EKOLOGIJA. 2006. Nr. 2. P. 44–50

Evidence and molecular analysis of *Rickettsia helvetica* and novel α-proteobacterium from the tick *Ixodes ricinus* in Poland

Anna Rymaszewska,

Bogumiła Skotarczak

Department of Genetics,
University of Szczecin, 71-065 Szczecin,
al. Piastow 40B, Poland
E-mail: ankas@univ.szczecin.pl

The ticks *Ixodes ricinus* collected from boreliosis-diagnosed dogs in Western Pomerania (Poland) were examined for pathogens of the genera *Anaplasma* and *Rickettsia* and for a possible presence of bacteria of the genus *Ehrlichia*. The bacteria were identified with PCR and sequencing of the following gene fragments: 16S rRNA, *gltA* and *msp2*. In addition, based on the results obtained, phylogenetic analysis for a number of *Rickettsia* species was carried out. The presence of *A. phagocytophilum* DNA was detected in 20% of isolates; 40% of isolates were found to contain DNA of an SFG rickettsia (*R. helvetica*), including a single case of co–infection with *A. phagocytophilum.* Moreover, 80% of the samples showed the presence of non-identified *Rickettsiales bacterium* symbiotic with *I. ricinus.* PCR products were sequenced and the sequence was submitted to GenBank (accession number: DQ105665 for Rickettsiales bacterium, and DQ105664 for *R. helvetica*) as the first Polish *Rickettsia* species. The Polish sequence of the *Rickettsiales bacterium* 16S rRNA gene was identical with the sequences from Italy and Denmark. The Polish sequence of the *Rickettsiales helvetica gltA* gene was identical with the sequences from Italy (a single sequence) and Switzerland, but differed from the sequences from the USA and Morocco, and Italy (two sequences), which made it possible to distinguish in the gene five haplotypes for *R. helvetica.* The Polish haplotype proved identical with that of *R. helvetica* from Italy and Switzerland. A phylogenetic analysis for some *Rickettsia* species, including two from Western Pomerania, was performed. Fragments of 16S rRNA (*Rickettsiales bacterium*) and *gltA* (*R. helvetica*) genes were used in phylogenetic analysis of the two *Rickettsia* species. The *R. bacterium* sequence from Poland, together with the Danish sequence, was grouped on a phylogenetic tree branch separate from that grouping the pathogenic *Rickettsia* species. The sequences of *R. helvetica* from Poland, Italy, and Switzerland form a joint clade.

Key words: *Rickettsia helvetica,* symbiotic rickettsiae, *Ixodes* ticks, phylogenetic analysis

INTRODUCTION

Rickettsiae are gram-negative bacteria which are subdivided into three groups: the spotted fever group (SFG), with almost 20 *Rickettsia* species, including *Rickettsia helvetica, R. sibirica, R. conorii, R. slovaca,* and *R. akari,* all occurring in Europe; the typhus group (TG) with two species: *R. typhi* and *R. prowazekii*; and the scrub typhus group (STG), represented by *R. tsutsugamushi.* The STG pathogens are particularly important in the Asia–Pacific region where they are endemic and where they may be a cause of death rate as high as 35% (Lee et al., 2003).

The SFG rickettsiae are present in Europe; they have been detected in patients suffering of tick-transmitted diseases. Swedish authors (Nilsson et al., 1999b) concluded that rickettsiae may be an important pathogen in the aetiology of perimyocarditis, which can result in sudden unexpected cardiac death in young people (Nilsson et al., 1999b).

Anaplasma phagocytophilum is an etiological factor of human granulocytic anaplasmosis (HGA), the first European case of which was reported in 1997 (Petrovec et al., 1997). Similarly to the SFG rickettsiae, the European vectors of *A. phagocytophilum* are ticks of the genus *Ixodes,* primarily *I. ricinus.* The ticks carry a number of other pathogens, e.g., *Borrelia burgdorferi* sensu lato, *A. phagocytophilum,* and *Francisella tularensis* (Wicki et al., 2000). In addition to pathogens, *I. ricinus* were found to support bacteria of unknown eco-

logy and pathogenicity, e.g., an *Ehrlichia*-like organism or *"candidatus Ehrlichia walkerii"* (Alekseev et al., 2001, Broqui et al., 2003; Schouls et al., 1999). The present study was aimed at detecting the presence of *Anaplasma* and *Rickettsia* pathogens in *I. ricinus* collected from dogs showing clinical symptoms of boreliosis as well determining whether the ticks support species related to monocytic *Ehrlichia.*

MATERIALS AND METHODS

Materials. The study involved DNA isolated from 10 ticks collected from clinical boreliosis-diagnosed dogs of various breeds, treated by the Veterinary Clinic in Szczecin (Western Pomerania, Poland). In addition, all the dogs were examined for the presence of tick-transmitted pathogens such as *Borrelia burgdorferi* sensu lato, *A. phagocytophilum,* and *Rickettsia* sp. The analytical methods and data on dog infestation were described by Wodecka et al. (in press).

