypical differences that were found after multi-spacer typing have been proposed for the creation of subspecies within *R. conorii* and *R. sibirica* complex (Zhu *et al.*, 2005; Fournier *et al.*, 2006a).

3. Epidemiology

Rickettsiae are emerging pathogens causing serious diseases in humans. The representatives of TG rickettsiae have always been linked to disastrous conditions, e.g. war, poverty, famine, catastrophe, etc. Economic decline in the standard of living and poor fundamental health choices favour their extension. *R. prowazekii* has been estimated to be responsible for several millions of deaths during and after World War I, acting in a devastating epidemic wave in the years 1917–1925 in Europe (Patterson, 1993). Epidemic
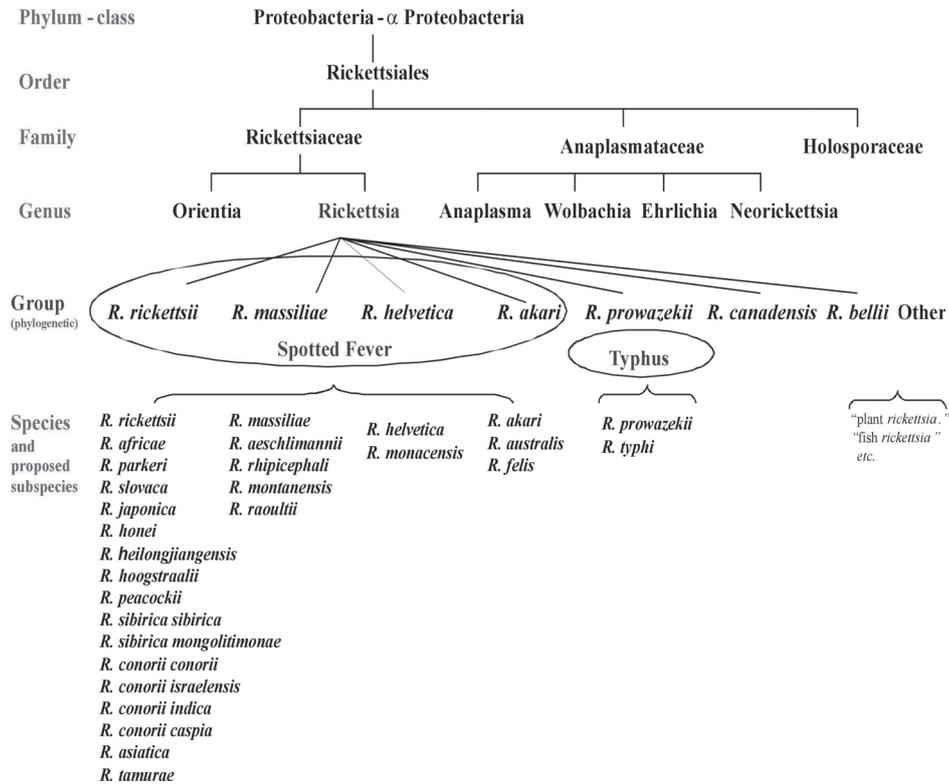
Fig. 1

Taxonomy of rickettsiae

typhus affected Napoleonˊs soldiers during the French retreat from Russia (Raoult *et al.*, 2006). Again, World War II saw this problem present itself again with epidemic typhus in northern Africa, southern Italy and central and Eastern Europe. Devastating outbreaks occurred in concentration camps (Weiss, 1988). At present, *R. prowazekii* is considered a potential albeit unlikely category B bioterrorism agent (Walker, 2009). The majority of cases are reported from places with compromised conditions of hygiene, e.g. rural highlands of Africa. The last large outbreak of epidemic typhus was reported in Burundi during the civil war in 1997 (Raoult *et al.*, 1997, 1998a). However, central and south America are also affected (Bechah *et al.*, 2008a).

A complication of this disease is that under stressful conditions or weakening of the immune system, infection can be reactivated in humans, years or decades after primary onset (PRICE, 1955). The disease can be reactivated causing a recrudescent infection. Affected individuals serve as a source for re-emergence of infection during times conducive for lice (Lutwick, 2001). This supplementary element of complexity in the epidemiology of *R. prowazekii* is called Brill-Zinsser disease. Several cases of such "dormitory stage" have recently been reported in Europe keeping medical staff on high alert

(Faucher *et al.*, 2012; Stein *et al.*, 1999; Turcinov *et al.*, 2000; Turcinov *et al.*, 2002).

R. typhi, the agent of murine typhus can affect homeless people in Europe or may be reported as travel associated zoonotic disease (Leshem *et al.*, 2011). Recently, in a French retrospective study, all murine typhus cases occurred in travellers and most of them had returned from Africa and South-east Asia (Walter *et al.*, 2012).

SFG rickettsie comprise the family of species with genetic based differences, causing diseases which usually reflect the continent from where the bacteria originated, e.g.: *R. rickettsii* causing Rocky Mountain spotted fever; *R. conorii* – Mediterranean spotted fever (MSF); *R. akari* – rickettsialpox; *R. japonica* – Japanese spotted fever; *R. sibirica sibirica*- North Asian tick typhus; *R. sibirica mongolitmonae*-lymphangitis-associated rickettsiosis; *R. raoultii* and *R. slovaca* –SENLAT (scalp eschar and neck lymphadenopathy following tick bites) *R. africae* – African tick bite fever, *R. australis* – Queensland tick typhus and *R. honei* – Flinders Island spotted fever, *R. marmionii* – Australian spotted fever, *R. heilongjiangensis* – Far Eastern spotted fever.

In times before the application of molecular biological methods for the detection of pathogens, MSF triggered by *R.*

conorii, and Siberian tick typhus caused by *R. sibirica* subsp. *sibirica*, were considered to be the only rickettsiosis prevalent in Europe (Blanco and Oteo, 2006). However, seven new autochthonous tick-borne rickettsial pathogens have been described in Europe over recent decades e.g. lymphangitis-associated rickettsiosis (LAR) caused by (a) *R. sibirica* subsp. *mongolitimonae* (Fournier *et al.*, 2006b), SENLAT (scalp eschar and neck lymphadenopathy following tick bites) (Angelakis *et al.*, 2010) [or tick – borne lymphadenopathy (TIBOLA) (Lakos, 2002, 2012) or *Dermacentor* spp.- borne necrosis – erythema lymphadenopathy (DEBONEL)] which is caused by (b) *R. slovaca* and (c) *R. raoultii* (Ibarra *et al.*, 2006), Israeli spotted fever by (d) *R. conorii israelensis* and Astrakhan fever by (e) *R. conorii* subsp. *caspia* (Tarasevich *et al.*, 1991), (f) *R. monacensis* induced disease (Simser *et al.*, 2002), and flu like symptoms based on the (g) *R. helvetica* (Beati *et al.*, 1993b). In addition, several new *Candidatus* rickettsia e.g. *Candidatus* Rickettsia barbariae (Mura *et al.*, 2008), "Candidatus *Rickettsia* AvBat" (Socolovschi *et al.*, 2012a) have been discovered. A special chapter in the epidemiological picture of population in Europe play tick-borne rickettsioses naturally occurring outside Europe, e.g. African tick-bite fever (ATBF) in patients returning from Africa (Jensenius *et al.*, 2004a;Raoult *et al.*, 2001;Rolain *et al.*, 2004), or Rickettsialpox, infection caused by *R. akari,* commonly detected in the United states which has been recently reported in Turkey (Ozturk *et al.*, 2003), Croatia (Radulovic *et al.*, 1996) and France.

4. Vectors

4.1 Lice

The body louse, *P. humanus corporis*, is a strictly haematophagous, blood-sucking ectoparasite, that lives in clothing throughout its life cycle. They need to feed five times per day with a lifespan of about 4–12 weeks, and deposit their infected faeces near the bite lesion. *R. prowazekii* can remain viable for 100 days in lice faeces (Raoult and Roux, 1999). Transovarian transmission in the louse does not occur since lice die several weeks after being infected.

