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REVIEW PAPER

Neoehrlichiosis: an emerging tick-borne zoonosis caused by *Candidatus* Neoehrlichia mikurensis

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Abstract *Candidatus* Neoehrlichia mikurensis is an emerging tick-borne pathogen causing a systemic inflammatory syndrome mostly in persons with underlying hematologic or autoimmune diseases. As it is neither well-known nor well-recognized, it might be misdiagnosed as recurrence of the underlying disease or as an unrelated arteriosclerotic vascular event. The pathogen is transmitted by hard ticks of the genus *Ixodes* and is closely associated with rodents in which transplacental transmission occurs. Transovarial transmission in ticks has not yet been shown. Infection rates vary greatly in ticks and rodents, but the causes for its spatiotemporal variations are largely unknown. This review summarizes the current state of knowledge on the geographical distribution and clinical importance of *Ca.* N. mikurensis. By elucidating the life history traits of this pathogen and determining more accurately its incidence in the human population, a better assessment of its public health relevance can be made. Most urgent research needs are the in vitro-cultivation of the pathogen, the development of specific serological tests, the determination of the full genomic sequence, the routine implementation of molecular diagnosis in diseased patients with a particular panel of underlying diseases, and promoting the knowledge about neoehrlichiosis among general practitioners, hospital physicians and the risk groups such as forest workers or immune-compromised people to raise awareness about this disease that can easily be treated when correctly diagnosed.

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Introduction

Less than a decade ago, the first human case of neoehrlichiosis was diagnosed in a German patient (von Loewenich et al. 2010). Since then, more than a dozen well documented cases have been published in Europe, and also recently cases in China (Li et al. 2012). Neoehrlichiosis can cause systemic inflammatory infections in immune-compromised persons that can be mistaken for the recurrence of an underlying disease or an unrelated arteriosclerotic vascular event. The causative agent, the intracellular bacterium *Candidatus* Neoehrlichia mikurensis, is considered an emerging tick-borne pathogen in Europe where it is transmitted by *Ixodes ricinus*, the most common hard tick species which acts as vector for several zoonotic tick-borne pathogens in Europe (Heyman et al. 2010). Transovarial transmission in the tick vector has neither been observed in epidemiological studies nor experimentally been proven (Burri et al. 2014). Recent evidence points towards *Ca*. N. mikurensis being very closely associated to several rodent species (Burri et al. 2014; Obiegala et al. 2014), and results from xenodiagnostic experiments with ticks have shown that rodents are competent reservoir hosts for *Ca*. N. mikurensis (Burri et al. 2014). Further, a recent study reported observations of transplacental transmission in rodents (Obiegala et al. 2014).

Even though considerable research efforts have been undertaken in recent years to elucidate the natural endemic cycle and geographical distribution of this pathogen, major parts of its life history traits, transmission mechanisms, host associations, and pathogenicity remain largely unknown. In this review, we summarize the current state of knowledge of *Ca*. N. mikurensis in terms of its distribution in ticks and wildlife, the clinical disease it causes, the diagnostic and treatment options, and its public health relevance. Further, we point out urgently needed research efforts for the future.

History of discovery

All members of the rickettsial family Anaplasmataceae are intracellular alpha-proteobacteria, most of which are transmitted by or infecting different arthropods or other invertebrates. They all have in common that they reside within membrane-enclosed vacuoles inside cells of their eukaryotic hosts (Dumler et al. 2001). Currently, the Anaplasmataceae include the genera *Anaplasma*, *Ehrlichia*, *Aegyptianella*, *Neorickettsia*, and *Wolbachia*, as well as two candidate genera, '*Candidatus* Neoehrlichia' and '*Candidatus* Xenohaliotis', the latter detected in abalone (*Haliotis* spp.) (Friedman et al. 2000; Kawahara et al. 2004). Several of them such as *Ehrlichia chaffeensis*, *Ehrlichia canis*, *Anaplasma phagocytophilum* and *Anaplasma marginale* may cause severe diseases in humans and/or animals (Aubry and Geale 2011; Bowman 2011; Dumler et al. 2007).

The history of discovery of *Ca*. N. mikurensis started in 1999 when data on a taxonomically ungrouped *Ehrlichia* DNA were published which had been detected in engorged *I. ricinus* ticks collected from roe deer in The Netherlands. It was first named after Corrie

Schot as the 'Schotti-variant' (Schouls et al. 1999). A retrospective investigation of museum-archived *I. ricinus* female ticks collected in Moldova during 1960 indicated that this pathogen has been around for much longer (Movila et al. 2013b).

