Borrelia miyamotoi and *Candidatus* Neoehrlichia mikurensis in *Ixodes ricinus* Ticks, Romania

Zsuzsa Kalmár, Hein Sprong, Andrei D. Mihalca, Călin M. Gherman, Mirabela O. Dumitrache, Elena C. Coipan, Manoj Fonville, Vasile Cozma

Author affiliations: University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania (Z. Kalmár, A.D. Mihalca, C.M. Gherman, M.O. Dumitrache, V. Cozma); National Institute of Public Health and Environment, Bilthoven, the Netherlands (H. Sprong, E.C. Coipan, M. Fonville)

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To the Editor: *Ixodes* spp. ticks are vectors for human and animal pathogens. *Ix. ricinus* ticks are widely distributed, frequently reported to feed on humans, and the main vector for a large variety of tickborne pathogens (*1*). The effect of ticks and tickborne diseases on public health, animal health and welfare, and animal production appears to be an increasing global problem, which will lead to considerable economic costs (*2*).

Borrelia miyamotoi is a spirochete that belongs to the relapsing fever group and causes symptoms similar to those of other relapsing fever group pathogens and Lyme borreliosis, including erythema migrans–like skin lesions (*3*). The geographic distribution of *B. miyamotoi* is sporadic; it has been detected in *Ixodes* spp. ticks in many countries in Europe and in North America and Asia. In Russia, the United States, and recently in the Netherlands, *B. miyamotoi* was detected in humans and confirmed to cause disease (*4*,*5*). In Romania, pathogens that cause Lyme borreliosis and reptile-associated borreliae were identified in different tick populations (*6*,*7*). However, no information is available on the presence of relapsing fever group borreliae in this country.

Candidatus Neoehrlichia mikurensis and *Anaplasma phagocytophilum* are obligate, intracellular, tickborne pathogens of the family *Anaplasmataceae*; both are emerging zoonotic agents. *Candidatus* N. mikurensis causes monocytotropic ehrlichiosis in canids and humans and granulocytic anaplasmosis in humans and domestic animals (*8*). These 2 pathogens are found throughout Europe in *Ix. ricinus* ticks (*8*). *A. phagocytophilum* has been reported in questing *Ix. ricinus* ticks, dogs, wild boars, hedgehogs, and tortoises in Romania (*9*). Recently, *Candidatus* N. mikurensis was detected in an *Ix. ricinus* tick that had bitten a human in Romania (*10*). This recently discovered tickborne agent was shown to be a risk for disease in humans and has been detected in questing *Ix. ricinus* ticks throughout Europe and in animal tissue samples and human patients (*8*).

Relapsing fever spirochetes and potential public health risks associated with tickborne pathogens are a serious medical problem. Thus, we assessed the presence of *B. miyamotoi*, *A. phagocytophilum*, and *Candidatus* N. mikurensis in questing *Ix. ricinus* ticks in Romania.

Questing *Ix. ricinus* ticks were available from previous studies conducted by our research group. A random sampling approach was used as described (*7*). To detect potentially pathogenic bacteria, 468 questing *Ix. ricinus* ticks were collected from 4 regions from Romania, randomly selected, and analyzed.

Detection of pathogens was performed by using multiplex quantitative PCRs (qPCRs) specific for the *flaB* and *ospA* genes of *B. miyamotoi*, the *msp2* gene of *A. phagocytophilum*, and the g*roEL* gene of *Candidatus* N. mikurensis. We used IQ Multiplex Powermix (Bio-Rad, Carlsbad, CA, USA) and a final reaction volume of 20 mL (*8*). For detection of *A. phagocytophilum* and *Candidatus* N. mikurensis, we also performed multiplex qPCR as described (*8*). For detection of *B. miyamotoi*, a specific region of the

Table. Prevalence of 3 bacterial species in questing *Ixodes ricinus* in 14 localities, Romania