Transmission of *R. prowazekii* occurs by contamination of bite sites, conjunctivae, and mucous membranes with the faeces or crushed bodies of infected lice. Infection through aerosols of faecal dust has also been reported (Andersson and Andersson, 2000; Raoult *et al.*, 2004). An ease of transmission provides the main risk of epidemic typhus contamination for utility staff, mainly physicians. Lice also suffer from *R. prowazekii* infection. After feeding on the infected person, bacteria multiply in the louse gut epithelium and are released on the rupture of infected epithelial cells. Blood enters the louse body cavity as a result of loss of the epithelial lining, the louse becomes red, and infected red lice die within 1 week (Houhamdi *et al.*, 2002).

4.2 Flea

The invertebrate vectors of *R. typhi* are rat or cat fleas both of which frequently dwell close to humans. Transmission to humans occurs through contaminated skin, conjunctiva, or via the respiratory route by aerosols of contaminated flea faeces (Raoult and Roux, 1997a), or through the flea bite itself. The normal transmission cycle for murine typhus is rat-flea-rat, but accidentally rat-flea-man (*X. cheopis*), or cat-flea-man (*C. felis*). These regular modes of dispersion can be replaced by the Virginia Opossum flea cycle. Successful attempts at experimental infection with *R. typhi* were performed in Slovakia (Rehacek *et al.*, 1976b). Fleas as possible vectors of rickettsial diseases have been studied extensively (Rehacek and Tarasevich, 1988; Rehacek *et al.*, 1975b). *R. typhi* is maintained transovarially in fleas where it multiplies in the epithelium of the flea midgut. Fleas are capable of multiple feeding, and thus potentially transmitting the *Rickettsia* to several hosts (Civen and Ngo, 2008; Mouffok *et al.*, 2011; Traub and Wisseman, 1978).

In addition, the cat flea, *Ctenocephalides felis*, is currently the only known biological vector of *R. felis*, the spotted fever agent. Recently, *R. felis* have been detected in several flea species, as well as in ticks, mites, mosquitoes and booklice suggesting a variety of arthropod hosts (Parola, 2011). Studies examining the transmission of *R. felis* using colonized cat fleas have shown a decrease in vertical transmission from one generation to other and stable horizontal transmission (Hirunkanokpun *et al.*, 2011). *R. felis* transmission has been suggested to be similar to the *R. typhi* mechanism of transmission (Raoult *et al.*, 1998b; Reif and Macaluso, 2009).

4.3 Ticks

Ecological characteristics of the tick vectors of rickettsiae influence the epidemiology and clinical aspects of tick-borne diseases (Parola and Raoult, 2001). In nature, vertebrate hosts infections may result in a rickettsemia that allows uninfected ticks to become infected and for the natural cycle to be perpetuated (Burgdorfer *et al.*, 1966). Ticks may acquire rickettsiae through transovarial passage, via transfer of bacteria from adult female ticks to the subsequent generation of ticks via the eggs. A transstadial passage, transfer of bacteria to another stage, is a necessary component for the vectorial competence of the ticks (Parola *et al.*, 2001). Sexual transmission of *R. rickettsii* from infected males to uninfected females cannot significantly propagate the infection in tick lineages (Schriefer. and Azad, 1994). Co-feeding transmis-
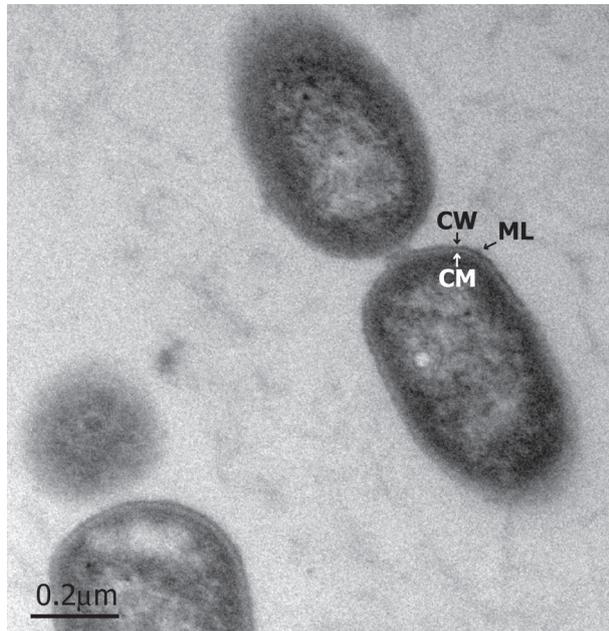
Fig.2

Electron micrograph of binary division of rickettsiae

R. slovaca: ML = microcapsular layer, CW = cell wall, CM = cytoplasmic membrane.

Fig. 3

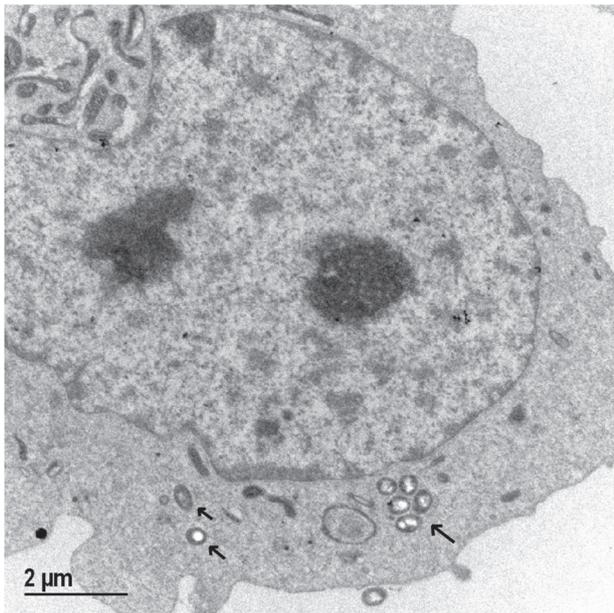**Ultrathin section of L929 cells infected with *R. slovaca***

Arrows are pointing to free bacteria in cytoplasm.

sion between different generations of ticks may obviate or lessen the requirement for transovarial maintenance of *R. conorii* (Philip, 1959).

4.4 Other arthropod vectors

Few other arthropods are known as vectors of rickettsial agents. A hematophagous mesostigmatid mite *Liponyssoides sanguineus,* the house mouse mite, is the vector of rickettsial-pox, *R. akari* agent. The transovarial and transstadial transmission of *R. akari* occurs in this *L. sanguineus* mite and suggests the potential role of reservoir in the wild besides various commensal and wild rodent species (Kiselev and Volchanetskaya, 1995). The prostigmatid trombiculid mites (chiggers) are the vector for the agent of scrub typhus, *Orientia tsutsugamushi,* a rickettsiosis of Eurasia and northern Australia.

Recently, *R. felis* was detected in *Anopheles gambiae* mosquito molecular form S, which represents the major African malaria vector, collected from Côte d'Ivoire and in *Aedes albopictus* mosquito collected from Gabon (Socolovschi *et al.*, 2012b,c). Mosquitoes of the family Culicidae are blood-sucking arthropods with a global distribution which are the main vector for infectious diseases. Mosquitoes are a potential vector of *R. felis* but more study is needed to confirm this hypothesis.

5. Morphology

Rickettsiae are usually coccobacillary in shape with dimensions ranging to about 0.7–1.0 μm in length, 0.3–0.5 μm in width. They are strict intracellular parasites, requiring host cells; even though attempts to propagate rickettsiae in axenic medium are taking place (personal communication). TG rickettsiae can be observed in the cytoplasm, SFG rickettsiae may be observed both in the nuclei and the cytoplasm of host cells (Boldis *et al.*, 2009; Silverman, 1991). Capsular material surrounding the cell wall can be seen in electron micrographs of fresh preparations as well as intracytoplasmic invaginations of the plasma membrane (mesosomes) and ribosomes. The bacterial surface exhibits a trilaminar construction composed of an inner (cytoplasmic membrane), outer leaflet (Tamura *et al.*, 1995), and microcapsular layer on the outside of the cell wall (Figs. 2, 3).