Similar DNA sequences were detected in *I. ricinus* and *I. persulcatus* ticks from Baltic countries (Alekseev et al. 2001). Additionally, between 1998 and 2001, DNA of an unknown organism was identified in engorged *I. ricinus* ticks collected from asymptomatic patients in Italy. This organism was suggested to be denominated *Ca*. Ehrlichia walkeri sp. nov. (Koutaro et al. 2005). In 2003, DNA sequences of this organism were also detected in *I. ricinus* ticks from Germany, and these investigations were followed by first explorations on possible reservoir hosts (Von Loewenich et al. 2003). Also in 2003, similar DNA sequences to the above mentioned were found in three brown rats (*Rattus norvegicus*) in China and were called the '*Rattus*-variant' (Pan et al. 2003). During 1998, 1999 and 2003, DNA of the same 'new' bacterium was detected in 7 out of 15 brown rats from the Japanese island Mikura (Kawahara et al. 2004). This organism was passaged by intraperitoneal injection of a spleen homogenate of the naturally infected rats in laboratory Wister rats, and first information on the ultrastructure of this organism as well as a phylogenetic analysis became available. Electron microscopy photographs presented them as inclusions in the endothelial cell lining of the splenic sinus in one of the Wister rats 2 months after inoculation (Kawahara et al. 2004). At present, the ultrastructural observations by Kawahara et al. (2004) remain the only ones available for this organism that the authors suggested to denominate '*Candidatus* Neoehrlichia mikurensis'. This taxonomy currently is still valid because the bacterium has not yet been cultured. The close genetic similarity of the *16S rRNA* and the *groEL* genes places *Ca*. N. mikurensis in the family Anaplasmataceae.

In a more recent investigation, gene sequences from all the above mentioned publications were compared to each other confirming the identity or very close relationship of the *Ehrlichia*-like organisms with the new proposed species *Ca*. N. mikurensis (Jahfari et al. 2012). It forms a separate cluster in the family Anaplasmataceae, together with the North American *Ca*. Neoehrlichia lotoris which had been detected in tick-infested raccoon populations (*Procyon lotor*) and was successfully isolated in the *Ixodes scapularis*-derived ISE6 tick cell line, and was formally described (Munderloh et al. 2007; Yabsley et al. 2008a). Interestingly, the culture was infectious for raccoons in further experiments but it did not produce a detectable infection in laboratory mice, rats or rabbits (Yabsley et al. 2008b). The analysis of several gene loci showed that *Ca*. N. lotoris is closely related to *Ca*. N. mikurensis, but can be differentiated on the basis of unique *16S rRNA* (98.4–98.8 % identity), *groESL* (90.3–90.6 % identity) or *gltA* (84.4 % identity) gene sequences (Munderloh et al. 2007).

Characteristics of the bacterium

Due to its intracellular character and its presumed endothelial cell tropism, it is highly likely that *Ca*. N. mikurensis will be included in the family Anaplasmataceae, but in a new genus. A full description of *Ca*. N. mikurensis similar to the one recently published for *Ca*. N. lotoris is currently still missing (Yabsley et al. 2008a). Kawahara et al. (2004) described *Ca*. N. mikurensis as a small (0.5–1.5 μm), gram-negative, pleomorphic coccus of the alpha-proteobacteria which grows in membrane-bound inclusions within the cytoplasm of endothelial cells. *Candidatus* N. lotoris was also described as morphologically similar to other members of the Anaplasmataceae, but the host cells in raccoons remain elusive. *Candidatus* N. lotoris is also gram-negative, pleomorphic with a size range from 0.5 to 4 µm. The bacterium has a thin periplasmic space that separates the outer unit membrane from the inner cell wall. This is a common characteristic of the family Anaplasmataceae (Yabsley et al. 2008a).

Ecology of *Candidatus* Neoehrlichia mikurensis

Occurrence and geographical distribution in ticks

Candidatus N. mikurensis has so far been detected in *I. ricinus* ticks from 16 European countries covering almost the entire continent (Fig. 1). Overall prevalence rates in *I. ricinus* or *I. persulcatus* ticks collected from the vegetation range from below 1 % to over 20 % (Table 1). Prevalences in *I. ricinus* seem to be somewhat higher than in *I. persulcatus* which usually are around or below 1 %, whereas prevalences in *I. ricinus* seem to range on average around 6–8 %. The prevalence in *I. ricinus* ticks collected from various host animals (including humans) in Europe seems to be actually somewhat lower than in questing ticks, but due to the different study designs, this cannot be quantified (Table 1). Furthermore, *Ca*. N. mikurensis was also detected in other tick species; however, these were usually collected while feeding on host animals (Table 2). Therefore, no evidence is provided thus far that tick species other than *I. ricinus* or *I. persulcatus* are involved in the transmission of *Ca*. N. mikurensis, because its detection in ticks from host animals may be blood meal artefacts. *Candidatus* N. mikurensis has been detected in one questing *Dermacentor reticulatus* tick (Richter et al. 2013), but this number is too low to draw any conclusion from it.

