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Candidatus Neoehrlichia Mikurensis—Recent Insights and Future Perspectives on Clinical Cases, Vectors, and Reservoirs in Europe

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Abstract

Purpose of Review Ticks are among the most important vectors of pathogens concerning animal and human health worldwide. *Candidatus* Neoehrlichia mikurensis (CNM) is a recently discovered intracellular bacterium of the order *Rickettsiales* associated with human clinical cases. In this review, we give an overview on the current knowledge of CNM in connection with diagnosis, clinical cases, and treatment and discuss the newest developments in the knowledge on potential vectors and reservoirs.
Recent Findings Small mammals and in particular rodents seem to be the most likely reservoir hosts for CNM in Europe. Ticks may be competent vectors in which the pathogen is transstadially transmitted. In both, vectors and reservoirs, vertical transmission is controversially discussed. Some recent studies suggested that CNM may be rather rodent- than tick-associated. As regards clinical cases, mainly immunosuppressed persons are affected but evidence of contact to CNM has also been established in some healthy people. Many other aspects such as important life history traits of CNM remain unknown and neglected in both research and diagnosis.
Summary CNM is a highly interesting tick-borne and rodent-associated pathogen that under the right preconditions can cause severe disease in human beings. The cultivation of this intracellular bacterium of the order *Rickettsiales* seems to be the most pressing task to tackle in the future research on this pathogen.

Keywords Neoehrlichiosis · *Ixodes ricinus* · Tick-borne pathogen · *Myodes glareolus*

Introduction

Ticks are among the most important vectors of pathogens of animal and human health concern worldwide. They can transmit pathogens of all diversity such as viruses and bacteria as well as protozoan and metazoan parasites. Amongst the most important zoonotic pathogens transmitted to humans in Central Europe are the tick-borne encephalitis virus and the spirochete *Borrelia burgdorferi* sensu lato [1]. However, in the recent decades, it became more and more evident that also intracellular bacteria of the order *Rickettsiales* can be important agents causing human and animal diseases [2]. Some of these pathogens and the related diseases are now becoming more familiar, such as the Mediterranean spotted fever [3, 4], but others still remain quite unknown, neglected in research, diagnosis, and largely also in their life history traits. Tick-borne pathogens in general are characterized by highly complex life cycles that involve the pathogenic agents, one or more tick species that transmit them, and vertebrate reservoir hosts that are necessary to keep the life cycle in nature. One of these recently discovered intracellular bacteria in connection with several human clinical cases is the *Candidatus* Neoehrlichia mikurensis (CNM) [5, 6]. In this review, we give an overview on the current knowledge of CNM in connection with diagnosis, clinical cases, and treatment and discuss the newest developments in the knowledge on potential vectors and reservoirs.

Clinical Symptoms and Clinical Cases of Neoehrlichiosis in Humans

Most human cases were reported in Europe thus far [7–9]. The first clinical cases of human Neoehrlichiosis was described in

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Germany where a 69-year-old patient who previously suffered from immunosuppressive therapy was diagnosed with Neoehrlichiosis [7]. Clinical cases were reviewed previously in detail [6]. Since then, the awareness of infections with CNM has risen amongst clinicians and practitioners. As a consequence, more and more cases were reported all over Europe. The preconditions for suffering from a clinical Neoehrlichiosis are mostly immunosuppression making CNM most likely an opportunistic pathogen [9]. However, there are also cases of Neoehrlichiosis reported in previously healthy persons [7, 10].

Most frequent symptoms in all reported clinical cases were mild fever, joint pain, and headache [9, 10]. In addition, skin rashes resembling erysipelas or erythema nodosum may occur. In fact, recent studies in patients from the Netherlands, Sweden, and Norway showed that a rash with previous tick-bite is rather caused by CNM than by *Borrelia burgdorferi* (s. l.) [11, 12•, 13•]. Neoehrlichiosis may show similarities to an infection with *Anaplasma* or *Ehrlichia* [5]. Laboratory analysis of blood parameters showed mostly high C-reactive protein levels, leukocytosis, anemia, and neutropenia [14, 15].