PCR, sequencing, phylogenetic analysis. The tick DNA was isolated using the MasterPure™ DNA Purification Kit (Epicentre, Madison, WI, USA) and kept at –70 ºC until analysed. The presence of *A. phagocytophilum* was detected with a 334 bp-long fragment of *p44* (msp2) gene, marked by msp2-3F and msp2-3R primers (Levin et al., 2002), and with a fragment of the 16S rRNA gene marked with 16SEHR-D and 16SEHR-R primers (Parola and Raoult, 2001). The 16SEHR-D and 16SEHR–R primers are known to amplify all the known *Ehrlichiae* species and some ana-plasmas (Parola and Raoult, 2001; Broqui et al., 2003). To detect the presence of *Rickettsia* DNA, the *gltA* gene-specific RpCS877 and RpCS1258 primers were used (Nilsson et al., 1999a). PCR was performed under conditions described by the authors whose primer sequences were utilized (Levin et al., 2002; Broqui et al., 2003). All the samples were sequenced for each gene, amplification being achieved by a set of primers identical to that used in the initial PCR, and the results were compared to corresponding sequences deposited at GenBank. Sequencing was performed at the Institute of Biochemistry and Biophysics, Polish Academy of Sciences (Warsaw, Poland). The multiple alignment analysis and distance matrix calculation

Table 1. **Nucleotide sequences used in this study**

Accesion number	Country	Source	Pathogen species	References
		16S rRNA		
DQ105665	Poland/ Western Pomerania	*Ixodes ricinus*	Uncultured *Rickettsia*	Rymaszewska, unpulished
D38628			*R. sibirica*	Ohashi et al., 1995
L36218	Former USSR	*Dermacentor nuttali*	*R. sibirica*	Roux & Raoult, 1995
L36212	Switzerland	*Ixodes ricinus*	*R. helvetica*	Roux & Raoult, 1995
L36221	USA	human	*R. typhi*	Roux & Raoult, 1995
U12463		*Pulex irritans*	*R. typhi*	Stothard & Fuerst, 1995
L36222	Japan	human	*R. tsutsugamushi*	Roux & Raoult, 1995
U25042	Armenia	*Rhipicephalus sanguineus*	*Rickettsia* sp.	Eremeeva et al., 1995
AY953290	USA	*Orchopeas howardi*	*Rickettsia* sp.	Reeves et al., unpulished
AY776167	Denmark	Roe deer	*Rickettsiales bacterium*	Skarphedinsson et al., 2005
AY02521	USA	human	*A. phagocytophilum*	Chen et al., 1994
		gltA		
DQ105664	Poland/ Western Pomerania	*Ixodes ricinus*	*R. helvetica*	Rymaszewska, unpulished
AJ427879	Italy	*Ixodes ricinus*	*Rickettsia* sp.	Beninanti et al., 2002
AJ427880	Italy	*Ixodes ricinus*	*Rickettsia* sp.	Beninanti et al., 2002
AY953289	USA	*Ctenocephalides felis*	*Rickettsia* sp.	Reeves et al., unpulished
U59722	Marocco	*Hyaloma marginatum*	*Rickettsia* sp. (*R. aeschlimanni)*	Raoult et al., 1997
U59723	Switzerland	*Ixodes ricinus*	*R. helvetica*	Raoult et al., 1997
AJ427878	Italy	*Ixodes ricinus*	*R. helvetica*	Beninanti t al., 2002
U59725	Slovakia	*Dermacentor marginatus*	*R. slovaca*	Raoult et al., 1997
U59734	Former USSR	*Dermacentor nuttali*	*R. sibirica*	Raoult et al., 1997
U59714	USA	human	*R. typhi*	Raoult et al., 1997

were performed, and the phylogenetic tree was constructed with the DNAMAN software (Lynnon BioSoft, 1994–2001, Canada). Distance matrices for the aligned sequences were calculated by the Kimura two-parameter method (Kimura, 1980), the maximum likelihood method being used to construct the phylogenetic tree.

Nucleotide sequence accession numbers used in the study. The list of the species used in the comparative analyses is given in Table 1.

Accession numbers of nucleotide sequences from Poland. The DNA sequences obtained from Western Pomerania (Poland) were deposited at GenBank under accession numbers DQ105665 (16S rRNA) and DQ105664 (*gltA*). In the tables and figures, they are denoted PZ (*Rickettsiales bacterium*) and PZ–S (*R. helvetica*), respectively.

