'*Candidatus* Neoehrlichia mikurensis' in Europe

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Abstract

'*Candidatus* Neoehrlichia mikurensis' is an uncultured emerging bacterium that is provisionally included in the family *Anaplasmataceae*. In Europe, it is transmitted by *Ixodes ricinus* ticks. Rodents are the reservoirs. It is widely distributed in mammals (both wild and domestic) and birds. It causes an inflammatory disease in humans with underlying diseases, but the microorganism also affects immunocompetent individuals in which asymptomatic infection has been recognized. A high degree of suspicion and the use of molecular tools are needed for the correct diagnosis. Efforts to cultivate it and to investigate its pathogenesis should be a priority.

Keywords: '*Candidatus* Neoehrlichia mikurensis', coinfection, *Dermacentor reticulatus*, doxycycline, Europe, *Ixodes ricinus*, neoehrlichiosis, reservoir, tick, tick-borne diseases
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Introduction

'*Candidatus* Neoehrlichia mikurensis' was first identified as a human pathogen in 2010. This bacterium was molecularly detected (using PCR assays) from the blood of a Swedish patient with chronic lymphocytic leukaemia who developed prolonged fever, an erysipelas-like rash and thromboembolic complications. Subsequently the patient recovered with doxycycline [1]. The finding of '*Ca.* N. mikurensis' contributed to increase the spectrum of human diseases transmitted by hard ticks in Europe, adding this microorganism to the list of *Borrelia burgdorferi* sensu lato, *Borrelia miyamotoi, Rickettsia* spp. (*R. conorii, R. helvetica, R. massiliae, R. sibirica* subsp. *sibirica, R. sibirica* subsp. *mongolitimonae, R. monacensis, R. aeschlimannii, R. slovaca,* '*Candidatus* R. rioja' and *R. raoultii*), *Anaplasma phagocytophilum, Francisella tularensis, Babesia divergens, Babesia microti, Bartonella henselae,* Eyach virus, tick-borne encephalitis virus and Crimean-Congo haemorrhagic fever virus [2]. Recent reviews about '*Ca.* N. mikurensis' have been published [3]; nevertheless, our aim was to compile and update, under a One Health scope, all available information about this microorganism that may be going unnoticed in our environment.

Basic aspects of the bacterium

'*Ca.* N. mikurensis' was found for the first time in spleen samples from rats on Mikura Island (Japan), as well as in ticks [4]. Genetic variants of *Ehrlichia* (e.g. *Ehrlichia*-like variant Schotii or '*Candidatus* Ehrlichia walkerii'), previously found in *Ixodes* spp. ticks or in rats, are synonyms of this species [4].

To date, it has not been possible to cultivate this microorganism. Nevertheless, on the basis of electron microscopic observations of the sinusoids of the spleen from infected rats by Kawahara et al. [4], it is assumed that '*Ca.* N. mikurensis' are small (0.5–1.5 μm), Gram negative, pleomorphic cocci (belonging to the class α-proteobacteria) that live in an interacting cycle affecting rodents and ticks. '*Ca.* N. mikurensis' grow in membrane-bound inclusions within the cytoplasm of endothelial cells, but the specific mechanism of infection remains

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unknown. According to 16S rRNA and *groEL* sequences, the phylogenetic analysis revealed a new cluster in the family *Anaplasmataceae,* close to other known tick-borne agents such as *Anaplasma* spp. or *Ehrlichia* spp [4].

Apart from '*Ca.* N. mikurensis,' the '*Candidatus* Neoehrlichia' genus includes at least four other members: '*Candidatus* Neoehrlichia lotoris' (detected in raccoons from North America) [5]; '*Candidatus* Neoehrlichia' sp. FU98 (closely related to '*Ca.* N. lotoris' and found in red foxes, a badger and one *Ixodes rugicollis* tick from Europe) [6,7]; and '*Candidatus* Neoehrlichia australis' and '*Candidatus* Neoehrlichia arcane,' both described in *Ixodes holocyclus* ticks from Australia [8]. None of them, except '*Ca.* N. mikurensis,' has been involved as a human pathogen.