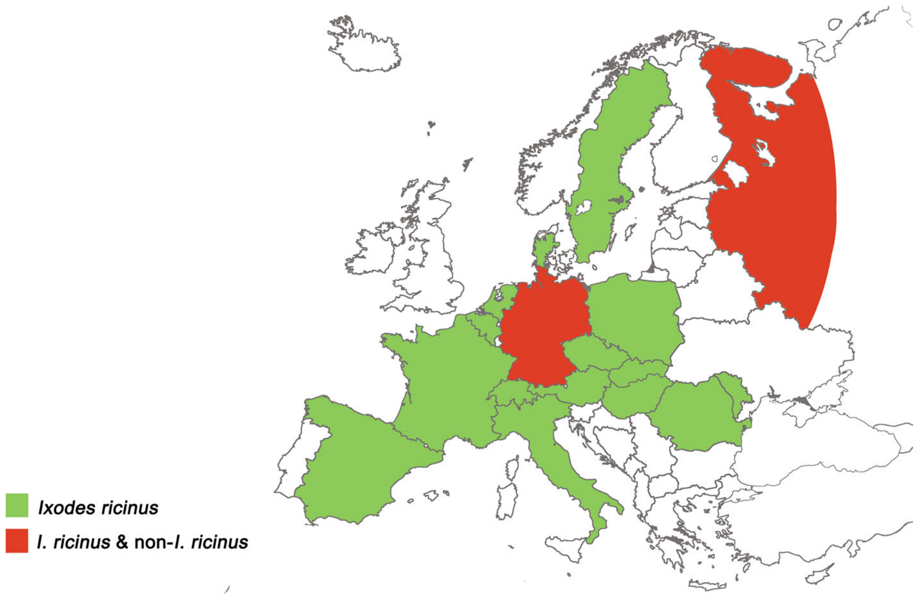

Fig. 1 *Candidatus* Neoehrlichia mikurensis in *Ixodes ricinus* in Europe. *Ixodes* species other than *I. ricinus* include *I. frontalis* in Russia and *I. hexagonus* and *I. trianguliceps* in Germany

Table 1 Detection of *Candidatus* Neoehrlichia mikurensis in *Ixodes ricinus* ticks in European countries

Country	Tick origin	Number positive/total number (% positive)	Sampling years	References
Austria	Vegetation	22/518 (4.2)	2002–2003	Glatz et al. (2014)
		19/86 (22.1)	na	Derdáková et al. (2014)
Czech Republic	Vegetation	2/20 (10)	na	Richter and Matuschka (2012)
		1473 [in pools] (MIR: 0.4–4.4)	2010	Venclikova et al. (2014)
		3/138 (2.2)	na	Derdáková et al. (2014)
Denmark	Vegetation	3/2625 (MIR: 0.1)	2011	Fertner et al. (2012)
		2350 [in pools] (MIR: 0.2–0.9)	2008–2012	Michelet et al. (2014)
France	Vegetation	1/60 (1.7)	na	Richter and Matuschka (2012)
		2350 [in pools] (MIR: 0.2–1.1)	2008–2012	Michelet et al. (2014)
Germany	Vegetation	189/782 (MIR: 24.2)	2008–2009	Silaghi et al. (2012)
		44/542 (8.1)	na	Richter and Matuschka (2012)
		51/2315 (2.2)	2009–2013	Obiegala et al. (2014)
	Rodents	15/234 (6.4)	2010–2011	Silaghi et al. (2012)
		33/965 (3.8)	2012–2013	Obiegala et al. (2014)
	Wild boar	1/16 (6.25)	2010–2013	Silaghi et al. (2014)
	Humans	9/111 (8.1)	na	Richter and Matuschka (2012)
	Dogs	32/773 (4.1)	2010–2011	Krücken et al. (2013)
		33/774 (4.3)	2010–2011	Schreiber et al. (2014)
Hungary	Vegetation	2004[a] [9/37 places]	2007	Hornok et al. (2013)
		3/34 (8.8)	2012	Szekeres et al. (2015)
Italy	Vegetation	20/193 (10.5)	2006–2008	Capelli et al. (2012)
	Humans	2/433 (0.5)	1995–2011	Otranto et al. (2014)
Moldova	Vegetation	1/126 (0.8)	1960	Movila et al. (2013b)
Poland	Vegetation	4/1325 (0.3)	2011	Welc-Faleciak et al. (2014a)
Romania	Human	1/1 (100)	2013	Andersson et al. (2014b)