Current Knowledge on Diagnosis of CNM in Humans

CNM could not be cultivated in vitro thus far, and this is why the preliminary status as a "*Candidatus*" species is still preserved. Thus far, laboratory Wistar rats were experimentally infected by intraperitoneal injection of a spleen homogenate obtained from previously naturally infected rats which revealed information on the ultrastructure of CNM. CNM was found as small inclusion bodies in endothelial cells from one of these Wistar rats after 2 months of infection [16]. According to similarities in the phylogenetic analysis of the *groEL* and the *16S rRNA* genes, CNM may be placed in the alpha-proteobacterial family *Anaplasmataceae*. Regarding serological features, the only known fact about CNM is the lack of cross-reactions with other *Anaplasmataceae* such as *Anaplasma*, *Ehrlichia*, or *Neorickettsia* [16]. CNM does not belong to the standardized panel of bacteria in clinical diagnostics. This may lead to underdiagnosis of Neoehrlichiosis and even if diagnosed correctly, it is often treated quite late. Usually, after negative testing for other pathogens by serological rapid tests, a conventional PCR is performed targeting the *16S rRNA* gene covering a broad spectrum of bacteria species followed by sequencing [17, 18]. However, a real-time PCR targeting the *groEL* gene as well as conventional PCR methods targeting the *groEL* and the *16S rRNA* genes of CNM are well established and make a faster diagnosis possible [19, 20, 21•].

Treatment of Neoehrlichiosis

Though Neoehrlichiosis is usually diagnosed late after infection, antimicrobial treatment with oral doxycycline (100 mg twice a day) is successful leading to a resolution of clinical symptoms within a few days [9, 22]. Moreover, a continuous decrease of all inflammation markers and normalization of platelet counts are reported in treated cases [22]. Nevertheless, *Neoehrlichia* DNA is still detectable in the patient's serum at high levels over a long course of time. This justifies that treatment should be discontinued after disappearance of CNM DNA in the patient's serum, which may take up to 6 weeks [8]. Rifampin (300 mg twice daily) may be used in patients with suspected hypersensitivity to doxycycline [5]. Treated patients did not relapse and were tested negative by PCR after treatment [9, 23]. To prevent CNM infection, it is important to prevent tick bites. However, patients do not always report a previous tick bite. This is why other sources of infection should be considered, e.g., blood transfusion.

Current Knowledge on Vectors and Reservoirs

Rodents

To date, small mammals and in particular rodents seem to be the most likely reservoir hosts for CNM in Europe [24–26]. In particular, rodent species belonging to the genera *Myodes*, *Microtus*, and *Apodemus* are regarded as main reservoirs [19, 26, 27]. A xenodiagnostic study showed evidence for the reservoir function of the yellow-necked mouse (*Apodemus flavicollis*), the bank vole (*Myodes glareolus*), and the wood mouse (*Apodemus sylvaticus*) [26]. Detailed prevalence rates for CNM in rodents from European countries are shown in Table 1.

The prevalence rates in rodents detected thus far are generally higher compared to prevalence rates in hard ticks, which are potential vectors of this bacterium [19, 25]. The vertical transmission of CNM in rodents is controversially discussed as Andersson et al. (2013) found a lack of CNM in juvenile rodents whose age was previously categorized by their body weight [39]. In contrast, Obiegala et al. (2014) reported 75% positive individuals that were the offspring from one positive *Myodes glareolus* female in Germany [25].

Thus far, no clinical cases were reported where patients had prior contact to rodents. However, a Polish study detected 1.6% CNM-positive immune-competent foresters who work in close contact to both, ticks and rodents [40].