RESULTS

Two of the ten *I. ricinus* isolates collected from dogs proved msp2–positive (samples 1, 2), thus establishing the presence of *A. phagocytophilum* DNA; eight isolates yielded a product with 16S rRNA-specific primers; and four samples revealed a product with RpCS877 and RpCS1258 primers, complementary to the citrate syntase gene fragment (*gltA*). Detailed data are shown in Table 2.

Table 2. **Occurrence of DNA of bacteria detected in the tick *Ixodes ricinus***

Gen \ Tick number	1	2	3	4	5	6	7	8	9	10
msp2	+	+	-	-	-	-	-	-	-	-
16S rRNA	+	+	-	+	+	+	+	+	+	-
gltA	+	-	-	-	+	-	+	-	+	-

According to Massung et al. (2003), the target gene marked with msp2–3F and msp2–3R primers amplifies only *A. phagocytophilum* DNA. Hence, after the preliminary analysis of data obtained for that gene, PCR with 16SEHR–D and 16SEHR–R primers was expected to yield DNA sequences characteristic of *A. phagocytophilum* in samples 1 and 2, DNA of other anaplasmas or *Ehrlichiae* species being expected in samples 4, 5, 6, 7, 8, and 9 (Table 2). Analysis of the sequences produced by the samples revealed a tick infestation pattern that differed from expectations. Sequencing of a fragment of 16S rRNA gene in 80% of the *I. ricinus* isolate samples (1, 2, 4, 5, 6, 7, 8, 9) yielded products that were identical (100% similarity) to the sequence of uncultured *R. bacterium* (AY776167), the similarity to other rickettsiae species ranging from 87.3% for *R. helvetica* (L36212) to 84.3% for *R. tsutsugamushi* (L36222) (Table 3). Due to a slight similarity to the Japanese *R. tsutsugamushi* (L36222) of TG, the latter was placed on a separate branch of the phylogenetic tree (Fig. 1), as opposed to the remaining groups of pathogenic rickettsiae, which is consistent with differences in the evolutionary history of pathogenic *Rickettsia* on the continent and on islands.

Moreover, analysis of the *glt*A gene fragment showed the presence of SGF rickettsiae in 40% of the samples (1, 5, 7, 9), co–infection with *A. phagocytophilum* being detected in one isolate (sample 1) (Table 2). The homology matrix shows the Western Pomeranian sequence, denoted PZ–S, to be 100% similar to the Italian *R. helvetica* sequence (AJ 427878) and to be 99.7% similar to the Switzerland sequence U59723. Similarity to other sequences of SFG rickettsiae and to *R. typhi* of TG was 95 and 91%, respectively. The sequence fragment similarity allows to assign the sequence to *R. helvetica* (Table 4). To confirm this, it is planned to analyse a larger number of ticks and to include other genes into the analysis.

Comparison of analogous *gltA* DNA sequence fragments of the bacteria assigned, in GenBank, to *Rickettsia* sp. and *R. helvetica* produced 26 polymorphic loci; most of them were transitions. This served to distinguish 5 haplotypes (Table 5). The Polish haplotype, denoted 1, proved identical with the Italian (AJ427878) and Switzerland (U59723) haplotypes, both sequences being classified in GenBank as *R. helvetica*. Haplotype 1 and the *Rickettsia* sp.–specific haplotypes 2, 3, 4,

Table 3. **Levels of similarity (%) among 16S rRNA gene sequences *Rickettsia* (Kimura, 1980), PZ-uncultured *Rickettsiales bacterium* from Poland (materials sequenced by the present authors, GenBank accession number DQ105665)**

	PZ	AY776167	AY953290	D38628	L36212	L36218	L36221	L36222	U12463	U25042	U02521
PZ	100.0										
AY776167	100.0	100.0									
AY953290	87.7	86.1	100.0								
D38628	87.3	85.8	99.5	100.0							
L36212	87.3	85.8	98.9	99.0	100.0						
L36218	87.3	85.8	99.5	100.0	99.0	100.0					
L36221	87.3	85.8	98.3	98.4	98.3	98.4	100.0				
L36222	84.3	83.5	90.2	90.5	90.1	90.5	90.5	100.0			
U12463	87.3	85.8	98.3	98.4	98.2	98.3	99.9	90.4	100.0		
U25042	87.7	86.1	99.6	99.1	99.1	99.9	98.4	90.4	98.4	100.0	
U02521	84.9	85.3	83.5	83.8	83.5	83.9	83.8	83.4	83.6	83.8	100.0

Table 4. **Levels of similarity (%) among *gltA* sequences *Rickettsia* (Kimura, 1980), PZ-S – *R. helvetica* from Poland (materials sequenced by the present authors, GenBank accession number DQ105664)**