The bacterial wall is chemically similar to that of gram-negative bacteria as it contains diaminopimelic acid and lacks teichoic acid (Silverman and Wisseman, Jr., 1978; Silverman *et al.*, 1978a,b). The presence of a lipopolysaccharide (LPS) layer, typical of gram negative bacteria (Wisseman, Jr., 1968) indicates high endotoxic potency of rickettsiae (Zahringer, 1994). The structural properties of LPS from *R. typhi* and *R. prowazekii* have revealed a chemical similarity to that of *Proteus vulgaris* OX19 (Amano *et al.*, 1998). Lipid A of *R. typhi* resemble classic hexaacyl lipids A forms known by enterobacteria (Fodorova *et al.*, 2011).

Confocal, scanning electron, and transmission electron microscopy has revealed induced polymerization of host cell

actin (Van Kirk *et al.*, 2000). So called actin-based motility (ABM) employed by SFG rickettsiae, promotes intracellular and cell-to-cell spread (Heinzen, 2003).

6. Isolation

Rickettsiae can be grown in vitro in tissue cell cultures (Vero, L929, HEL, XTC-2, or MRC5 cells), using the centrifugation shellvial technique (Vestris *et al.*, 2003), or maintained in living host cells (animal mouse models or embryonated eggs). Isolation attempts must only be carried out in biosafety level 3 laboratories. They can be performed in various modes. A minimum of 5 ml of blood collected prior to antimicrobial therapy, routinely by using buffy coat preparations of heparinized or EDTA-anticoagulated whole blood (decanted plasma, collected on heparin or citrate anticoagulant) can be applied. Skin biopsy specimens (inoculation eschar being the best to use) are an excellent source of rickettsiae, and of course vectors, arthropods (crushed ticks are mostly used). Culturing may be time consuming as new isolates need time to adapt to laboratory growth conditions, and a certain percentage of samples may be contaminated by other pathogens. Only one third of isolation attempts are successful. Although they possess a membrane resembling a gram-negative bacterium, they are usually not stained with Gram techniques, but frequently with

Gimenez staining method (Gimenez, 1964). Light microscopy has shown that the maximum accumulation of SFG rickettsiae is between days 5–7 and cell death was apparent 7 – 10 days post infection. Later stages of infection were characterized by necrotic effect of the infected cultures.

In common with many other intracellular pathogens, rickettsiae utilize a "zipper" mechanism to mediate their cellular internalization (Ham *et al.*, 2011). Rickettsiae exploit the cytoskeleton to enter and spread within the host cell. Although these bacteria are able to make all metabolites necessary for growth, they have an ATP transport system that allows them to use host ATP. Thus, they are energy parasites as long as ATP is available from the host (Winkler and Daugherty, 1984). They divide by binary fission and metabolize host-derived glutamate via aerobic respiration and the citric acid cycle (Coolbaugh *et al.*, 1976; Rees, Jr. and Weiss, 1968). Differences in cytopathic effects are known, however parameters of mechanisms of oxide injury are not completely elicited (Eremeeva *et al.*, 2001).

7. Genetic coding for intracellular activities

The rickettsiae have guanine plus cytosine (G + C) content of 29% to 32.6%, and a genomic size varying between 1.1 Mb and 1.59 Mb (*Rickettsia* endosymbiont of *I. scapularis* was
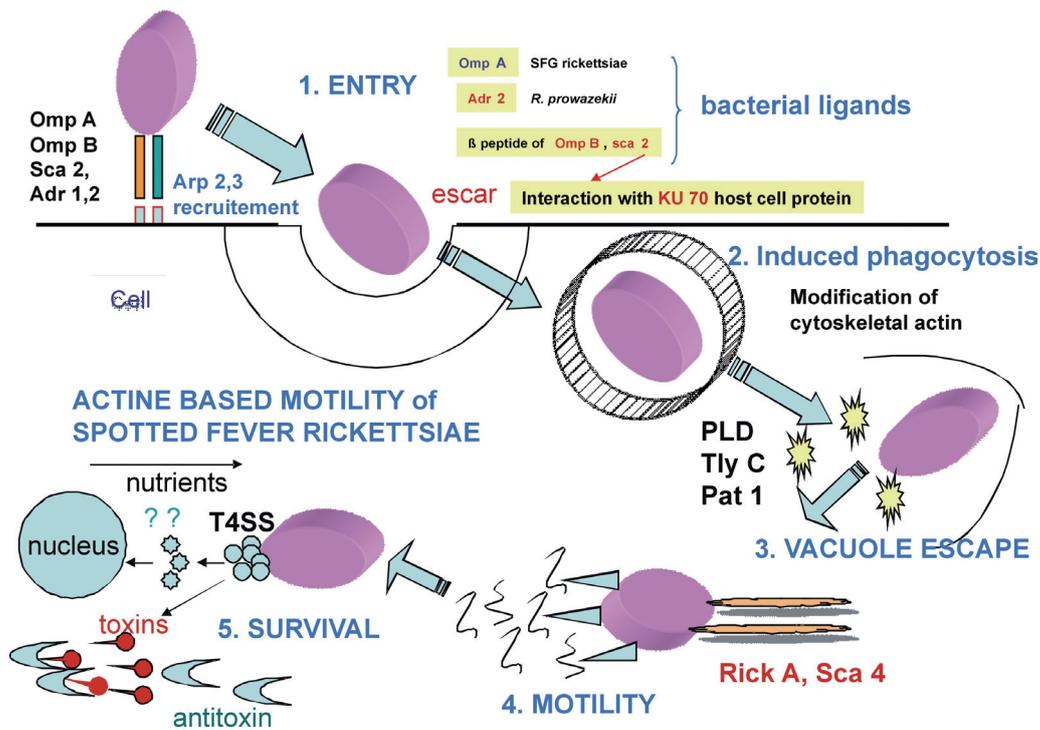

Fig. 4

Intracellular behavior of rickettsiae

described to be 2.1). Knowledge of genomes has provided new insight into the functions that are required by rickettsiae for their survival in the animal host (Palmer, 2002) (Fig. 4). It has been considered that gene loss can be a critical feature of the evolution of intracellular pathogenic bacteria (Andersson and Andersson, 1999; Andersson and Kurland, 1998; Blanc *et al.*, 2007; Darby *et al.*, 2007). All rickettsiae share two outer membrane proteins (*OmpB* and *Sca4*) and LPS biosynthesis machinery. The genome of *R. typhi* contains four potentially membranolytic genes (*tlyA, tlyC, pldA*, and *pat-1*) and five autotransporter genes, *sca1, sca2, sca3, ompA*, and *ompB*. Each of these genes plays a crucial role in life of rickettsiae within cells.

Genetic alterations, e.g. the disruption of rickettsial outer membrane proteins (*rOmpA* and *rOmpB*), coding for so called "attachment to cells proteins" were shown to play vital roles in adhesion and invasion of rickettsiae into mammalian cells, providing an essential condition for further spread of infection (Chan *et al.*, 2009; Li and Walker, 1998). The cell surface antigen *sca4* of *Rickettsia* co-localizes with vinculin in cells at sites of focal adhesions in sca4-transfected cells. *Sca4* binds to and activates vinculin through two vinculin binding sites that are conserved across all Rickettsia (Park *et al.*, 2011). Traversal of host cell membrane is probably enabled by *tlyA* gene coding for Hemolysin A (Walker and Yu, 2005). S*ca2* gene has been associated with adherence (Cardwell and Martinez, 2009) and actin-based motility (Kleba *et al.*, 2010). Disruption of this gene led to no fever status after the *R. rickettsii* infection.

Following binding of rickettsiae to host cells and associated cytoskeletal rearrangements, the bacterium is engulfed by a cellular vacuole. Rickettsiae can penetrate nonphagocytic cells by induced phagocytosis (Teysseire *et al.*, 1995). Once phagocytised, *Rickettsia* spp. tends to rapidly escape the vacuole entering the cytoplasm of the host cell (Teysseire *et al.*, 1995). It is likely that this rapid escape is instrumental to the survival of these microbes, prior to the fusion of lysosomes. Phagosomal membrane escape is due to *thyC* and *pld* genes with membranolytic activity, coding for Hemolysin C and Phospholipase D (Olano, 2005; Renesto *et al.*, 2003; Walker *et al.*, 2001), respectively.