Other Mammals

Small mammals belonging to the genera *Sorex* and *Crocidura*, which are insectivores but not rodents, were tested negative

Table 1 Prevalence of *Candidatus* Neoehrlichia mikurensis in small mammals in European countries as potential reservoirs

Reservoir species	Country	Number of positive individuals/number of individuals tested (prevalence in %)	References
Myodes glareolus	France	5/276 (1.8)[5]	[28]
Apodemus flavicollis	Germany	48/91 (52.7)[1]	[24]
Myodes glareolus			
Apodemus agrarius			
Apodemus flavicollis		36/254 (14.2)[4]	[29]
Myodes glareolus			
Apodemus agrarius			
Microtus arvalis			
Microtus agrestis			
Apodemus flavicollis		181/631 (28.7)[1]	[25]
Myodes glareolus			
Apodemus sylvaticus			
Microtus arvalis			
Microtus agrestis			
Apodemus flavicollis	Hungary	6/177 (3.4)[1]	[30]
Apodemus agrarius		6/348 (1.7)[1]	
Myodes glareolus	Italy	1/34 (2.9)[2]	[31]
Microtus oeconomus Apodemus flavicollis	Poland	0/104 (0)*	[32]
Myodes glareolus			
Microtus spp.	Russia (Siberia)	1/38 (2.6)[3]	[33]
Apodemus peninsulae Myodes rufocanus		3/236 (1.3)[3]	
		1/606 (0.2)[3]	
Apodemus spp.	Slovakia	31/286 (10.8)[5]	[34]
Myodes glareolus		0/36 (0)[1]	[35]
Apodemus spp.			
Myodes glareolus			
Apodemus flavicollis		41/604 (6.8)[1]	[27]
Myodes glareolus			
Micromys minutus			
Microtus arvalis			
Apodemus agrarius		16/997 (1.6)[1]	[36]
Apodemus flavicollis			
Myodes glareolus			
Myodes glareolus	Sweden	50/261 (19)[1]	[37••]
Apodemus flavicollis		68/771 (8.8)[2,5]	[38]
Apodemus sylvaticus			
Microtus agrestis			
Myodes glareolus			
Myodes glareolus	Switzerland	4/103 (3.9)[2]	[26]
Apodemus sylvaticus			
Apodemus flavicollis			
Myodes glareolus	The Netherlands	11/79 (13.9)[1]	[19]
Apodemus sylvaticus			
Microtus arvalis			

[1] Detection method: qPCR targeting the *groEL* gene (102 bp)

[2] Detection method conventional PCR targeting the *16S rRNA* gene (452–1426 bp)

[3] Detection method: nested PCR targeting the *groESL* operon (1320 bp)

[4] Detection method: qPCR targeting the *16S rRNA* gene (257 bp)

[5] Detection method: conventional PCR targeting the *groEL* gene (1024–1233 bp)

*Method not mentioned

and are therefore currently not considered as main reservoir hosts [24, 25, 38]. On the other hand, *Erinaceus roumanicus* was also reported to be positive [41]. Moreover, tested wild cervids (*Capreolus capreolus*, *Dama dama*, *Cervus elaphus*) were also all negative for CNM by PCR [42]. In contrast, the same study revealed prevalence rates ranging from 1 to 33% in European badgers (*Meles meles*), chamois (*Rupicapra rupicapra*), mouflon (*Ovis gmelini musimon*), and brown bears (*Ursus arctos arctos*) [42]. Foxes (*Vulpes vulpes*) and European squirrels (*Sciurus vulgaris*) were thus far all tested

Table 2 Prevalence of *Candidatus* Neoehrlichia mikurensis in ticks from European countries as potential vectors