	U59723	U59725	U59734	U59414	AJ427878	AJ427879	AJ427880	AJ427889	U59722	PZ-S
U59723	100.0									
U59725	96.8	100.0								
U59734	96.9	99.8	100.0							
U59414	92.0	92.9	92.7	100.0						
AJ427878	100.0	95.0	95.3	91.2	100.0					
AJ427879	95.3	96.2	96.5	92.7	95.3	100.0				
AJ427880	95.6	95.9	96.2	92.4	95.6	99.7	100.0			
AJ427889	95.2	95.2	95.4	91.1	95.6	95.6	95.3	100.0		
U59722	96.8	99.3	99.4	92.8	95.3	96.5	96.2	95.4	100.0	
PZ-S	99.7	95.1	95.3	91.8	100.0	95.3	95.6	95.8	95.3	100.0

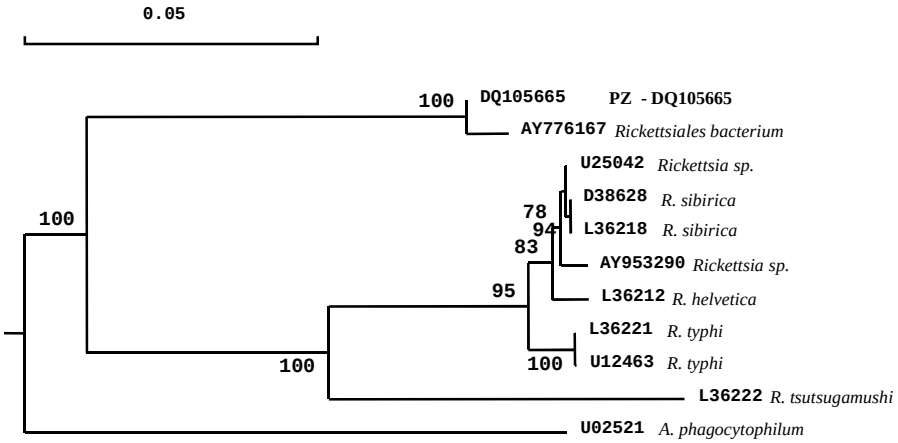

Fig. 1. Phylogenetic tree of rickettsiae based on 16S rRNA gene (*Rickettsiales bacterium,* PZ, from Poland and various *Rickettsia* spp.); the scale bar = 5% difference in nucleotide sequences; the maximum likelihood method was used to construct the phylogenetic tree by using the DNAMAN program (bootstrap 1000)

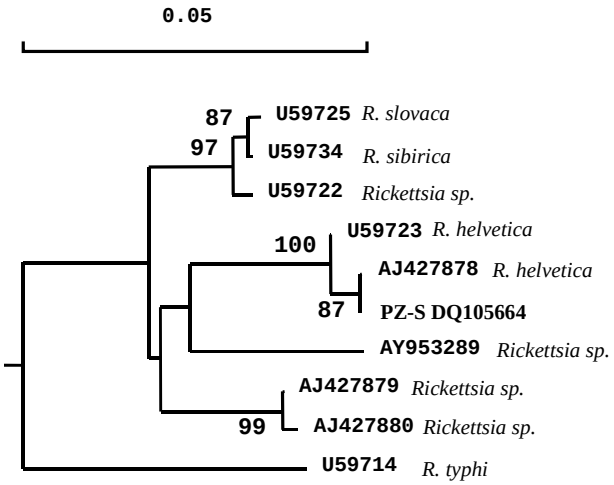

Fig. 2. Phylogenetic tree of rickettsiae based on *gltA* gene (*R. helvetica,* PZ-S, from Poland and various *Rickettsia* spp.); scale bar = 5% difference in nucleotide sequences; the maximum likelihood method was used to construct the phylogenetic tree by using the DNAMAN program (bootstrap 1000)

and 5 were found to differ. However, a detailed protein analysis of the gene fragment showed the polymorphism to concern DNA only, as most of the changes were neutral and did not affect the protein amino acid sequence.

On the basis of 16S rRNA and *gltA* gene fragments, phylogenetic analyses were performed for some rickettsia species, including two from Western Pomeranian (Figs. 1, 2). The 16S rRNA gene-based analysis involved an analogous *A. phagocytophilum* DNA sequence fragment (U02521). Higher bootstrap values were obtained in the nucleotides of 16S rRNA-based trees for the relationships between the Polish sequences and the Danish sequence (AY776167) detected in roe deer (bootstrap value: 1 000), and also between these and other *Rickettsia* species (Fig. 1). The *A. phagocytophilum* 16S rRNA model sequence (U02521), as an external group, forms a separate branch.

The topologies of the *gltA*-based phylogenetic trees demonstrate a high degree of similarity between the Polish rickettsiae and pathogenic populations of *R. helvetica* from Italy and Switzerland (Fig. 2). Bootstrap analysis of the data was performed to measure the reliability of the branching order proposed by each analysis. The values for the nodes were higher than 88% for *R. helvetica* of SFG, which confirms the accuracy of the analysis.