Once within the cytoplasm, SFG rickettsiae demonstrate actin-based motility (ABM), probably facilitating their spread. RickA is essential for interaction with the multisubunits of the eukaryotic Arp 2/3 complex, among other genes, regulating the actin cytoskeleton responsible for ABM (Balraj *et al.*, 2008). Members of TG: *R. prowazakii*, *R. typhi*, and *R. peacockii* of the SFG do not possess functional rickA protein (Teysseire *et al.*, 1995). Hence, motility may take part in, but cannot explain per se the differences in pathogenicity. It has been shown, that the disruption and gene loss defective process can contribute to the avirulence of ricketsiae (Ellison *et al.*, 2008). On the other hand, a massive comparative genomic analysis of gene loss in intracellular bacteria, underlined the hypothesis that such a state enables a pathogen to enhance virulence (Darby *et al.*, 2007; Merhej *et al.*, 2009). Comparison of *R. africae* and *R. rickettsii* has pointed to regulatory genes as essential factors in the development of pathogenicity (Fournier *et al.*, 2009).

The first genome of a tick-transmitted rickettsia (*R. conorii* strain Seven) was fully sequenced in 2001 (Ogata *et al.*, 2001). At present a complete genome sequence of 24 validated species is known, and several other rickettsial genomic projects are running. Genomes portray the key features of rickettsial intracellular survival (Walker *et al.*, 2005). Comparative genomics enables unveiling of functions of their construction units, and opens up a new, genomic era (Table 1).

8. Detection

Guidelines for the diagnosis of tick-borne bacterial diseases in Europe were proposed by Brouqui *et al.* (2004). Clinical symptoms and epidemiological history together with laboratory confirmation should all be taken into consideration in order to make the correct diagnosis. The complement fixation test of the past times serology was replaced by a test of first choice microimmunofluorescence (Kelly *et al.*, 1992b), followed by the latex agglutination, indirect hemagglutination, immunoperoxidase assay, and enzyme-linked immunosorbent assay (Kovacova and Kazar, 2000; Kovacova *et al.*, 2006). Five ml of blood should be collected early in the course of the disease, followed by a second sample taken 2 weeks later. If possible, a third sample should be collected 4–6 weeks later. Sera can be preserved at -20°C or lower for several months without degradation of the antibodies. A harvest of a few drops of blood onto blotting paper screened for positive reaction was proposed as an alternative method (Fenollar and Raoult, 1999). MIF is widely accepted as the reference method (La Scola and Raoult, 1997). IgG titres of 64 or more and/or IgM titres of 32 or more are considered to be indicative of infection by other *Rickettsia* species in other rickettsioses. IgM and IgG antibodies are detectable separately in acute and convalescent sera. IgG titres of 128 or above and/or IgM titres of 64 or above are considered to be indicative of infection by *R. conorii conorii* in suspected cases of MSF. Both IgM and IgG antibodies are usually detectable between 7 and 15 days after the onset of the disease. However, response to *R. africae* is delayed, a seroconversion in patients was detected 28 and 25 days after the onset of symptoms (Fournier *et al.*, 2002).

The interpretation of serological data can be confounded by the cross-reactivity that occurs between SFG rickettsiae and/or other bacteria (Parola *et al.*, 2005; Raoult and Dasch, 1995). Therefore, western blotting was suggested as a method of choice which will detect false positive results. Neverthe-

Table 1. Rickettsia organisms´ genome overview, genomic projects annotation report

No.	Organism	Strain, subspecies name	Causative agent	Size	NCBI link	Related publication
1.	*Rickettsia prowazekii*	Madrid E	Epidemic typhus	1,11	AJ235269	Bechah *et al.* (2010); Andersson *et al.* (1998)
		BuV67-CWPP			CP003393	
		Chernikova			CP003391	
		Dachau			CP003394	
		RpGvF24			CP003396	
		Rp22			CP001584	
		GvV257			CP003395	
		Katsinjan			CP003392	
2.	*R. typhi*	Wilmington	Murine typhus	1,11	AE017197	McLeod *et al.* (2004)
		B9991CWPP			CP003398	
		TH1527			CP003397	
3.	*R. rickettsii*	Iowa ASM1744v1	Rocky mountain spotted fever	1,27	CP000766	Ellison *et al.* (2008b)
		Hlp#2			CP003311	
		Arizona			CP003307	
		Brazil			CP003305	
		Colombia			CP003306	
		Hauke			CP003318	
		Hino			CP003309	
		ShelaSmith			CP000848	
4.	*R. conorii complex*	*conorii* Malish 7	Mediterranean spotted fever	1,27	AE006914	Sentausa *et al.* (2012b,c,d); Ogata *et al.* (2000, 2001b)
		caspia A-167	Astrakhan fever	1,26	AJUR00000000	
		indica ITTR	Indian tick typhus	1,25	AJHC00000000	
		israelensis ISTT CDC1	Israeli spotted fever	1,25	AJVP00000000	
5.	*R. sibirica* complex	*sibirica* 246	Siberian tick typhus	1,25	AABW00000000	Sentausa *et al.* (2012a,e); Malek *et al.* (2004)
		sibirica BJ-90			AHIZ00000000	
		mongolitimonae HA-91	Lymphangitis-associated rickettsiosis	1,25	AHZB00000000	
6.	*R. slovaca*	13-B	SENLAT	1,28	CP002428	Fournier *et al.* (2012)
		D-CWPP			CP003375	
7.	*R. massiliae*	MTU5	Spotted Fever	1,38	CP00683	Blanc *et al.* (2007a)
		AZT80		1,28		
8.	*R. africae*	ESF-5	African tick bite fever	1,29	CP00612	Fournier *et al.* (2009b)
9.	*R. helvetica*	C9P9	Spotted fever	1,42	AICO00000000	Dong *et al.* (2012a)
10.	*R. montanensis*	OSU 85-930		1,28	CP003340	–
11.	*R. rhipicephali*	3-7-female6-CWPP		1,31	CP00342	–
12.	*R. parkeri*	Portsmouth	Spotted fever	1,30	CP003341	–
13.	"*R. philipii*"	364D	Spotted fever	1,29	Cp003308	–
14.	*R. japonica*	YH	Japanese or oriental spotted fever	1,28	Ap011533	–
		YH*			AMRT00000000	
15.	*R. heilongjiangensis*		Far Eastern tick-borne rickettsiosis	1,28	CP002912	Duan *et al.* (2011)
16.	*R. peacockii*	Rustic	–	1,31	CO001227	Felsheim *et al.* (2009)
17.	*R. honei*	RB	Flinders Island spotted fever	1,27	AJTT00000000	Xin *et al.* (2012)
18.	*R. felis*	URRWXCal2	Spotted Fever	1,59	CP000053	Ogata *et al.* (2005)
19.	*R. akari*	Hartford	Rickettsialpox	1,23	CP000847	–
20.	*R. australis*	Cutlack	Queensland Tick Typhus	1,32	CP003338	Dong *et al.* (2012b)
		Phillips		1,32	AKVZ00000000	
21.	*R. bellii*	RML369-C10.2	–	1,52	CP000087	Ogata *et al.*(2006)
		OSU 85-389		1,53	CP000849	
22.	*R. canadensis*	McKiel	–	1,16	CP000409	
		CA410	–	1,15	CP003304	
23.	*Candidatus* "*R. amblyommii*"	GAT-30V	–	1,48	NC017028	Burkhardt *et al.* (2011); Baldridge *et al.* (2010)
24.	*Rickettsia* endosymbiont of *Ixodes scapularis*		–	2,1	CM000770	–

SENLAT: scalp eschar and neck lymphadenopathy after tick-bite.

less this technique is suited to reference laboratories only (Jensenius *et al.*, 2004b).