Table 1 continued

Country	Tick origin	Number positive/total number (% positive)	Sampling years	References
Baltic region	Birds	1/135 (0.7)	2009	Movila et al. (2013a)
Slovakia	Vegetation	2/68 (2.9)	2006	Špitalská et al. (2008)
		16/670 (2.39)	2008–2010	Pangrácová et al. (2013)
		4/378–14/121 (1.1–11.6)	na	Derdáková et al. (2014)
Spain	Cattle	2/200 (1)	2013	Palomar et al. (2014)
Sweden	Vegetation	57/949 (6)	2010–2011	Andersson et al. (2014a)
Switzerland	Vegetation	52/818 (6.4)	2009–2010	Lommano et al. (2012)
		1916 (3.5–8)	2009	Maurer et al. (2013)
	Rodents	15/575 (2.6)	2011–2012	Burri et al. (2014)
	Birds	1205 (3.3)	2007–2010	Lommano et al. (2014)
The Netherlands	Vegetation	300/5343 (5.6)	2000–2009 & 2006–2010	Coipan et al. (2013)
		2350 [in pools] (MIR: 2.4–3.5)	2008–2012	Michelet et al. (2014)
The Netherlands and Belgium	Vegetation, European mouflon, wild boar, sheep	166/2375 (7)	2009–2010	Jahfari et al. (2012)

MIR minimum infection rate, *na* not available

[a] Number of positives not available

Table 2 Detection of *Candidatus* Neoehrlichia mikurensis in non-*Ixodes ricinus* tick species

Country	Tick origin	Tick species	Number positive/total number (% positive)	Sampling years	References
Germany	Rodents	*Dermacentor reticulatus*	9/117 (7.7)	2010–2011	Silaghi et al. (2012)
		Ixodes spp.	2/2 (100)		
		Unidentified larva	1/1 (100)		
	Rodents	*Ixodes trianguliceps*	1/40	2012–2013	Obiegala et al. (2014)
	Vegetation	*D. reticulatus*	1/1237 (0.08)	2010–2011	Krücken et al. (2013)
	Dogs	*Ixodes hexagonus*	10/151 (6.6)		
	Dogs	*I. hexagonus*	?/152 (5.9)	2010–2011	Schreiber et al. (2014)
Baltic region	Birds	*Ixodes frontalis*	1/4 (25)	2009	Movila et al. (2013a)
Russia (Asiatic part)	Vegetation	*Ixodes persulcatus*	2/53 (3.8)	2002	Shpynov et al. (2006)
Russia (Eastern Siberia & Far East)	Vegetation	*I. persulcatus*	5/2590 (0.2)	2003–2008	Rar et al. (2010)
China	Vegetation	*I. persulcatus*	8/516 (1.6)	2010	Li et al. (2012)
		Haemaphysalis concinna			
Japan	Vegetation	*Ixodes ovatus*	?/164 (?)	2000–2001	Kawahara et al. (2004)
Nigeria	Dogs	*Rhipicephalus sanguineus*, *Haemaphysalis leachi*	258 [in pools: 4/76] (5.3)	2011	Kamani et al. (2013)