DISCUSSION

Phylogenetic analyses are frequently based on the conservative 16S rRNA gene. The relatively low variability of the gene within a genus, and even within a family, makes it possible – with appropriate sets of

Table 5. **Comparison of *gltA* gene fragments of *R. helvetica* from Poland (PZ-S) with sequences of *R. helvetica* and *Rickettsia* sp. deposited in GenBank (nucleotides numbered with respect to 1234 bp-long from sequences U59723)**

	807	832	845	850	889	898	901	904	907	931	934	937	961	964	967	982	987	988	992	997	1000	1003	1006	1009	1012	1099	HAPLOTYP
U59723	C	T	A	C	G	A	A	C	C	C	T	A	T	T	G	A	G	A	C	A	C	C	G	C	A	G	1
AJ427878	C	T	A	C	G	A	A	C	C	C	T	A	T	T	G	A	G	A	C	A	C	C	G	C	A	G	1
AJ427879	C	C	A	C	G	A	G	T	T	T	C	G	G	C	A	A	A	A	T	A	T	T	A	T	A	A	2
AJ427880	C	C	A	C	G	A	G	T	C	T	C	G	G	C	A	A	A	A	T	A	T	T	A	T	A	A	3
AJ953289	C	C	A	T	A	G	G	C	T	C	T	C	G	C	A	G	G	A	C	A	C	C	A	C	C	A	4
U59722	T	C	T	T	A	A	G	T	T	T	C	G	G	C	A	A	G	G	C	C	C	C	G	C	A	G	5
PZ-S	C	T	A	C	G	A	A	C	C	C	T	A	T	T	G	A	G	A	C	A	C	C	G	C	A	G	1

primers – to simultaneously detect a number of species that can be identified correctly after a subsequent sequencing. This property has been used by a number of authors, because it allows simultaneous detection of various closely related bacteria; occasionally, unknown bacteria are detected in this way as well. When analyzing the 16S rRNA gene, Schouls et al. (1999) detected, in the ticks *I. ricinus* collected in the Netherlands, *Ehrlichia*-like species known in the literature as the *Shotti variant* (AF104680). The bacterium, a close relative of monocytic species, forms a new group within the genus *Ehrlichia* and is classified with the family Ana-plasmataceae. Broqui et al. (2003), too, found *I. ricinus* to harbor bacteria of the genus *Ehrlichia* whose pathogenicity is unknown. As demonstrated by the analyses of rRNA and *gltA* genes, the *candidatus Ehrlichia walkeri*, a new microorganism from northern Italy, although far removed from the monocytic ehrlichias, belongs to the same clade (Broqui et al., 2003). In Japan, analysis of DNA isolated from *I. ovatus* with a set of primers identical to that used in this study established the presence of a monocytic *Ehrlichia* species of 98.6% similarity to *E. chaffeensis* and *E. muris.* Hence, this study was expected to yield similar results.

So far, no monocytic *Ehrlichia*, or other anaplasm species, has been detected in Poland, although tick-transmitted parasites such as *B. burgdorferi*, *A. phaghocytophilum*, and *Babesia* sp. have been reported. (Skotarczak et al., 2003). No endemic rickettsiosis centres have been uncovered in Poland, either. Although *R. helvetica* DNA was occasionally found in the ticks *Ixodes ricinus* and *Dermacentor reticulatus* in the northern part of the country (Stańczak, 2004a; Stańczak et al., 2004b), no information exists on human or animal infestation incidences.

Bacteria that reside within the cells of arthropods are common in nature. Many of these bacteria have been shown to be parasites, while others have been shown to have an obligate, mutualistic relationship with their hosts, for example *Rickettsia* sp. or *Wolbachia* sp. (Beninati et al., 2004; Harlet et al., 2004; Noda et al., 1997).

This paper presents results of DNA identification using primers of the 16S rRNA gene of a wide spectrum of applications. The aim was to detect the tick-transmitted bacteria. However, the sequence analysis detected, in the Polish *I. ricinus* individuals, the presence of DNA of bacteria which have not been identified so far. *Ricketsiales bacterium* is an unidentified bacterium whose biology, ecology, and genetic structure have not been completely elucidated so far. A sequence identical to the Polish one was deposited at GenBank (AY776167) in 2004, originating from a rickettsia found in deer in Denmark (Skarphedinsson et al., 2005). To identify the Polish bacteria, similarity between them and the *Rickettsia* bacteria was analysed using the DNA sequences available at GenBank. The homology between the fragments (345pz) of those sequences and the sequences of STG-*Rickettsia* and TG-*Rickettsia* was found to range within 84–87% (Table 3). The 16S rRNA gene fragment-based phyloge-