Distinct differences in DNA based composition between the typhus and SFG of rickettsiae were established long time ago (Tyeryar, Jr. *et al.*, 1973). Application of molecular biology techniques, a sensitive specific and rapid tool to detect and identify rickettsiae, caused the emergence of several new species. This technique can be used to find the agent in vectors (ticks), in EDTA-anticoagulated blood, in eschar/crust swab or skin biopsies. Samples can be taken directly from eschar ("tâche noire") being the most useful specimen. The usefulness of noninvasive cutaneous swab specimens for detecting rickettsiae was recently analyzed in animal model (guinea pigs) and in several humans (Bechah *et al.*, 2011). Specimens from eschars were positive for rickettsiae as long as lesions were present. Optimal storage temperature for specimens was 4°C for 3 days. In a prospective study in Algeria on autochthonous rickettsioses and in a retrospective study on travellers from South Africa (Mouffok *et al.*, 2011; Socolovschi *et al.*, 2012d), eschar/crust swab from inoculation eschar showed the same sensibility and specificity as skin biopsy sample. In addition, this test can be used at bedsides or in outpatient clinics and could be useful for epidemiologic, clinical studies and is also preferred by patients.

A current trend applied in most screening laboratories is on PCR, amplifying sequences of species specific genes. *OmpA* (Fournier *et al.*, 1998), *ompB* (Roux and Raoult, 2000), *gltA* (Roux *et al.*, 1997b), and gene D (*sca4*) (Sekeyova *et al.*, 2001) are reliable determinations of rickettsiae on the species level. Primer sets targeting rickettsial genes can be used in any laboratory with suitable facilities (Fig. 5). Technical progress determined the next steps of rickettsial detection. The multi-locus sequence typing (MLST) scheme was proposed as an alternative proportional system successfully used to detect variability among the Rickettsiae (Vitorino *et al.*, 2007a). Multi-spacer typing based on three intergenic spacers achieved identification of rickettsiae at species level also (Fournier and Raoult, 2007).

A real-time quantitative PCR has increased sensitivity of detection. A positive correlation between the bacteria copies and culture, the isolation success in skin biopsies and ticks, has recently been described (Angelakis *et al.*, 2012). Culture remains critical for strain analysis.

Genomic era, a comparison of whole genomes is more likely to become a comparative method in the future but for now can only be performed in specialized laboratories.

9. Pathology

The clinical presentation of rickettsial diseases can vary from mild to very severe. The case-fatality rate for highly virulent rickettsiae ranges from 2% to more than 30%

(Azad, 2007; Raoult and Maurin, 2002; Raoult and Roux, 1997a). Epidemic typhus *R. prowazekii* usually manifests after an incubation period of 10–14 days. Typhus group rickettsiae can cause severe pathological changes in an organism leading to ruthless disorders and if untreated, inevitably resulting in a fatal outcome (Bechah *et al.*, 2008b). Rash can be apparent only in half of the patients suffering from the murine typhus disease in contrast with epidemic typhus when patients suffer from purpuric rash over the course of the illness. An extensive multi system disease can develop; fever, cough, headache, diarrhoea, muscle pain, rash, local lymphadenopathy (in some cases) splenomegaly, conjunctivitis and myalgia (disseminated in later stages), affecting the disorders of brain functionality, lymphohistocytic vasculitis of the central nervous system, lung, liver, kidney, heart (endothelia), leading to diffuse alveolar damage and haemorrhage, interstitial pneumonia, pulmonary oedema, interstitial myocarditis and nephritis, portal triaditis, and cutaneous, mucosal, and serosal haemorrhages (Raoult *et al.*, 1986, 1997a, 1998b; Walker, 2007; Walker and Ismail, 2008). Nonspecific haematological and biochemical findings include thrombocytopenia, leucocyte count abnormalities and elevated hepatic enzyme levels (Brouqui *et al.*, 2004).

However, clinical signs vary depending on the rickettsial species involved. As for SFG rickettsiae, incubation time of 6–14 days after the bite of infected vector necessitates development of the disease. Typically, the clinical symptoms of SFG rickettsiae include a characteristic inoculation eschar ("tâche noire") at the site of the bite. The systemic infection leads to vasodilatation, which can be present in 60–80% of patients only. The rash can be (a) macular (in case of evolution of the disease into perivascular oedema), maculo-papular (in MSF). (b) Vesicular form can be seen in rickettsialpox or African tick-bite fever. (c) Profuse or composed of a few cutaneous elements as has been reported in a lymphangitis-associated type of rickettsiosis. Disruption of vascular integrity leads to (d) petechial rash for *R. rickettsii* infection. The rash can also be (e) nonspecific. New clinical signs and features have recently been described in rickettsioses such as: lymphangitis in *R. sibirica mongolitimonae* infection (LAR), cervical lymphadenopathy for *R. raoultii* and *R. slovaca* infection (SENLAT), and multiple eschars in MSF.

10. Treatment

The differences on the species level are not the only explanation for the disease outcome. Host associated factors, e.g. age, occupational bias, reduced access to health care facilities or antibiotic treatment are part of the building blocks determining the course of the illness. In vitro studies
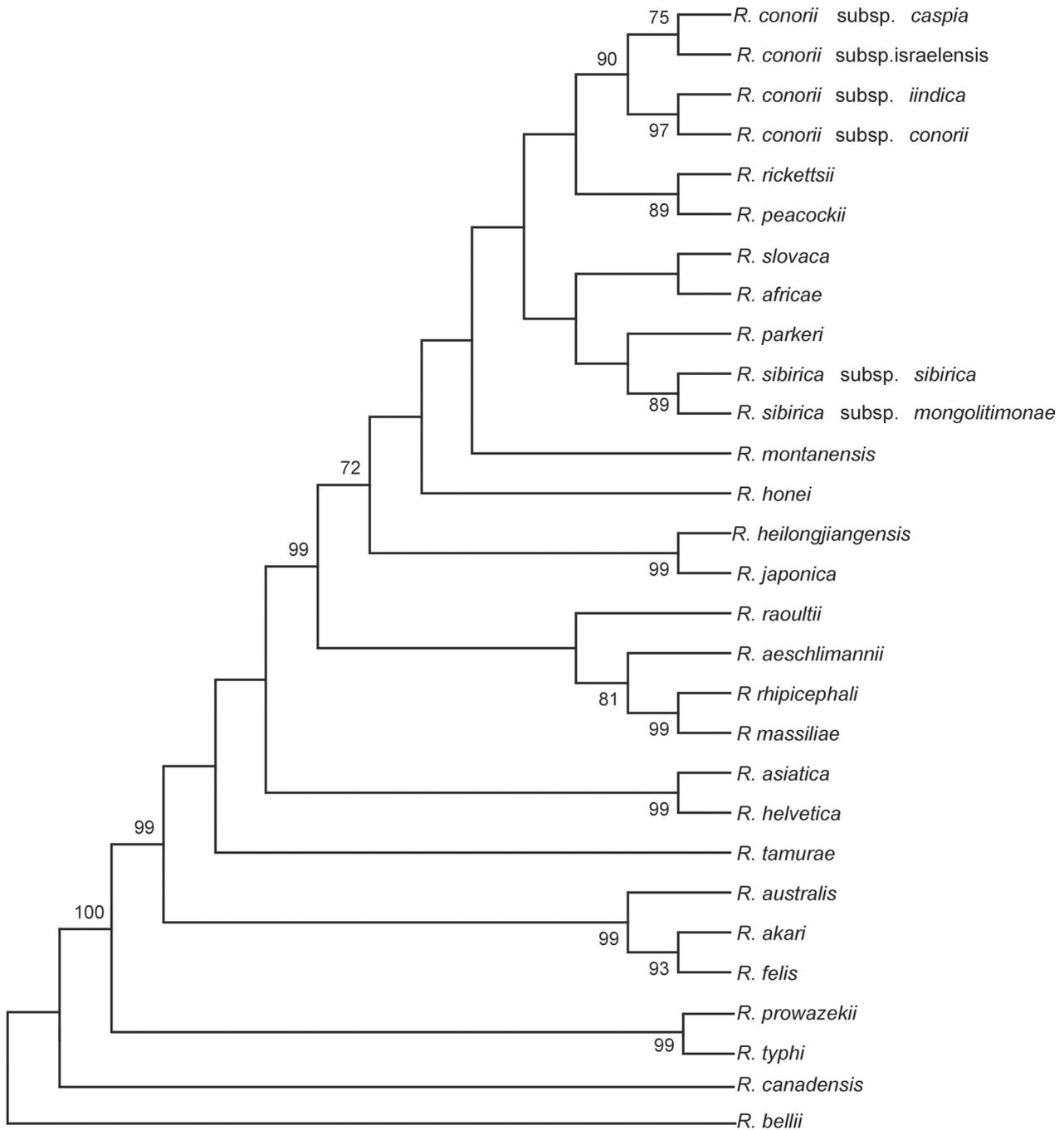
Fig. 5