Epidemiology

The main vector of '*Ca.* N. mikurensis' is *Ixodes ricinus,* a tick species that frequently bites humans in Europe. This tick is distributed throughout Europe and is involved in the transmission of a high number of human infectious diseases (Lyme borreliosis, human anaplasmosis, rickettsiosis, babesiosis, tick-borne encephalitis, *B. miyamotoi* infection and neoehrlichiosis) [2]. To date, '*Ca.* N. mikurensis' has been detected in *I. ricinus* ticks from 20 European countries (Table 1). It has been found in *I. ricinus* from the vegetation in 18 European countries, with a prevalence of infection that ranges from 0.1% to 24.3% (Denmark and Hungary, respectively) and sometimes

TABLE 1. Detection of *Candidatus* Neoehrlichia mikurensis in ticks from different sources in European countries

Country	Hard tick species (source)	Prevalence (%)	Sampling years	Coinfection	Reference
Austria	*Ixodes ricinus* (vegetation)	4.2–22.1	2002–13	*Babesia* spp., *Borrelia afzelii, Borrelia burgdorferi, Babesia divergens, Babesia venatorum, Rickettsia* spp., *Rickettsia helvetica, Rickettsia raoultii*	[9–11]
Belgium	*I. ricinus* (vegetation)	0.4	2012–14		[12]
	I. ricinus (hedgehogs)	2.7	2014–15		[13]
	Ixodes hexagonus (hedgehogs)	0.09	2014–15		[13]
Czech Republic	*I. ricinus* (vegetation)	0.4–10	2010–14		[10,14–16]
	I. ricinus (sheep)	30.7	2013–14		[16]
Denmark	*I. ricinus* (vegetation)	0.1–0.9	2008–12		[17,18]
Estonia	*I. ricinus* (vegetation)	1.3	2006–13		[19]
France	*I. ricinus* (vegetation)	0.2–1.7	2008–12		[14,18]
Germany	*I. ricinus* (vegetation)	2.2–24.2	2008–13	*Babesia* spp., *Anaplasma phagocytophilum, R. helvetica*	[14,20,21]
	I. ricinus (rodents)	3.8–6.4	2010–13		[20,21]
	I. ricinus (dogs)	4.1–4.3	2010–11		[22,23]
	I. ricinus (humans)	8.1	NA		[14]
	I. ricinus (wild boar)	6.25	2010–13		[24]
	Dermacentor reticulatus (vegetation)	0.08	2010–11		[22]
	D. reticulatus (rodents)	7.7	2010–11		[20]
	Ixodes trianguliceps (rodents)	2.5	2012–13		[21]
	Ixodes spp. (rodents)	100	2010–11		[20]
	Unidentified larva (rodents)	100	2010–11		[20]
	I. hexagonus (dogs)	5.9–6.6	2010–11		[22,23]
Hungary	*I. ricinus* (vegetation)	8.8–24.3	2007–12		[25,26]
Italy	*I. ricinus* (vegetation)	10.5	2006–8		[27]
	I. ricinus (rodents)	5.3	2011–13		[28]
	I. ricinus (humans)	0.5	1995–2011		[29]
Moldova	*I. ricinus* (vegetation)	0.8	1960		[30]
Norway	*I. ricinus* (vegetation)	5.9	1998–99	*B. afzelii, B. burgdorferi* sensu stricto	[31]
Poland	*I. ricinus* (vegetation)	0.3	2011		[32]
	I. ricinus (dogs)	8.1	2013–14		[33]
	I. hexagonus (dogs)	0.7	2013–14		[33]
Romania	*I. ricinus* (vegetation)	5.3–14.6	2013–14	*Borrelia* spp., *Rickettsia* spp.	[34,35]
	I. ricinus (humans)	100	2013	*B. afzelii*	[36]
Russia-Baltic region	*I. ricinus* (birds)	0.7	2009		[37]
	Ixodes frontalis (birds)	25	2009		[37]
Serbia	*I. ricinus* (vegetation)	4.2	NA		[38]
Slovakia	*I. ricinus* (vegetation)	1.1–11.6	2006–13	*A. phagocytophilum*	[10,39–42]
	I. ricinus (rodents)	0.3–1.3	2011–14	*Babesia microti*	[41,42]
	I. trianguliceps (rodents)	2.7	2011–13		[41]
Spain	*I. ricinus* (cows)	1	2013		[43]
Sweden	*I. ricinus* (vegetation)	6	2010–11	*B. afzelii*	[44]
	I. ricinus (birds)	2.1	2009		[45]
Switzerland	*I. ricinus* (vegetation)	3.5–8	2009–10	*B. afzelii*	[46,47]
	I. ricinus (rodents)	2.6	2011–12		[48]
	I. ricinus (birds)	3.3	2007–10		[49]
Netherlands	*I. ricinus* (vegetation)	2.4–11.7	2000–12	*B. afzelii, R. helvetica*	[18,50,51]
	I. ricinus (humans)	5.4	2007–8		[52]
Netherlands and Belgium	*I. ricinus* (vegetation)	7	2009–10		[53]
	I. ricinus (birds)	4.9	2012–14	*B. burgdorferi* sensu lato, *Borrelia miyamotoi, R. helvetica*	[54]
	I. ricinus (red deer)	6.3	2009–10		[53]
	I. ricinus (European mouflon)	4.3	2009–10		[53]
	I. ricinus (wild boar)	8.3	2009–10		[53]
	I. ricinus (sheep)	12.5	2009–10		[53]