netic tree features the rickettsiae from Poland (Western Pomerania) and Denmark forming a joint clade that can be grouped separately from pathogenic species (Fig. 1). The 80% similarity between the Polish and Danish sequences to the SGF and TG species suggests that the bacteria found are not pathogenic to humans and other mammals, but are probably symbiotic with the *Ixodes* ticks. This suggestion is supported by the similarity between the sequences from Pomerania (Poland) to DNA sequences of the bacteria detected in *I. ricinus* in Italy (AJ566640). The only difference between the sequence fragments concerned is seen at base 306 (743 with respect to the full gene sequence) where a T-to-A base transversion occurred (data not shown). According to Beninati et al. (2004), the bacteria in question live in the cytoplasm and mitochondria of ovarian cells and are tick symbionts. The authors quoted suggest the bacteria to be widely distributed within the European *I. ricinus* population. Transmission of the endosymbiotic organism is transstadial and transovarial, and is 100% successful; most likely the males lose the bacteria as they develop. However, neither the ecology nor genetics of the bacteria are fully known.

The *Ixodes* ticks, similarly to, e.g., gonads of *Culex pipens*, were found to support endosymbiotic bacteria of the genus *Wolbachia*. The *Wolbachia* spp. of insects are closely related phylogenetically to the *Rickettsia, Anaplasma* and *Ehrlichia* species transmitted to ticks (Noda et al., 1997). DNA sequences from the Polish *I. ricinus* were compared to the endosymbiotic *Wolbachia* species; the similarity between the analysed fragments of 16S rRNA gene amounted to 82.1–85% (data not shown). A phylogenetic analysis was performed as well; the diagram obtained showed the monophyletic species from Italy, Denmark, and Poland to form a joint clade with the *Rickettsia sp.* endosymbiotic in *I. scapularis*, while the *Wolbachia* species formed separate clades (data not shown). The formation of a separate clade by endosymbiotic bacteria in phylogenetic analyses with alpha–proteobacterial genera, e.g., *A. phagocytophilum, Rickettsia* (Fig. 1) or *Wolbachia* (data not shown) suggests that the novel clade comprises at least one new genus (Beninati et al., 2004).

In addition to *I. ricinus*-symbiotic bacteria, the ticks examined revealed DNA of *R. helvetica,* an SFG bacterium. The presence of the bacteria belonging to that group was recorded in the neighbouring countries, i.a., in Slovaka. Špitalska and Kocianova (2002) found the presence of DNA of *Rickettsia* sp. belonging to the spotted fever group in 10.9% of *I. ricinus* from south–western Slovakia. *Rickettsia helvetica* was detected in the *Ixodes* ticks in such European countries as Slovenia, Portugal, Denmark, Italy, Switzerland, and Sweden (Fournier et al., 2004, Nielsen et al., 2004). The estimated 2–36.8% *I. ricinus* ticks in Europe are infected with this *Rickettsia* species (Beati et al., 1994, Beninati et al., 2004, Fournier et al. 2000, Nielsen et al., 2004). To date, the pathogenetic role of *R. helvetica* is unclear but has been suspected in acute perimyocarditis, unexplained febrile illness or sarcoidosis (Fournier et al., 2000; Nilsson et al. 1999b; Nilsson et al., 2002). This may be important from the epidemiological standpoint, as *R. helvetica* is an important human pathogen and a significant etiological agent in animal rickettsioses. Although no SFG rickettsioses have been reported in Poland, this may be due to inadequate knowledge, among relevant medical and veterinary agencies, on the threats posed by tick-transmitted diseases and by incorrect diagnosis of a disease incident. Developing diagnostic tests based on, i.a., molecular analyses may contribute to improved detection of rickettsiae-caused diseases.

This study was the first to document the presence of bacteria symbiotic with the Polish population of *I. ricinus*; in addition, it provided a detailed *gltA* gene fragment-based characteristics of *R. helvetica.* Thus, another bacteria, this time belonging to the SFG rickettsiae, can be placed on the Polish list of potentially dangerous pathogens transmitted by *I. ricinus.*