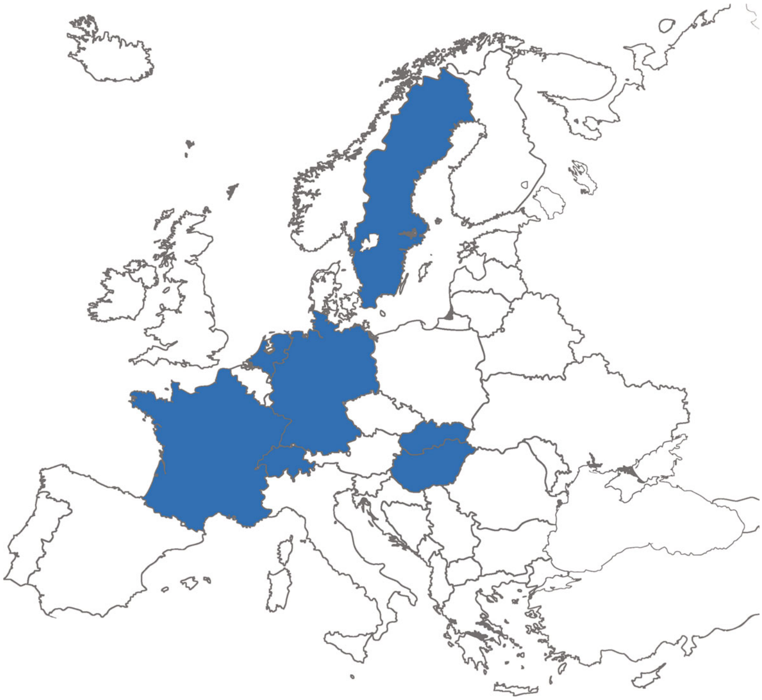

Fig. 2 *Candidatus* Neoehrlichia mikurensis-infected small mammals as determined by PCR in Europe

Occurrence and geographical distribution in rodents and other host animals

Similar to other tick-borne diseases, the emergence of human clinical cases corresponds to the distribution of vectors and reservoirs. Studies investigating rodents as well as questing ticks showed that prevalences in rodents were about 2–10 × higher than in ticks (Obiegala et al. 2014; Silaghi et al. 2012). A reservoir function of rodents seems highly likely and was indeed proven in recent xenodiagnostic studies (Burri et al. 2014). Both human cases and naturally infected rodents have been documented in Germany, Sweden and Switzerland whereas so far only infected rodents have been identified elsewhere in Europe (Fig. 2). In Europe, *Ca*. N. mikurensis has been detected in the six rodent species *Apodemus agrarius*, *A. flavicollis*, *A. sylvaticus*, *Myodes glareolus*, *Microtus agrestis* and *Mi. arvalis* (Table 3) and 10 species in Asia (Table 4). Infection rates vary considerably (8.3–52.7 %) among rodent species. *Myodes glareolus* was the most frequently infected species with an average of 9.1 % positive voles. The prevalence in this species varied from 1.8 % in France, up to 52.7 % in Germany. Thus, *My. glareolus* could represent an indicator species in monitoring protocols. Besides *My. glareolus*, other species indicating the presence of *Ca*. N. mikurensis belong to the genera *Apodemus* and *Microtus*. It seems that insectivores do not play a role in the transmission or maintenance of *Ca*. N. mikurensis as so far none of the investigated insectivores were positive in Germany, Sweden and The Netherlands (Andersson and Raberg 2011; Jahfari et al. 2012; Silaghi et al. 2012).

DNA of *Ca*. N. mikurensis has also been detected in *I. ricinus* adults feeding on red deer (*Cervus elaphus*), wild boar (*Sus scrofa*, Silaghi et al. 2014), European mouflon (*Ovis*

Table 3 Detection of *Candidatus* Neoehrlichia mikurensis in rodents in European countries

Country	Rodent species	Number positive/total number (% positive)	Sampling years	References
France	*Myodes glareolus*	5/276 (1.8)	2008	Vayssier-Taussat et al. (2012)
Germany	*Apodemus flavicollis*, *A. agrarius*, *My. glareolus*	48/91 (52.7)	2010–2011	Silaghi et al. (2012)
	A. flavicollis, *A. agrarius*, *My. glareolus*, *Microtus arvalis*, *Mi. agrestis*	36/254 (14.2)	2010–2011	Krücken et al. (2013)
	My. glareolus, *A. sylvaticus*, *A. flavicollis*, *Mi. arvalis*, *Mi. agrestis*	181/631 (28.7)	2012–2013	Obiegala et al. (2014)
Hungary	*A. flavicollis*	6/177 (3.4) [in spleen]	2010–2013	Szekeres et al. (2015)
	A. agrarius	6/348 (1.7) [in skin]		
Slovakia	*Apodemus* spp., *My. glareolus*	(10.75)	2006–2011	Vichova et al. (2014)
Sweden	*My. glareolus*, *Mi. agrestis*, *A. sylvaticus*, *A. flavicollis*	68/771 (8.8)	2008	Andersson and Raberg 2011
	My. glareolus	50/261 (19)	2008	Andersson et al. (2014a)
Switzerland	*A. sylvaticus*, *A. flavicollis*, *My. glareolus*	4/103 (3.9)	2011–2012	Burri et al. (2014)
The Netherlands	*Apodemus* sylvaticus, *Mi. arvalis*, *My. glareolus*	11/79 (13.9)	2007–2010	Jahfari et al. (2012)

Table 4 Detection of *Candidatus* Neoehrlichia mikurensis in wild mammals in Asian countries