Locality (county)	No. ticks tested (nymphs, males, females)	No. (%) ticks positive, by bacterial species		
		Borrelia miyamotoi	*Anaplasma phagocytophilum*	*Candidatus* Neoehrlichia mikurensis
Cugir (Alba)	19 (8, 4, 7)	0	2 (10.53)	1 (5.26)
Vladimirescu (Arad)	17 (0, 5, 12)	0	2 (11.76)	0
Bicaci (Bihor)	23 (12, 5, 6)	0	4 (17.4)	0
Bistrița (Bistriţa-Năsăud)	30 (0, 10, 20)	0	0	0
Poiana Mărului (Braşov)	66 (0,10, 56)	2 (3.03)	0	0
Vultureni (Cluj)	44 (3, 10, 31)	1 (2.27)	3 (6.82)	2 (4.55)
Micești (Cluj)	62 (0, 15, 47)	1 (1.62)	0	2 (3.23)
Reșița (Caraș-Severin)	21 (0, 10, 11)	0	0	0
Corund (Harghita)	59 (7, 17, 37)	0	1 (1.7)	4 (6.78)
Bistra (Maramureș)	26 (1, 10, 15)	0	0	0
Icland (Mureș)	37 (6, 5, 26)	2 (5.41)	4 (10.81)	8 (21.62)
Mediaș (Sibiu)	12 (2, 4, 6)	1 (8.33)	0	2 (16.67)
Rătești (Satu Mare)	22 (7, 10, 15)	0	0	1 (4.55)
Lugoj (Timiș)	30 (0, 11, 19)	0	0	5 (16.67)
Total	468 (46, 126, 296)	7 (1.5)	16 (3.42)	25 (5.34)

flab gene was targeted by using multiplex qPCR according to a previous described protocol (*1*). For quality control of qPCRs, we included positive and negative controls. Sequences of qPCR products were analyzed and compared with sequences available in GenBank.

B. miyamotoi was detected in 7 ticks: 2 (1.59%) of 126 males, 2 (0.68%) of 296 females, and 3 (6.52%) of 46 nymphs. *A. phagocytophilum* was detected in 16 ticks: 1 (0.79%) of 126 males, 11 (3.72%) of 296 females, and 4 (8.70%) of 46 nymphs. *Candidatus* N. mikurensis was detected in 25 ticks: 5 (3.97%) of 126 males, 18 (6.08%) of 296 females, and 2 (4.35%) of 46 nymphs. Overall prevalences were 1.50% for *B. miyamotoi*, 3.42% for *A. phagocytophilum*, and 5.34% for *Candidatus* N. mikurensis. Prevalences of each pathogen in specific varied by locality (Table). No co-infections were detected.

We analyzed *flab*, *msp2*, and *groEL* gene sequences obtained by qPCR. These sequences showed 99%–100% identities with gene sequences of *B. miyamotoi* (GenBank accession no. KJ847050), *A. phagocytophilum* (accession no. KP164415), and *Candidatus* N. mikurensis (accession no. FJ966365).

In Romania, the density of *Ix. ricinus* ticks is high and their host diversity is extensive (*7*). However, data for effects of tickborne pathogens on public health are scarce in this country. In this study, we detected *B. miyamotoi*, *A. phagocytophilum*, and *Candidatus* N. mikurensis in questing *Ix. ricinus* ticks in Romania, which confirms the emerging trend of these pathogens in Europe. Because of the scarcity of information on human infections with these pathogens in Romania, serologic and molecular investigations and their implementation are needed for diagnosis, which might help in assessing the effect of these pathogens on public health.