Received 25 October 2005
Accepted 26 January 2006

References

1. Alekseev A. N., Dubinina H. V., Van de Pol I., Schouls L. M. 2001. Identification of *Ehrlichia* spp. and *Borrelia burgdorferi Ixodes* ticks in the Baltic regions of Russia. *J. Clin. Microbiol*. Vol. 39. P. 2237–2242.
2. Beati L., Humair P. F., Aeschlimann A., Raoult D. 1994. Identification of spotted fever group rickettsiae isolated from *Dermacentor marginatus* and *ixodes ricinus* ticks collected in Switzerland. *Am. J. Trop. Med. Hyg.* Vol. 51. P. 138–148.
3. Beninati T., Lo N., Sacchi L., Genchi C., Noda H., Bandi C. 2004. A novel alpha-proteobacterium resides in the mitochondria of ovarian cells of the ticks *Ixodes ricinus. Appl. Envirom. Microbiol.* Vol. 70. P. 2596–2602.
4. Brouqui Ph., Sanogo Y. O., Caruso G., Merola F., Raoult D. 2003. A new *Ehrlichia* detected in *Ixodes ricinus* tick collected from asymptomatic humans in Nothern Italy. *Ann. N.Y. Acad. Sci*. Vol. 990. P. 1–7.
5. Fournier P. E., Allombert C., Supputamongkol Y., Caruso G., Broqui P., Raoult D. 2004. Aneruptive fever associated with antibodies to *Rickettsia helvetica* in Europe and Tailand. *J. Clin. Microbiol*. Vol. 42. P. 816–818.
6. Fournier P. E., Grunnenberger F., Jaulhac B., Gastinger G., Raoult D. 2000. Evidence of *Rickettsia helvetica* infection in humans, eastern France. *EID*. Vol. 6. P. 389–986.
7. Harlet K., Oehme R., Frank H., Brockmann S. O., Hassler D., Kimmig P. 2004. Pathogens and symbionts in ticks: prevalence of *Anaplasma phagocytophilum* (*Ehrlichia* sp.), *Wolbachia* sp., *Rickettsia* sp., and *Babesia* sp. In Southern Germany. *Int. J. Med. Microbiol*. 293. Suppl. 37. P. 86–92.
8. Kimura M. 1980. A simple method for estimatimg evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol*. Vol. 16. P. 111–120.

9. Lee J. H., Park H. S., Jang W. J., Koh S. E., Kim J. M., Shim S. K., Park M. Y., Kim Y. W., Kim B. J., Kook Y. H., Park K. H., Lee S. H. 2003. Differentiation of Rickettsiae by *groEL* gene analysis. *J. Clin. Microbiol*. Vol. 41. P. 2952–2960.
10. Levin M. L., Nicholson W. L., Massung R. F., Sumner J. W., Fish D. 2002. Comparison of the reservoir competence of medium-sized mammals and *Peromyscus leucopus* for *Anaplasma phagocytophilum* in Connecticut. *Vec. Borne and Zoonot. Dis*. Vol. 2. P. 125–136.
11. Massung R. F., Slater K. 2003. Comparasion of PCR assays for detection of the agent of human granulocytic ehrlichiosis, *Anaplasma phagocytophilum*. *J. Clin. Microbiol*. Vol. 41. P. 717–722.
12. Nielsen H., Fournier P. E., Pedersen I. S., Krarup H., Ejlertsen T., Raoult D. 2004. Serological and molecular evidence of *Rickettsia helvetica* in Denmark. *Scand. J. Infect. Dis*. Vol. 36. P. 559–563.
13. Nilsson K., Lindquist O., Liu A. J., Jaenson T. G. T., Friman G., Påhlson C. 1999. *Rickettsia helvetica* in *Ixodes ricinus* ticks in Sweden. *J. Clin. Microbiol.* Vol. 37. P. 400–403.
14. Nilsson K., Lindquist O., Påhlson C. 1999. Association of *Rickettsia helvetica* with chronic perimyocarditis in sudden cardiac death. *Lancet*. Vol. 345. P. 1169–1173.
15. Nilsson K., Påhlson C., Lukinius A., Eriksson L., Nilsson L., Lindquist O. 2002. Presence of *Rickettsia helvetica* in granulomatous tissue from patients with sarcoidosis. *J. Infect. Dis*. Vol. 185. P. 1128–1138.
16. Noda H., Munderloh U. G., Kurtti T. J. 1997. Endosybionts of ticks and their relationship *Wolbachia* spp. and tick-borne pathogens of humans and animals. *Appl. Environ. Microbiol*. Vol. 63. P. 3926–32.
17. Parola P., Raoult D. 2001. Tick and tick–borne bacterial diseases in humans: an emerging infectious threat. *Clin. Infect. Dis.* Vol. 32. P. 897–928.
18. Petrovec M., Lotric Furlan S., Zupanc T. A., Strle F., Brouqui Ph., Roux V., Dumler J. S. 1997. Human disease in Europe caused by a granulocytic *Ehrlichia* species. *J. Clin. Microbiol*. Vol. 35. P. 1556–1559.
19. Schouls L. M., van de Pol I., Rijpkema S. G. T., Schot C. S. 1999. Detection and identification of *Ehrlichia, Borrelia burgdorferi* sensu lato, and *Bartonella* species in Dutch *Ixodes ricinus* ticks. *J. Clin. Microbiol*. Vol. 37. P. 2215–2222.
20. Skarphédinsson S., Jensen P. M., Kristiansen K. 2005. Survey of tickborne infections in Denmark. *EID.* Vol. 11. P. 1055–1061.
21. Skotarczak B., Rymaszewska A., Wodecka B., Sawczak M. 2003. Molecular evidence of coinfection of *Borrelia burgdorferi* sensu lato, human granulocytic ehrlichiosis agent, and *Babesia microti* in ticks from North–Western Poland. *J. Parasitol*. Vol. 89. P. 194–196.
22. Špitalskã E., Kocianovã E. 2002. Agents of *Ehrlichia phagocytophila* group and other microorganisms co-infecting ticks in southwestern Slovakia. *Acta Virol.* Vol. 46. P. 49–50.
23. Stańczak J. 2004. The first report of occurrence of a spotted fever group rickettsia from *Ixodes ricinus* in Poland. *Wiad. Parazytol*. Sup. 50. P. 112.
24. Stańczak J., Gabre R. M., Krumnis–Łozowska W., Racewicz M., Kubica-Biernat B. 2004. *Ixodes ricinus* as a vector of *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum* and *Babesia microti* in urban and suburban forests. *AAEM*. Vol. 11. P. 109–114.
25. Wicki R., Sauter P., Mettler C., Natsch A., Enzler T., Pusterla N., Kuhnert P., Egli G., Bernasconi M., Lienhard R., Lutz H., Leutenegger C. M. 2000. Swiss army survey in Switzerland to determine the prevalence of *Francisella tularensis*, members of the *Ehrlichia phagocytophila* genogrup, *Borrelia burgdorferi* sensu lato, and tick-borne encephalistis virus in ticks. *Eur. J. Clin. Microbiol. Infect. Dis*. Vol. 19. P. 427–432.
26. Wodecka B., Rymaszewska A., Sawczuk M., Skotarczak B. Detectability of *Borrelia burgdorferi* in the blood of dogs undergoing treatment for boreliosis (in press).