Country	Rodent species	Number positive/total number (% positive)	Sampling years	References
China	Rodents	8/211 (3.8)	2010	Li et al. (2012)
	Apodemus agrarius, *A. peninsulae*, *Eothenomys custos*, *Myodes rufocanus*, *Niviventer confucianus*, *Rattus norvegicus*, *Tamias sibiricus*	34/841 (4)	2005–2009	Li et al. (2013)
Japan	*R. norvegicus*	7/15	1998–1999 and 2003	Kawahara et al. (2004)
	Rodents	17/111 (15.3)		Naitou et al. (2006)
	Ap. specious	5/55 (9)	2000–2004	Tabara et al. (2007)
	Ap. argenteus	2/7 (28.6)		
Russia (Siberia and Far East)	*Microtus* spp., *A. peninsulae*, *My. rufocanus*	5/1458	2003–2008	Rar et al. (2010)

orientalis musimon), sheep (*Ovis aries*) (Jahfari et al. 2012) and cows (Palomar et al. 2014). These findings raised the question on the role of other animals than rodents in the ecology of *Ca*. N. mikurensis. A survey from the Trento region (Italy) revealed *Ca*. N. mikurensis in *My. glareolus* but not in roe deer (*Capreolus capreolus*). Thus, it seems that *Ca*. N. mikurensis cannot infect wild Cervidae (Beninati et al. 2006; Beck et al. 2014b). This assumption was additionally supported by the fact that *Ca*. N. mikurensis has not been found in roe deer (n = 48), red deer (n = 107) and fallow deer (n = 13) in a survey including Slovenia, Croatia and Bosnia and Herzegovina (Beck et al. 2014b). The same study revealed that, in addition to small rodents, *Ca*. N. mikurensis-DNA was present in 30 % of Brown bears (*Ursus arctos arctos*) (3/10), 33 % of European badgers (*Meles meles*) (10/30), 13 % of chamois (*Rupicapra rupicapra*) (7/54) and 1 % of European mouflons (1/62). Interestingly, all 74 investigated red foxes (*Vulpes vulpes*) were negative for *Ca*. N. mikurensis. In a study of northern white-breasted hedgehogs (*Erinaceus roumanicus*) from parks in central Budapest, Hungary (Földvári et al. 2014), *Ca*. N. mikurensis-DNA was present in 2.3 % (2/88) ear tissue samples. Another study from Hungary reported no *Ca*. N. mikurensis detection in birds (Hornok et al. 2014).

Clinical cases in humans

In Europe, clinical symptoms caused by *Ca*. N. mikurensis infections have mainly been described in immune-compromised patients (Grankvist et al. 2014). Only one case of neoehrlichiosis in a previously healthy male patient was thus far reported (von Loewenich et al. 2010). Fourteen cases of neoehrlichiosis were reported, with the patients having a median age of 61, being mostly male, and originating from Sweden, Switzerland, Germany, and the Czech Republic (Table 5). Based on the current knowledge of the geographical distribution of *Ca*. N. mikurensis in questing *I. ricinus* (Table 1), neoehrlichioses in (immune-compromised) patients are expected to occur basically all over Europe (Hansford et al. 2014; Jahfari et al. 2012). Less than half of these patients recalled tick exposure, making this a poor predictor of neoehrlichiosis. Most patients had ongoing or recent immune suppressive treatment or were asplenic. The most frequent symptoms were fever, localized pain in muscles and/or joints, vascular and thromboembolic events (Grankvist et al. 2014). Typical laboratory findings were elevated C-reactive protein, leukocytosis with neutrophilia, and anemia.

Generally, patients recovered upon standard treatment with doxycycline. *Candidatus* N. mikurensis is apparently able to infect immune competent people as well; a recent study from China reported seven cases of *Ca*. N. mikurensis infection after screening blood samples from 622 febrile patients by PCR (1.1 %). All patients had a relatively mild disease: fever, headache, and malaise were reported for all seven patients, and none had a history of underlying immune-compromising conditions (Li et al. 2012). It was not reported whether the patients recovered upon treatment with antibiotics. Five cases of asymptomatic *Ca*. N. mikurensis infections in immune-competent individuals (1.6 %) were found by screening blood samples from 316 foresters in Poland by PCR (Welc-Faleciak et al. 2014b). How long *Ca*. N. mikurensis may circulate in healthy individuals is unknown. It is also unclear whether *Ca*. N. mikurensis infections may be transmitted via blood transfusion as described in eight cases of *A. phagocytophilum* infection (Townsend et al. 2014) and one case of ehrlichiosis (Regan et al. 2013). To what extend co-infections of *Ca*. N. mikurensis with other tick-borne pathogens, e.g. *Borrelia burgdorferi* s.l., can