Phylogenetic organization of rickettsia species and subspecies inferred from the comparison of sequences from the *sca1* gene using the maximum likelihood method Numbers at the nodes are boostrap values exceeding 70%

of antibiotic susceptibilities have been done in order to find the right treatment (Rolain *et al.*, 1998).

Doxycycline remains the first-line antibiotic therapy for patients with rickettsial diseases. The conventional antibiotic regimen for SFG rickettsioses is a 7–14-day oral course of doxycycline, 200 mg daily (Raoult *et al.*, 2002). In severely diseased patients, the intravenous route should be used first to administer doxycycline at 200 mg daily, and prolonged

duration, up to 3 days following apyrexia, should be considered (Brouqui *et al.*, 2007).

Doxycycline and azithromycin are safe and effective for the treatment of SENLAT. Starting early treatment is highly recommended (Ibarra *et al.*, 2005). Tetracyclin are the most effective antibiotic against *R. rickettsii* and *R. conorii*. However, they are contraindicated during pregnancy (Raoult. *et al.*, 2002). Short-course therapy with doxycycline (one dose of 200 mg) has been reported to be as effective for MSF as a 10-day course and is well tolerated, even in children (Bella-Cueto *et al.*, 1987; Lochary *et al.*, 1998). Indeed, only the long-term or repeated use of doxycycline remains contraindicated in children younger than 8 years of age because of tooth coloration; short course of treatment can therefore be used safely. Interestingly, Cascio et al., reported no more dental defects in children <8 years treated 3 weeks with minocycline compared to controls (Cascio *et al.*, 2004).

Josamycin may represent an alternative to tetracyclines in pregnant women, but clinical data is lacking. Fluoroquinolones, including pefloxacin, ofloxacin and ciprofloxacin, have been shown to be effective for the treatment of MSF (Raoult and Maurin, 1998) and a safe alternative to tetracycline. However, it was demonstrated in vitro that ciprofloxacin, although effective, induced a deleterious effect in cells infected with *R. conorii* due to the up-regulation of the toxin-antitoxin module *vapC-vapB* (Botelho-Nevers *et al.*, 2012). Recently, in a retrospective study, fluoroquinolone treatment increased significantly and independently MSF severity and was associated with a significantly longer hospital stay (Botelho-Nevers *et al.*, 2011). Chloramphenicol (administered for at least 1 week) has long been considered the main alternative for rickettsial infections, however, the potential risk of aplastic anaemia and acute haemolytic anaemia in patients with the Mediterranean form of G6PD deficiency, and its contrain-

Rickettsia name	Erythromycin	Rifampin
R. sibirica	R	S
R. mongolotimonae	R	S
R. parkeri	R	S
R. africae	R	S
R. Conorii complex	R	S
R. slovaca	R	S
R. raoultii	R	R
Thai-tick typhus rickettsia	R	S
R. honei	R	S
R. rickettsii	R	S
R. japonica	R	S
Bar 29	R	R
R. massiliae	R	R
R. rhipicephali	R	R
R. aeschlimanii	R	R
R. montana	R	R
R. helvetica	R	S
R. australis	R	S
R. acari	R	S
R. typhi	S	S
R. prowazekii	S	S
R. canada	R	S
R. bellii	R	S

Resistance to rifampin has been shown to occur also in: *Mycobacterium tuberculosis*, *Mycobacterium leprae*, *Escherichia coli*, and *Neisseria meningitis*

Mutations in the *rpoB* gene encoding the beta subunit of the RNA polymerase

High levels of susceptibility to macrolides

Fig. 6

Antibiotic sensitivity and resistance to rickettsiae

dication in pregnant women, reduced the possibility of its application ((Brouqui *et al.*, 2007)). Macrolide compounds may represent a safe alternative for children and pregnant women. However, sensitivity or resistance has to be taken into consideration with all antibiotics (Fig. 6). A genome comparison analysis of molecular mechanisms of resistance to antibiotics in the *Rickettsia* genus was studied (Rolain and Raoult, 2005) yet, this topic is still open. The mode of resistance is similar to other bacteria. There are certain antibiotics that are contraindicative for treatment. Co-trimoxazole is not only unhelpful for the treatment of rickettsioses, but may potentially worsen the outcome of the disease (Beltran and Herrero, 1992).

11. Rickettsiae in Slovakia

11.1 Arthropod vectors in Slovakia

Five genera (*Argas, Dermacentor, Haemaphysalis, Ixodes, Hyalomma*) and 21 species of soft and hard ticks have been recognized in fauna of Slovakia. *I. ricinus*, *D. marginatus*, *D. reticulatus*, *H. concinna*, *H. punctata*, and *H. inermis* are main exophillic tick species occurring in studied localities (Cerny, 1972;Rehacek *et al.*, 1975a, 1991). *I. ricinus* ticks are widely distributed throughout the whole country, whereas *D. reticulatus* and *D. marginatus* ticks are limited along rivers, places of steppe, forest steppe and the karst region, respectively. *D. marginatus* is very common in uncultivated pastures, from where it spreads on the fallow, bushy ridges between fields and field paths (Bullova *et al.*, 2009; Nosek, 1972). Its presence was determined along the rivers in the South-east (Latorica) as well as in the South-west (March, Danube). However at present, *D. reticulatus* has extended the range of its former habitats to higher altitude. It can be found at least 200km further north. *H. concinna* occurs in the swampy woods along the river March, in the hornbeam-oak forests of the Little Carpathians and in swampy woods in the area Bratislava-Dvorniky-Štúrovo. It is especially abundant in the forests between Bratislava and Gabčíkovo and in the oak woods and *Robinia pseudaccacia* plantations in the neighbourhood of Jarok-near-Nitra. *H. inermis* occurs on the Kováčské Hill, and in the west of the Štiavnické Mountains, where it lives in climatically favourable localities in the river-basins of Štiavnička and Hron (in the environs of Benadik). The distribution of *H. punctata* is approximately the same as that of *D. marginatus* (Nosek and Grulich, 1967).

Recently, the abundance and distribution of fleas (Siphonaptera) on small mammals (rodents and insectivores) in central and eastern Slovakia have been studied (Stanko *et al.*, 2006). Thirteen flea species were collected on 9 host species and the most abundant was *Ctenophthalmus agyrtes* Heller

on *Apodemus flavicollis* Melchior. In addition, infestation as well as parasitisation of humans with flea *Ctenocephalides felis,* cat fleas, was recorded in the basements of flat blocks in the central area of Košice (Peťko, 1993).

In 45 winter nests of the common mole (Kocianova and Kozuch, 1988) from the Záhorska lowland (western Slovakia), 7 flea species with the eudominant species *Ctenophthalmus assimilis* were found in them and the other species were subrecedent. The acarinium of the mole winter nests was composed of 19 mite species. Predominant were euryphagous species *Haemogamasus nidi, H. hirsutus, Eulaelaps stabulari*s, and *Androlaelaps fahrenholzi* (Kocianova *et al.*, 1988).