Anna Rymaszewska, Bogumiła Skotarczak

RICKETTSIA HELVETICA EGZISTAVIMAS BEI MOLEKULINIAI TYRIMAI IR NAUJA α-PROTEOBAKTERIJA *IXODES RICINUS* ERKĖSE LENKIJOJE

Santrauka

Ixodes ricinus erkės, surinktos nuo borelijomis užsikrėtusių šunų Vakarų Pomeranijoje (Lenkija), buvo ištirtos dėl *Anaplasma* ir *Rickettsia* genties ligų sukėlėjų bei galimo užsikrėtimo *Erlichia* genties bakterijomis. Bakterijos buvo identifikuotos atlikus PGR bei sekvenuojant 16S rRNA, *gltA* ir *msp2* genų fragmentus. Remiantis gautais filogenetinės analizės rezultatais, buvo nustatytos kelios *Rickettsia* rūšys. *A. phagocytophilum* DNR buvo aptikta 20% išskirtų mėginių, SFG rickettsia (*R. helvetica*) DNR – 40% mėginių, įskaitant vienintelį atvejį, kai buvo aptikta mišri infekcija su *A. phagocytophilum.* Be to, net 80% mėginių buvo aptikta anksčiau nenustatytų, simbiotinių *I. ricinus* erkių bakterijų, *Rickettsiales bacterium*. Amplifikuoti fragmentai buvo susekvenuoti, gautos sekos deponuotos Genų Banke (registracijos numeriai: DQ105665 – *Rickettsiales bacterium* ir DQ105664 – *R. helvetica*). Tai pirmi nustatyti riketsiozės atvejai Lenkijoje. Lenkiškos *R. bacterium* 16S rRNA sekos buvo identiškos sekoms iš Italijos ir Danijos. Lenkiškos *R. helvetica gltA* geno sekos atitiko sekas iš Italijos (viena seka) ir Šveicarijos, bet skyrėsi nuo sekų iš JAV, Maroko ir Italijos (dvi sekos). Remiantis šiais duomenimis, buvo išskirti penki *R. helvetica* haplotipai. Lenkijoje išskirtas haplotipas buvo identiškas *R. helvetica* haplotipams iš Italijos ir Šveicarijos. Buvo atlikta kai kurių *Rikettsia* rūšių, įskaitant ir minėtąsias dvi iš Vakarų Pomeranijos, filogenetinė analizė. Filogenetinėje analizėje buvo naudoti dviejų *Rickettsia* rūšių, 16S rRNR (*R. bacterium*) ir *gltA* (*R. helvetica*) genų fragmentai. *R. bacterium* sekos iš Lenkijos kartu su sekomis iš Danijos filogenetiniame medyje sudarė atskirą šaką, atskirtą nuo patogeniškų *Rikettsia* rūšių. *R. helvetica* sekos iš Lenkijos, Italijos ir Šveicarijos filogenetiniame medyje suformavo vieningą atskirą grupę.

Raktažodžiai: *Rickettsia helvetica,* simbioziniai rickettsiae, *Ixodes* erkės, filogenetiniai tyrimai