Table 5 Reported human cases of neoehrlichiosis (until December 2014)

Year	Location	Case			References
		Gender	Age (years)	Cause of immune suppression	
2007	Germany	Male	69	Immunosuppressive therapy	von Loewenich et al. (2010)
2008	Germany	Male	57	Previously healthy	von Loewenich et al. (2010)
	Czech Republic	Female	55	Mantle cell lymphoma	Pekova et al. (2011)
2009	Sweden	Male	77	Chronic lymphocytic leukemia, asplenic	Welinder-Olsson et al. (2010)
	Switzerland	Male	61	Coronary artery bypass grafting	Fehr et al. (2010)
	Czech Republic	Male	58	Liver transplantation and splenectomy	Pekova et al. (2011)
2011	Switzerland	Male	68	Chronic lymphocytic leukemia	Maurer et al. (2013)
	Sweden	Male	77	B cell chronic lymphocytic leukemia, splenectomy	Grankvist et al. (2014)
		Female	67	Follicular lymphoma, (inborn) asplenic	Grankvist et al. (2014)
2012	Switzerland	Male	58	Follicular lymphoma	Maurer et al. (2013)
2013	Sweden	Female	67	T-cell large granular lymphoma splenectomy	Grankvist et al. (2014)
		Male	54	Psoriasis, immunosuppressive therapy	Grankvist et al. (2014)
		Male	59	Rheumatoid arthritis, splenectomy	Grankvist et al. (2014)
2014	Sweden	Female	71	Immunosuppressive therapy	Andréasson et al. (2015)

All but one patient had immune-compromised preconditions. The table is compiled from tables in Jahfari et al. (2012) and Grankvist et al. (2014)

affect disease severity has not been investigated so far (Horowitz et al. 2013; Lantos and Wormser 2014).

Candidatus N. mikurensis infection and clinical cases in domestic animals

Candidatus N. mikurensis has been identified as one of the most prevalent microorganism in *I. ricinus*, indicating frequent exposure of animals to this potentially pathogenic species (Michelet et al. 2014). So far, one single case of the disease in an 8-year-old female dog has been described by Diniz et al. (2011). After ovariohysterectomy and mastectomy, the dog became lethargic and developed a profuse subcutaneous haemorrhage around the surgery site. Three days after surgery, the dog developed a subcutaneous hematoma. Laboratory findings included thrombocytopenia and mild anaemia, whereas serum chemistry profile and coagulation time showed normal results. Antibodies against vector-borne pathogens were not detected whereas PCR, performed 5 days after surgery, was positive for *Ehrlichia/Anaplasma* spp. Unfortunately, sequencing was not performed but treatment with doxycycline resulted in clinical stabilisation and resolution of the thrombocytopenia. Two months later, the dog was neutropenic, and this finding persisted over the next 2 months despite repeated treatments with doxycycline for 4 weeks. At that moment, *Ca*. N. mikurensis was confirmed by sequencing, and the treatment resulted in the elimination of the organism from the bloodstream. Haematological changes from the first and the second infection varied, and it was not possible to conclude whether *Ca*. N. mikurensis had caused thrombocytopenia, mild anaemia or neutropenia.

Based on the described findings in this dog, it is rather difficult to compare the clinical signs with the changes described in the human cases. Neutropenia persisted despite the elimination of *Ca*. N. mikurensis and deferred from the first findings. Based on the facts that a febrile stage was absent and there were non-specific haematological changes, it may be concluded that *Ca*. N. mikurensis was not the single cause of disease in this particular case. Recently, in a retrospective study with archival tissues from 19 dogs which died from haemolytic anaemia, *Ca*. N. mikurensis was detected and confirmed by sequencing in a 3.5 months old puppy (Beck et al. 2014a). This finding represents the first case in which *Ca*. N. mikurensis may have been pathogenic for younger dogs. There is no evidence of pathogenicity in other domestic animal species. A big progress in the knowledge of several canine vector-borne diseases has been made with frequent use of fast tests in small animal clinics. Such a test for detecting infection with *Ca*. N. mikurensis does not exist so far. Therefore, the role of *Ca*. N. mikurensis in disease development in companion animals remains unclear.