11.2 SFG rickettsiae detected and/or isolated in Slovakia

The study of rickettsiae has had a long tradition. They have been in the "crosshairs" scope of the Department of Rickettsiology at the Institute of Virology in Slovakia since 1956. Occurrence of rickettsiae has been continuously monitored and the presence of six rickettsial species has been confirmed, mostly in localities of the southern part of Slovakia (Fig. 7), including five species transmitted by ticks: *R. slovaca, R. raoultii, R. monacensis* strain IRS3 and IRS4, *R. helvetica* and *R. conorii conorii;* one rickettsial species usually transmitted by ticks *R. africae* but identified in fleas in Slovakia. In addition in 1975, rickettsiae of the SFG were also found in fleas and mites by smears method of detection of rickettsia in arthropods using the immunofluorescence technique and antibody response in mice inoculated with infected arthropods which was found to be the most effective in these studies (Rehacek *et al.*, 1975b).

11.2.1 Rickettsia slovaca

R. slovaca was the first species found to be common in Slovakia (Brezina *et al.*, 1969). The isolate originating from *D. marginatus* ticks was collected in central Slovakia in 1968. It was first classified as a species closely related to *R. sibirica* (indicated as "B" or "D") but ten years later it was identified as a new species (Urvolgyi and Brezina, 1978). Several studies were provided in the next 20 years after its initial discovery (Rehacek *et al.*, 1990). Ever since this species has been detected in many other countries, e.g. in Armenia (Rehacek *et al.*, 1977), Ukraine (Urvolgyi *et al.*, 1978), Yugoslavia (Manor *et al.*, 1992), Crimea (Balayeva *et al.*, 1993), France (Beati *et al.*, 1993a), Switzerland (Beati *et al.*, 1994), Portugal (Bacellar *et al.*, 1995) etc. there appear to be no boundaries to stop this agent (Tian *et al.*, 2012). In 1998, on the basis of the distinctive clinical, epidemiological, phenotypic features and genotypic features, *R. slovaca* was finally accepted as a separate taxonomic species (Sekeyova *et al.*, 1998). The prevalence of *R. slovaca* in Slovakia has been continuously monitored, confirmed in *D. marginatus*, and in other ticks (Rehacek *et al.*, 1976a; Spitalska and Kocianova,
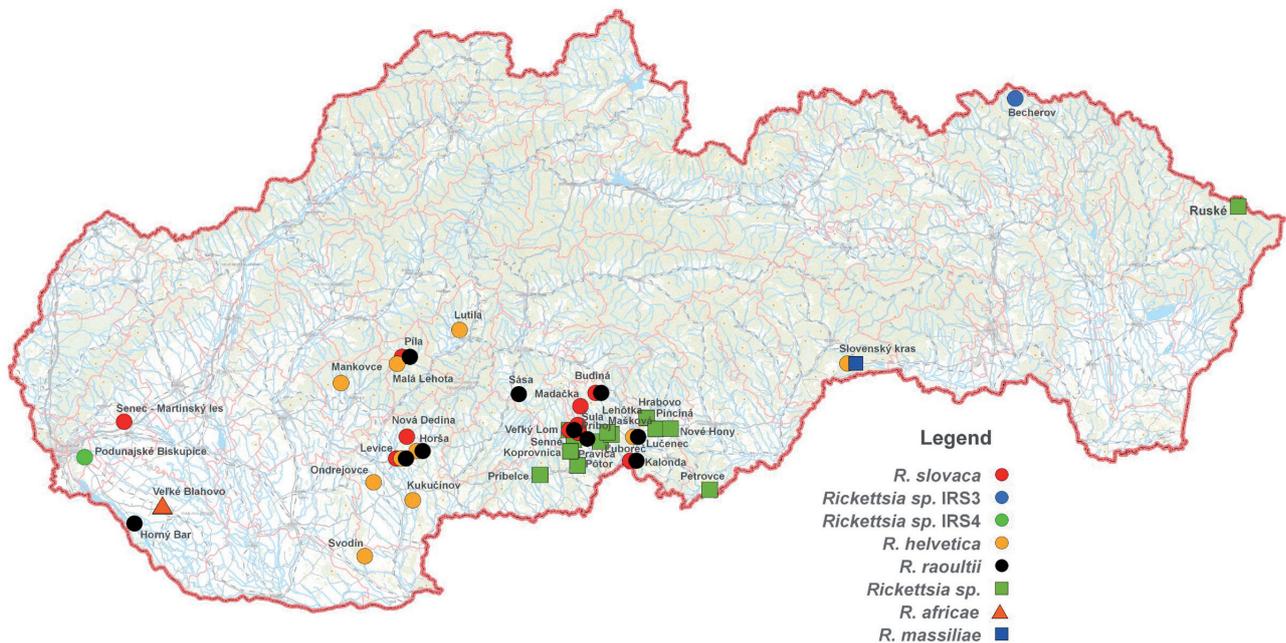
Fig. 7

Map of rickettsiae in Slovakia

2002; Spitalska *et al.*, 2012). The role of dogs in the circulation of *R. slovaca* has been defined (Kovacova *et al.*, 2006), and even contact with horses as a risk factor for dispersion of *R. slovaca* has recently been described (Lakos *et al.*, 2012).

R. slovaca is a human pathogen. A variety of names have been attributed to the disease it causes e.g. scalp eschar and neck lymphadenopathy after tick bites (SENLAT) (Angelakis *et al.*, 2010), tick-borne lymphadenopathy (TIBOLA) (Lakos, 1997, 2002; Lakos *et al.*, 2012), or Dermacentor-borne necrosis erythema and lymphadenopathy (DEBONEL) (Ibarra *et al.*, 2006; Oteo *et al.*, 2004; Raoult *et al.*, 1997a; Selmi *et al.*, 2008). This illness commonly occurs in Slovakia (Sekeyova *et al.*, 2012c).

A study of this bacterium is continuously being carried out. Boldiš *et al.* (2008) provided a detailed picture of the infection cycle of *R. slovaca* including ultrastructural changes within host cel. PCR-RFLP assay based on enzymatic digestion of a623 bp of *sca4* gene with *HaeIII* was suggested as one of the best tools for fast differentiation of *R. slovaca* from other rickettsiae (Spitalska *et al.*, 2008). Growth kinetics of *R. slovaca* in static (L929 and Vero cells) and dynamic (*D. marginatus* and *I. ricinus* ticks) cultivation systems have helped us better characterize the biological properties of this species (Boldis and Spitalska, 2010).

11.2.2 Rickettsia raoultii

Rickettsia sp. genotypes DnS14, DnS28, and RpA4, were first identified as new subspecies of the *R. massiliae* genogroup in 1999 (Roux *et al.*, 1995). A few years later, a DNA gene sequenc-

ing of *gltA* and *ompA* genes extracted from *D. nutallii* ticks collected in Siberia, and *Rh. pumilio* ticks collected in Astrakhan, helped to confirm *R. raoultii* as a new species (Rydkina *et al.*, 1999). Since then, it has been detected in *Dermacentor* ticks throughout Europe, the European part of Russia (Shpynov *et al.*, 2001), Germany (Dautel *et al.*, 2006), Portugal (Vitorino *et al.*, 2007b), the Netherlands (Nijhof *et al.*, 2007), France, Croatia (Mediannikov *et al.*, 2008), Poland (Chmielewski *et al.*, 2009), and Spain (Marquez, 2008). In Mongolia it was detected in *D. nuttalli* ticks (Speck *et al.*, 2012). *Rickettsia raoultii*-like bacteria was found in *Dermacentor* spp. ticks in Tibet, China (Wang *et al.*, 2012). The most recent isolate of its kind is available from Dutch *D. reticulates* ticks (Alberdi *et al.*, 2012). A human case was recently reported in Poland (Switaj *et al.*, 2012).

R. raoultii is commonly found in *D. marginatus* and *D. reticulatus* ticks collected in Slovakia, of which molecular evidence was provided (Boldis *et al.*, 2008). The prevalence of *R. raoultii* was found to be from 9% to 22–27% (Spitalska *et al.*, 2012), and an occurrence of the disease triggered by this bacteria confirmed (Sekeyova *et al.*, 2012c). Infection with this agent appears to be less pathogenic in humans than with *R. slovaca* (Parola et al. 2009). Interestingly, this rickettsia has been proved to be resistant to rifampicin (Mediannikov *et al.*, 2008).