NA, not available.

coinfecting with other tick-borne agents (Table 1). '*Ca.* N. mikurensis' has also been reported in *I. ricinus* collected on a variety of wild and domestic vertebrates, including rodents (0.3–6.4%), dogs (4.1–8.1%), birds (0.7–4.9%), hedgehogs (2.7%), sheep (12.5–30.7%), wild boar (6.25–8.3%), cattle (1%), red deer (6.3%) and European mouflon (4.3%) (Table 1). Moreover, studies about the presence of '*Ca.* N. mikurensis' in *I. ricinus* that had fed on humans have been reported from Germany, Italy, Romania and the Netherlands (Table 1). '*Ca.* N. mikurensis' has also been detected in other *Ixodes* spp., such as *I. hexagonus, I. trianguliceps* or *I. frontalis,* from various sources (vegetation, rodents, dogs, hedgehogs and birds) (Table 1). In addition, it is worth mentioning that '*Ca.* N. mikurensis' has been found in *Dermacentor reticulatus,* a tick species that frequently bites humans (Table 1). Transovarial transmission of '*Ca.* N. mikurensis' in ticks does not seem to occur [53].

The role of rodents as reservoir hosts of '*Ca.* N. mikurensis' was suggested as a result of the higher prevalence rates of the bacterium found in ticks from rodents than in questing ticks [20,21]. Xenodiagnoses on rodents confirmed their capacity to transmit '*Ca.* N. mikurensis' to ticks, and therefore their role as reservoir [48]. Transplacental transmission in rodents was also observed [21]. In Europe, '*Ca.* N. mikurensis' has been detected in at least seven rodent species belonging to the genera *Apodemus* (*A. flavicollis, A. agrarius, A. sylvaticus*), *Myodes* (*My. glareolus*) and *Microtus* (*M. arvalis, M. agrestis, M. minutus*) [20–22,42,55]. It has been also detected in other mammals such as wild boar, bears, badgers, chamois and mouflons [56], hedgehogs [57] and dogs [58–61] (Fig. 1). To our knowledge, despite having been investigated, '*Ca.* N. mikurensis' has never been found in cats, wild cervids, martens, jackals or foxes [22,56,60,61], perhaps as a result of low or intermittent bacteraemia or the low tropism of the microorganism in tested tissues.

Neoehrlichiosis/'*Ca.* N. mikurensis' infection in humans

Clinical manifestations

Since the first European case of '*Ca.* N. mikurensis' infection in a Swedish patient with a chronic lymphocytic leukaemia with prolonged fever, erysipelas-like rash and thromboembolic