Public health relevance

Based on current findings, incidences and estimations of the disease burden caused by *Ca*. N. mikurensis infections cannot be given—they will certainly be much lower than for Lyme borreliosis, tick-borne encephalitis, or Mediterranean spotted fever, but higher than for human granulocytic anaplasmosis in Europe, with the ubiquitous prevalence of the causing agent in ticks but almost no reported human cases. The public health relevance of *Ca*. N. mikurensis is currently not caused by high mortality or morbidity rates in the general population, but probably rather by the obliviousness of most health professionals for neoehrlichiosis (Grankvist et al. 2014; Maurer et al. 2013). Medical specialists would recognize the atypical symptoms of patients with neoehrlichiosis, but are currently unable to provide an etiological diagnosis. As a consequence, these patients may not get an adequate antibiotic treatment, or only very late, or they may get 'treatment' based on a different diagnosis, e.g. Lyme borreliosis. Increasing awareness (alone) may not be sufficient. The development of clinical case definitions for 'probable' and 'possible' neoehrlichiosis, together with laboratory diagnostics, will be highly supportive for medical specialists in order to gain 'confirmed' cases and thus counts necessary to judge the impact of this disease on public health.

In The Netherlands alone, it can be estimated that approximately 60,000 persons are annually bitten by ticks infected with *Ca*. N. mikurensis, compared to 183,000 that are bitten by ticks infected with *B. burgdorferi* sensu lato, the causative agent of Lyme borreliosis (Fonville et al. 2014; Jahfari et al. 2012). Thus, exposure of people to ticks infected with *Ca*. N. mikurensis is relatively high, and is expected to be on the rise. In The Netherlands, the incidence of (reported) tick bites and erythema migrans has increased three- to fourfold over the past 2 decades, which could be explained partially by a concomitant increase in diagnostic requests and/or the total number of questing *I. ricinus* (Hofhuis et al. 2015; Sprong et al. 2012). Environmental (e.g. landscape management and climate change), socio-economic and demographic factors (e.g. population aging and life-styles) synergistically increase the risk of acquiring tick-borne diseases (Godfrey and Randolph 2011; Medlock et al. 2013; Sprong et al. 2012; Stefanoff et al. 2012). Furthermore, an ageing population and advanced treatments for patients with haematological, oncologic and rheumatological diseases increase the numbers of immune-compromised persons in the general population who have a higher risk for developing neoehrlichiosis (Cascio et al. 2011).

Gaps of knowledge and need for future research

Until now, *Ca*. N. mikurensis has not been cultured. Its closest relative, *Ca*. Neoehrlichia lotoris, is cultivatable in a tick cell line and this might be a promising approach for *Ca*. N. mikurensis too (Yabsley et al. 2008a). A culture is still the key to the development of molecular and serological diagnostics tools, as well as tests of the specific sensitivity to antibiotics of this bacterium (Raoult 2014). These gaps in knowledge indicate the necessity of research for revealing the impact on human and animal health. The majority of published research deals with the ecology of *Ca*. N. mikurensis and detection in human patients. Further research should also focus on determining the role of *Ca*. N. mikurensis in clinical cases in animals. In addition, it is necessary to determine the role of *Ca*. N. mikurensis in disease development with concurrent infections. To determine the full genome sequence would be widely beneficial: it could be used to devise specific molecular diagnostic tests and it could lead to the identification of protein-coding regions that might be used as recombinant proteins to develop serological diagnostics in case the attempts to isolate *Ca*. N. mikurensis in vitro continue to fail. The genomic information may likewise be useful to identify regions that are involved in pathogenicity during host cell infection. Finally, genomic regions with considerable levels of heterogeneity may be identified, which in turn may allow strain differentiation, phylogenetic analysis or identification of particular transmission cycles.

Although a considerable amount of field data has been gathered over the last decade, no systematic study solely on *Ca*. N. mikurensis has been performed, neither in a natural environment nor using different rodent species in the laboratory, in order to follow the time course of infection on the individual and the population level. These efforts would help determine the real threat that *Ca*. N. mikurensis poses to human and animal health, develop serological methods, and investigate genetic diversity focusing on isolates that cause clinical signs in humans.

Conclusion

Candidatus N. mikurensis is one of those examples where the pathogen was detected before the disease it causes. This course of events is expected to be rather the rule than the exception in the future (Tijsse-Klasen et al. 2014). Several important aspects of *Ca*. N. mikurensis have recently been assessed: rodents are its vertebrate reservoir hosts, it is widely distributed in the vector tick species *I. ricinus* and *I. persulcatus* and it may cause severe diseases in immune-compromised patients. Above all, the isolation of *Ca*. N. mikurensis should be attempted in order to develop diagnostic tools and to experimentally investigate its transmission cycle and pathogenic properties in controlled laboratory experiments.

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Conflict of interest The authors declare that they have no conflict of interest.

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