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Detection of spotted fever group rickettsiae and family *Anaplasmataceae* in *Ixodes ricinus* ticks from Republic of Moldova and Eastern Ukraine

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INTRODUCTION

Spotted fever group (SFG) rickettsioses and *Anaplasmataceae* are obligate intracellular Gram-negative bacteria belonging to the order *Rickettsiales*. They are now recognized as important emerging vector-borne human infections worldwide. Recently, various *Rickettsia* and *Anaplasma* species have been found in *Ixodes ricinus* ticks from different European countries [1].

In the Republic of Moldova and Ukraine, the data about SFG rickettsiae and *Anaplasma* spp. in ticks are scarce. In the present study, the prevalence of SFG rickettsiae and members of the family *Anaplasmataceae* in *I. ricinus* ticks from the Republic of Moldova and Ukraine was investigated.

MATERIALS AND METHODS

The flagging of vegetation for questing *I. ricinus* ticks was performed by blanket dragging in Republic of Moldova (Central part) and Ukraine (Kharkiv region) in spring 2006. All collected ticks were identified by standard taxonomic keys and transferred to 70% ethanol. The DNA in one half of each tick was extracted using the QIAamp DNA mini kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. Rickettsial DNA was identified on the basis of PCR amplification of 396-bp and 475-bp partial fragments of the *gltA* gene and *ompA* gene, respectively. The DNA was also screened with primers that amplify a 345-bp fragment of the 16S rRNA gene of bacteria within the family *Anaplasmataceae*. All the PCR conditions were carried out according to the previously described protocols [2]. All positive PCR products were purified using the QIAquick PCR purification kit (Qiagen, GmbH, Germany) and then directly sequenced in order to identify the species and strains.

Pathogen-infected tick samples were tested by PCR with a primer set amplifying a 307-bp fragment of the mitochondrial 12S rRNA gene, as previously described [3].

RESULTS AND DISCUSSION

PCR amplification and sequencing of a 307-bp fragment of the 12S rDNA of the ticks was performed, and yielded a sequence that was 99–100% similar to the corresponding 12S rDNA sequence of *I. ricinus*. Altogether, 156 (from Moldova) and 84 (from Ukraine) *I. ricinus* ticks were analysed for *Rickettsia* sp., *Anaplasma* sp. and *Ehrlichia* sp. The prevalences of rickettsial DNA in *I. ricinus* ticks were 17.3% and 6.0% in the Republic of Moldova and Ukraine, respectively. The partial sequences of the *gltA* gene had 99% similarity with those of *Rickettsia helvetica* and 98–100% with different strains of *Rickettsia monacensis*. The *ompA* results showed that all strains were *R. monacensis*, with 99–100% similarity. *R. monacensis* is widely distributed in *I. ricinus* ticks in both countries. The prevalences in Moldova and Ukraine were 15.4% and 4.8%, respectively. Human pathogenic *R. helvetica* was detected in only 1.9% and 1.2% of tested ticks from Moldova and Ukraine (Table 1).

Members of the family *Anaplasmataceae* were detected in 26.1% (Moldova) and 3.6% (Ukraine) of tested ticks. Analysis of partially sequenced 16S rRNA PCR products confirmed these organisms as *Anaplasma phagocytophilum* and Candidatus *Nicolleae massilliensis* in 5.1% and 21%, respectively, with 98%–100% sequence similarity to Moldavian ticks. The prevalence of the *Anaplasmataceae* in Ukranian ticks was 3.6% (three ticks). BLAST analysis confirmed that all were *A. phagocytophilum*, with 99% sequence similarity (Table 1).

One tick from Moldova was simultaneously infected by both *A. phagocytophilum* and *R. helvetica*.

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Table 1. Prevalence of spotted fever group rickettsiae and family *Anaplasmataceae* in *Ixodes ricinus* ticks from Republic of Moldova and Eastern Ukraine

Country	No. of positive ticks/%	Total number of examined ticks	Gene	Identification
Republic of Moldova	3/1.9	156	*GltA*	*Rickettsia helvetica*
	24/15.4		*GltA/ompA*	*Rickettsia monacensis*
	8/5.1		16S	*Anaplasma phagocytophilum*
	33/21		16S	'Candidatus *Nicolleae massilliensis*'
Eastern Ukraine	1/1.2	84	*GltA*	*R. helvetica*
	4/4.8		*GltA/ompA*	*R. monacensis*
	3/3.6		16S	*A. phagocytophilum*
	0/0		16S	'Candidatus *N. massilliensis*'

Our results represent the first demonstration of SFG rickettsiae in Moldavian and Ukrainian *I. ricinus* ticks on the basis of molecular biological identification techniques. One bacterium, *R. helvetica*, occurs in several parts of Europe and has been implicated as a human pathogen [1]. *R. monacensis* has only recently been discovered in *I. ricinus* from central and western Europe. Whether they are pathogenic is not known, but as other rickettsiae of previously unknown pathogenicity have subsequently been shown to be associated with disease, this bacterium could be pathogenic.

These data represent the first evidence of *Anaplasmataceae* family members in the ticks from Ukraine. Several studies on anaplasmoses in Moldova have been conducted in the past, and they all reported *A. phagocytophilum* as the causative agent [4]. The *A. phagocytophilum* prevalence in ticks reported herein was very similar to the infectious rate in ticks from some areas of Russia, ranging from 1% to 8% [5]. Candidatus *N. massilliensis* has never been detected in the studied regions. The pathogenicity for humans and animals of this species is unknown.

Our survey revealed the occurrence of SFG rickettsiae and *Anaplasmataceae* family members in ticks from the Republic of Moldova and Ukraine. Further studies are needed to determine the epidemiological and clinical importance of these diseases in these regions.

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