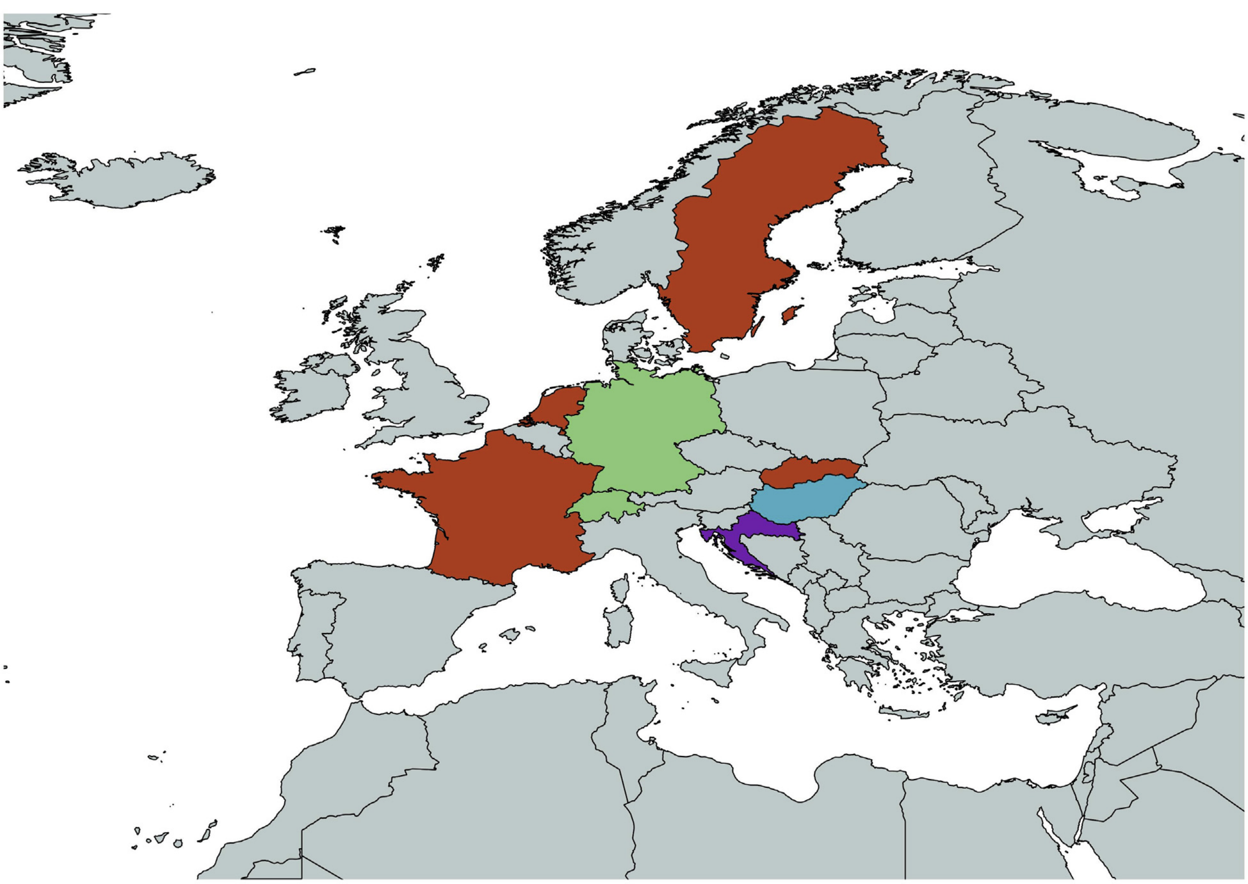

FIG. 1. European mammals in which '*Candidatus* Neoehrlichia mikurensis' has been molecularly detected. Red, rodents; green, rodents and dogs; blue, rodents and hedgehogs; purple, rodents, dogs, wild boars, bears, badgers, chamois and mouflons.

TABLE 2. Clinical background of 18 patients with neoehrlichiosis reported in European countries