11.2.3 Rickettsia monacensis

In 2000, two rickettsial strains: *Rickettsia* sp. "IRS3" and "IRS4" were detected in *I. ricinus* ticks collected in north eastern and south western Slovakia (Sekeyova *et al.*, 2000,

2003). Sequences of the 16S rRNA, citrate synthase (*gltA*) and outer membrane protein *rOmpA* encoding genes of both strains were shown to be nearly identical but distinct from those of all other known rickettsiae. Phylogenetic analysis inferred from *gltA* and 16S rDNA sequence comparisons showed that this rickettsial genotype is most closely related to *R. helvetica*. This rickettsia can be commonly found in *I. ricinus* ticks, in vegetation of Slovakia, as in other European countries, e.g. in Italy (Sanogo *et al.*, 2003), Spain (Fernandez-Soto *et al.*, 2004), Bulgaria (Christova *et al.*, 2003), Serbia (Tomanovic *et al.*, 2012). In 2002, it was isolated, characterized and named *R. monacensis* in Germany (Simser *et al.*, 2002). It was confirmed to be frequently occurring in Germany (Schicht *et al.*, 2012) and Switzerland (Lommano *et al.*, 2012)in co-infections with other rickettsial species. It was found in ticks removed from humans in Turkey (Gargili *et al.*, 2012), or those collected from domestic dogs in the Ukraine (Hamel *et al.*, 2012). It was find not only in Europe, but also in China (Fernandez-Soto *et al.*, 2004;Huang *et al.*, 2006) and North Africa (Jado *et al.*, 2007).

R. monacensis is a human pathogen, detected as a cause of Mediterranean spotted fever-like illness in Italy (Madeddu *et al.*, 2012) and Spain (Jado *et al.*, 2007). In addition to fever and flu-like symptoms, the inoculation eschar was identified only in an Italian patient (left calf) but generalized rash, including palms and soles, only in both Spanish patients. The patients recovered without sequelae with doxycycline treatment.

11.2.4 *Rickettsia helvetica*

R. helvetica was first isolated from *I. ricinus* ticks in Switzerland (Burgdorfer *et al.*, 1979). It has been generally accepted that *I. ricinus* is the main vector of *R. helvetica*. However, *D. reticulatus* ticks were found to be infected with *R. helvetica* in Croatia (Dobec *et al.*, 2009). Serosurveys have found antibodies reactive to *R. helvetica* in 1.9%–12.5% of the population and PCR positive cases were confirmed in France (Davoust *et al.*, 2012), Italy (Corrain *et al.*, 2012), Austria (Sonnleitner *et al.*, 2012), Poland (Chmielewski *et al.*, 2011) and Sweden (Nilsson *et al.*, 2010). *R. helvetica* was also detected in Africa and Asia (Parola *et al.*, 2005).

In Slovakia, *R. helvetica* was identified by molecular biology in *I. ricinus* ticks [adults captured from vegetation (Spitalska *et al.*, 2008), collected from free-living green lizards (Vaclav *et al.*, 2011), or nymphs picked from *P. modularis* birds (Spitalska *et al.*, 2011)], and in roe deer (Stefanidesova *et al.*, 2008). Human cases were confirmed (Sekeyova *et al.*, 2012c), and the first isolation of *R. helvetica* yield from ticks collected in Slovakia has recently been provided (Sekeyova *et al.*, 2012b). No human strain of *R. helvetica* exists worldwide.

11.2.5 *Rickettsia conorii conorii*

R. conorii conorii is responsible for the MSF, also known under various other names, as Boutonneuse fever. The disease is transmitted to humans by brown dog ticks *R. sanguineus* (Raoult *et al.*, 1997a). MSF is widely distributed throughout the Mediterranean basin and the Middle East (Gilot *et al.*, 1990; Raoult *et al.*, 1986). *R. sanguineus* ticks rarely feed on people unless its preferred host (the domestic dog) is not available.

This kind of tick is not domestic in Slovakia however; the existence of sporadic intrusion of this tick into south parts however cannot be excluded. The presence of *R. conorii conorii* in Slovakia was confirmed by serological examination, in dogs (3.1 %) and in humans (2.9 %) (Kovacova *et al.*, 2006). However, molecular evidence of the occurrence of *R. conorii conorii* in Slovakia is not available.

11.2.6 *Rickettsia africae*

R. africae, the etiologic agent of African tick bite fever (ATBF), was discovered twice: the first time isolated in 1930 by Pijper but unfortunately this isolate was lost and the second time in 1992 by the Raoult team from the blood of a 36-year-old Zimbabwean woman (Kelly *et al.*, 1992a). However, the clinical signs of ATBF were described in 1911 in Mozambique and South Africa (McNaught, 1911). Numerous cases have been described recently, mainly in international travellers (Kernif *et al.*, 2012; Socolovschi *et al.*, 2012d). An epidemiologic success of the dispersion of this disease is due to various factors, including the increase of tourism, mode of attack (multiple at once), host-seeking behavior of its vector ticks, *Amblyomma* spp., and the elevated prevalence of *R. africae* in these ticks, with infection rates of up to 100% (Parola *et al.*, 2001). This bacterium has been frequently identified in areas with warm climates, such as the West Indies, where it was found in Guadeloupe, Martinique, St Kitts and Nevis, and the Antigua islands (Parola *et al.*, 2005). Cases in Europe are only occasionally introduced, sporadically described, mostly detected in travellers returning from Africa. No human case has ever been recorded in Slovakia however; *R. africae* was detected in *Ceratophyllus garei* flea collected from Reed warbler (*Acrocephalus scirpaceus*), a migrating bird captured upon arrival from Africa (Sekeyova *et al.*, 2012a).

11.2.7 *Rickettsia spp.*

Unspecified types of *Rickettsia* spp. were found in nymph from the passerine bird, European robin *Erithacus rubecula*, ticks recovered from humans, or dogs, in the Carpathian region (Spitalska *et al.*, 2006).

12. Conclusion

In recent decades, the identification and description of rickettsial species have increased dramatically from various arthropod species worldwide driven largely by the use of

molecular techniques and cell culture. Some rickettsial species that were considered non-pathogenic for decades are now associated with infection of humans, such as *R. slovaca* isolated in 1968 in Slovakia (Brezina *et al.*, 1969) and recognized as human a pathogen only in 1997 in France (Brezina *et al.*, 1969) or *R. monacensis* detected in ticks in 2000 in Slovakia (Sekeyova *et al.*, 2000) and recognized as a human pathogen in 2007 in Spain (Jado *et al.*, 2007; Raoult *et al.*, 1998b). Novel *Rickettsia* species of undetermined pathogenicity continue to be detected in or isolated around the world such as recently isolated *Rickettsia* sp. AvBat from *Argas vespertilionis*, bat ticks in Southern France (Socolovschi *et al.*, 2012a). In addition, new discoveries such as the detection of rickettsial agents in mosquito vectors (Socolovschi *et al.*, 2012b,c); the high level of rickettsial etiology in febrile patients in rural sub-Saharan Africa, Afganistan, or Nepal (Maina *et al.*, 2012; Pradhan *et al.*, 2012; Bailey *et al.*, 2011); the possibility of the plant-mediated transmission route of endosymbiotic *Rickettsia* (Caspi-Fluger *et al.*, 2012) open new issues in the rickettsial field. In the case of rickettsial projects in Slovakia, preparation and prevention are the main tasks for future. We would like to test other arthopod species and to follow up on human rickettsioses in Slovakia. The potential for bioterrorism through the deliberate release of an infectious agent in an area not previously affected adds a further dimension to the emergence of infectious disease and its control. Multidisciplinary research activities with focused goals that span over to international constellations of scientists are a reliable indication and warranty of bio safety prevention in Slovakia and Europe.

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