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References

1. Hansford KM, Fonville M, Jahfari S, Sprong H, Medlock JM. *Borrelia miyamotoi* in host-seeking *Ixodes ricinus* ticks in England. Epidemiol Infect. 2015;143:1079–87. http://dx.doi.org/10.1017/S0950268814001691
2. Jongejan F, Uilenberg G. The global importance of ticks. Parasitology. 2004;129(Suppl):S3–14. http://dx.doi.org/10.1017/S0031182004005967
3. Platonov AE, Karan LS, Kolyasnikova NM, Makhneva NA, Toporkova MG, Maleev VV, et al. Humans infected with relapsing fever spirochete *Borrelia miyamotoi*, Russia. Emerg Infect Dis. 2011;17:1816–23. http://dx.doi.org/10.3201/eid1710.101474
4. Crowder CD, Carolan HE, Rounds MA, Honig V, Mothes B, Haag H, et al. Prevalence of *Borrelia miyamotoi* in Ixodes ticks in Europe and the United States. Emerg Infect Dis. 2014;20:1678–82. http://dx.doi.org/10.3201/eid2010.131583
5. Fonville M, Friesema IH, Hengeveld PD, Docters van Leeuwen A, Jahfari S, Harms MG, et al. Human exposure to tickborne relapsing fever spirochete *Borrelia miyamotoi*, the Netherlands. Emerg Infect Dis. 2014;20:1244–5. http://dx.doi.org/10.3201/eid2007.131525
6. Kalmár Z, Cozma V., Sprong H, Jahfari S, D'Amico G, Mărcuțan DI, et al. Transstadial transmission of *Borrelia turcica* in *Hyalomma aegyptium* ticks. PLoS ONE. 2015;10:e0115520. http://dx.doi.org/10.1371/journal.pone.0115520
7. Kalmár Z, Mihalca AD, Dumitrache MO, Gherman CM, Magdaş C, Mircean V, et al. Geographical distribution and prevalence of *Borrelia burgdorferi* genospecies in questing *Ixodes ricinus* from Romania: a countrywide study. Ticks Tick Borne Dis. 2013;4:403–8. http://dx.doi.org/10.1016/j.ttbdis.2013.04.007
8. Jahfari S, Fonville M, Hengeveld P, Reusken C, Scholte EJ, Takken W, et al. Prevalence of *Neoehrlichia mikurensis* in ticks and rodents from north-west Europe. Parasit Vectors. 2012;5:74. http://dx.doi.org/10.1186/1756-3305-5-74
9. Matei IA, Kalmár Z, Magdaş C, Magdaş V, Toray H, Dumitrache MO, et al. *Anaplasma phagocytophilum* in questing *Ixodes ricinus* ticks from Romania. Ticks Tick Borne Dis. 2015;6:408–13. http://dx.doi.org/10.1016/j.ttbdis.2015.03.010
10. Andersson M, Zaghdoudi-Allan N, Tamba P, Stefanache M, Chitimia L. Co-infection with *'Candidatus* Neoehrlichia mikurensis' and *Borrelia afzelii* in an *Ixodes ricinus* tick that has bitten a human in Romania. Ticks Tick Borne Dis. 2014;5:706–8. http://dx.doi.org/10.1016/j.ttbdis.2014.05.013

Address for correspondence: Zsuzsa Kalmár, University of Agricultural Sciences and Veterinary Medicine, Calea Mănăștur 3–5, Cluj-Napoca 400372, Romania; email: zsuzsa_kalmar@yahoo.com

Suspected Rabies in Humans and Animals, Laikipia County, Kenya

Mark Obonyo, James M. Akoko, Austine B. Orinde, Eric Osoro, Waqo Gufu Boru, Ian Njeru, Eric M. Fèvre

Author affiliations: Ministry of Agriculture, Livestock and Fisheries, Nairobi, Kenya (M. Obonyo, A.B. Orinde); Field Epidemiology and Laboratory Training Program, Nairobi (M. Obonyo, W.G. Boru); International Livestock Research Institute, Nairobi (J.M. Akoko, E.M. Fèvre); Kenya Zoonotic Disease Unit, Nairobi (A.B. Orinde, E. Osoro); Ministry of Health, Kenya (W.G. Boru, I. Njeru); University of Liverpool Institute of Infection and Global Health, Liverpool, UK (E.M. Fèvre)

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To the Editor: Dog bites are a serious public health problem because of the associated risk for rabies virus exposure in countries to which the virus is endemic (*1*,*2*).