Country	Year	Gender	Age	Medical condition	Reference
Czech Republic	2008	F	55	Mantle cell lymphoma, asplenic	[62]
	2009	M	58	Liver transplantation, sclerosing cholangitis, splenectomy	[62]
Germany	2007	M	69	Chronic inflammatory demyelinating polyneuropathy	[63]
	2008	M	57	Previously healthy	[63]
Sweden	2009	M	77	B cell chronic lymphocytic leukaemia, asplenic	[1]
	2011	M	75	B cell chronic lymphocytic leukaemia, splenectomy	[64]
	2011	F	67	Follicular lymphoma, systemic lupus erythematosus, (inborn) asplenic	[64]
	2013	F	67	T cell large granular lymphoma, psoriasis arthropathy, splenectomy	[64]
	2013	M	54	Psoriasis, immunosuppressive therapy	[64]
	2013	M	59	Diffuse large cell B cell lymphoma, rheumatoid arthritis splenectomy	[64]
	2014	F	71	Rheumatoid arthritis, recurrent fever, immunosuppressive therapy	[65]
	2015	M	78	Rheumatoid arthritis	[66]
	2015	M	55	Granulomatosis with polyangiitis	[66]
	2015	M	57	Pre–B cell acute lymphocytic leukaemia	[66]
	N.A.	F	65	Autoimmune haemolytic anaemia	[67]
Switzerland	2009	M	61	Coronary artery bypass grafting, septicaemia	[68]
	2011	M	68	Chronic lymphocytic leukaemia, asplenic	[47]
	2012	M	58	Follicular lymphoma	[47]

F, female; M, male; N.A., Not available.

complications that recovered with doxycycline [1], a total of 18 cases have been reported from European countries, more often in patients with immunocompromised conditions associated with haematologic neoplasias (Table 2). All patients were older than 54 years (mean, 64 years). Seventy-two percent were men and 28% were women. Immunosuppressive conditions were present for 88.9% (16/18), and 50% (9/18) had haematologic neoplasia. Half (9/18) were splenectomized or asplenic, and 78% (14/18) had received immunosuppressive therapy (e.g. 8/14 with rituximab) for the treatment of lymphomas or rheumatic diseases. Fever was present in the 14 cases with available data, and nine of them experienced myalgia and arthralgia. Vascular events such as deep vein thrombosis, thromboembolic events, aneurysm and transitory ischemic accidents were reported for 56% of patients (10/18). At least 22% of the patients (4/18) had skin manifestations, such as erythema nodosum or erysipelas-like rashes. A tick bite was reported by 39% (7/18) patients. Nevertheless, in our opinion, only data from four patients were valid because clinical manifestations had begun between 6 and 16 weeks after the tick bites. Although some patients had recalled previous tick bites, who was going to think about a tick in a patient who had developed deep thrombosis and fever of unknown origin 2 years after a tick bite? When available (14/18 patients), C-reactive protein levels were elevated in all patients (100%), and most (n = 10) had leukocytosis (neutrophilia) and anaemia. Whenever it was analysed, the delay in diagnosis resulted in very high values. Thus, for 11 studied patients, the median number of days from the onset of symptoms to diagnosis was 60 [64].

Moreover, '*Ca.* N. mikurensis' has been found in the blood of asymptomatic people from Poland. The study was carried out in healthy foresters bitten by ticks, and '*Ca.* N. mikurensis' was amplified from blood in five (1.6%) of 316 subjects [32]. With the purpose of studying possible coinfections in immunocompetent patients with tick-borne diseases, some recent studies have investigated the presence of '*Ca.* N. mikurensis' in patients with erythema migrans (Table 3). Thus, in Sweden, the bacterium was detected in two (1.9%) of 102 immunocompetent people bitten by ticks. Both had an erythematous rash; one was infected with *B. burgdorferi,* and the other one seroconverted to

TABLE 3. Clinical picture of European patients with '*Candidatus* Neoehrlichia mikurensis' infection, with or without coinfecting pathogens, after receipt of tick bite

Country, no. infections/no. patients (prevalence)	Year	Gender	Age (years)	Medical condition (no. patients)	Reference
Poland, 5/316 (1.6%)	2012	4 M, 1 F	44.1 (mean)	Asymptomatic, previously healthy (foresters with high risk of tick bites)	[32]
Norway, 7/70 (10%)	2014–15	1.6F:1M (ratio)	55 (mean)	EM (7), fatigue (1)	[69]
Sweden, 2/102 (1.9%)	2015	F	68	EM, tick bite, *Borrelia*-specific IgM and IgG antibodies	[70]
		F	57	EM, tick bite, *Anaplasma phagocytophilum*–specific IgG antibodies	
Netherlands, 7/626 (1.1%)	2007–8	F	63	Tick bite, arthralgia	[52]
		M	79	Tick bite	
		M	40	EM	
		F	60	EM	
		F	61	EM, headache, myalgia, pain in limbs	
		M	48	EM, tingling in limbs	
		M	71	Tick bite	

EM, erythema migrans; M, male; F, female.

A. phagocytophilum [70]. In the Netherlands, '*Ca.* N. mikurensis' was detected in EDTA blood samples from four (1.4%) of 291 patients with erythema migrans. Two of them did not report any additional symptom, whereas the remaining two reported pain or tingling in the limbs, in one case associated with headache and myalgia [52]. The same authors also found '*Ca.* N. mikurensis' prevalences of 0.9% (3/335) in humans bitten by ticks and higher than 5% (17/314) in ticks removed from participants [52]. In Norway, '*Ca.* N. mikurensis' was found in seven (10%) of 70 blood specimens (whole blood and/or plasma/pellet fraction) from recently tick-bitten persons with erythema migrans. The authors assumed that all of them were immunocompetent, and only one patient showed fatigue as a symptom [69]. It seems that coinfections with *B. burgdorferi* s.l. and '*Ca.* N. mikurensis' do not alter the clinical picture of Lyme disease patients, although more studies are necessary.

Diagnosis

To date, the diagnosis of '*Ca.* N. mikurensis' is based on molecular techniques, specifically PCR assays and sequence analysis of 16S rRNA (pan-bacterial, *Anaplasmataceae* specific and/or '*Ca.* N. mikurensis' specific) and *groEL* genes [1,47,62,63,65,68,70]. Positive PCR results for '*Ca.* N. mikurensis' were obtained using EDTA blood (plasma, serum, whole blood, and blood culture flask contents) and bone marrow [47,64–66] as human specimens. The microorganism has never been amplified from cerebrospinal fluid samples [64].

Serologic tests are not good tools. No cross reactions with other *Anaplasmataceae* (*A. phagocytophilum* or *Ehrlichia chaffeensis*) have been reported. The absence of cross reactions due to the immunosuppressive condition of patients or due to the low level of bacteraemia in immunocompetent people is plausible [63,69]. Only one immunocompetent patient seroconverted to *A. phagocytophilum,* but the tick was not studied [70]. No serologic assay to detect specific antibodies against '*Ca.* N. mikurensis' is available to date.

Other molecular techniques such as multilocus sequence analysis and PCR–reverse line blot hybridization have occasionally been used for epidemiologic studies in Europe [11,66]. Multilocus sequence analysis was applied to investigate the genetic diversity of '*Ca.* N. mikurensis' DNA obtained from immunocompromised patients. The data were based on the study of five housekeeping genes in addition to the 16S rRNA gene, although the technique was limited by the amount of available DNA [66]. Moreover, PCR–reverse line blot hybridization allowed the detection of simultaneous bacteria in *I. ricinus* ticks from Austria, suggesting a significant co-occurrence of '*Ca.* N. mikurensis' and *Babesia* spp. [11].

Treatment

Doxycycline (100 mg twice a day) has been used in nearly all published cases (Table 2). One patient with a suspected allergy to doxycycline was successfully treated with rifampin (300 mg twice a day) [71]. A combination of rifampin (450 mg twice a day) plus doxycycline (100 mg twice a day) was also prescribed in another case [68]. The optimal duration of treatment still remains unknown, but in most cases it lasted for 3 weeks [62,63]. The mean time to resolution of symptoms was 5 days. All patients recovered, and PCR analysis after treatment yielded negative results for '*Ca.* N. mikurensis.'

Future key questions

Studies about the distribution of '*Ca.* N. mikurensis' in nature, as well as reports about the clinical manifestations, diagnosis and treatment of patients infected with this bacterium, have increased in recent years. Nevertheless, there are still several key questions about this pathogen that need to be answered: What are the human target cells of '*Ca.* N. mikurensis'? It seems that leukocytes and endothelial cells are the human targets. Structures with coccoid forms were observed in circulating leukocytes from patients [62]. In addition, apart from humans, is '*Ca.* N. mikurensis' pathogenic for other mammals, such as dogs? Is there a possibility of outbreaks by '*Ca.* N. mikurensis'? And finally, how will climate change affect the distribution of this tick-borne disease?

Conflict of interest

None declared.

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