Tick species	Country	Number of positive ticks/number of ticks collected (prevalence in %)	Tick origin	References
Ixodes ricinus	Austria	22/518 (4.2)^{5}	Vegetation	[49]
		19/86 (22.1)^{2}		[50]
Ixodes ricinus	Belgium	6/373 (1.6)^{2}	Vegetation	[19]
		2/73 (2.8)^{2}	Hedgehog	[51]
Ixodes hexagonus		1/1132 (0.1)^{2}		
Ixodes ricinus	Bosnia and Herzegovina	0/30 (0)*	Vegetation	[52]
Dermacentor reticulatus		0/54 (0)*		
Dermacentor marginatus		0/3 (0)*		
Ixodes ricinus	Bulgaria	1/1291 (0.1)^{2}	Vegetation/various hosts	[53]
Hyalomma spp.		0/60 (0)^{2}	Vegetation/various hosts	
Rhipicephalus spp.		0/150 (0)^{2}		
Haemaphysalis punctata		0/2 (0)^{2}		
Dermacentor marginatus		0/2 (0)^{2}		
Ixodes ricinus	Czech Republic	2/20 (10)^{3}	Vegetation	[54]
		1473 (in 54 pools)		[55]
		(MIR^{1}. 0.4–4.4)^{3}		
		3/138 (2.2)^{2}		[50]
Ixodes ricinus	Denmark	3/2625 (MIR^{1}, 0.1)^{3}	Vegetation	[56]
		2350 (pooled) MIR^{1}, 0.2–0.9^{3}		[57]
		7/661 (1)^{3}	Dogs	[58]
Ixodes ricinus	Estonia	7/548 (1.3)^{4}	Vegetation	[59]
Ixodes persulcatus		0/228 (0)^{4}		
Ixodes ricinus	Finland	0/3158 (0)^{2}	Vegetation	[60]
		0/11 (0)^{2}	Dog ($n = 1$)	
Ixodes ricinus	France	1/60 (1.7)^{2}	Vegetation	[57]
		2350(pooled) (MIR^{1}, 0.2–1.1)^{3}		[54]
Ixodes ricinus	Germany	189/782 (MIR^{1}, 24.2)^{2}	Vegetation	[24]
		15/234 (6.4)^{2}	Rodents	
		44/542 (8.1)^{3}	Vegetation	[54]
		9/111 (8.1)^{3}	Humans	
		51/2315 (2.2)^{2}	Vegetation	[25]
		32/918 (3.5)^{2}	Rodents	
Dermacentor reticulatus		1/40 (2.5)^{2}		
Ixodes ricinus		33/774 (4.3)^{3}	Dogs	[61]
		32/773 (4.1)^{5}	Dogs	[29]
		1/16 (6.25)^{5}	Wild Boar	
Ixodes ricinus	Great Britain	0/954 (0) ^{2}	Vegetation	[62]
		0/338 (0)^{2,3}	Vegetation/various hosts	[19]
Dermacentor reticulatus		0/61 (0)^{2}	Vegetation	[63]
		0/63 (0)^{3}	Vegetation/various hosts	[19]
Haemaphysalis punctata		0/100 (0)^{3}	Vegetation	[63]
Ixodes ricinus	Hungary	2004* (9/37 places)^{5}	Vegetation	[64]
		3/34 (8.8)^{2}		[30]
Dermacentor reticulatus		0/64 (0)^{2}		
Haemaphysalis concinna		0/62 (0)^{2}		
Ixodes ricinus	Italy	20/193 (10.5)^{6}	Vegetation	[65]
		2/433 (0.5)^{2}	Humans	[66]
		10/357 (2.8)^{3}	Humans	[67]
		19/358 (5.3)^{2}	Rodents	[68]
Ixodes hexagonus		0/15 (0)^{2}	Dogs	
Ixodes ricinus	Moldova	1/126 (0.8)^{2}	Vegetation	[69]
Ixodes ricinus	Norway	8/341 (2.3)^{3}	Vegetation	[70]
Ixodes ricinus	Poland	4/1325 (0.3)^{4}	Vegetation	[40]
		0/40 (0)^{3}		[54]
		10/127 (8.1)^{2}	Dogs, cats	[71]
Ixodes hexagonus		1/137 (0.8)^{2}		
Ixodes ricinus	Romania	1/1 (100)^{6}	Humans	[72]
		25/468 (5.3)^{2}	Vegetation	[73]

Table 2 (continued)

Tick species	Country	Number of positive ticks/number of ticks collected (prevalence in %)	Tick origin	References
		7/538 (1.3)^{2}		[74]
Ixodes persulcatus	Russia (Asiatic part and Eastern Siberia)	8/3552 (0.2)^{4}	Vegetation	[75]
		5/2590 (0.2)^{4}		[33]
		2/53 (3.8)^{4}		[47]
Ixodes ricinus		21/295 (7.1)^{3}		[76]
Ixodes frontalis/*I. ricinus*		2/139 (1.4)^{2}	Birds	[48]
Ixodes ricinus	Serbia	3/71 (4.2)^{3}	Vegetation	[77]
Ixodes ricinus	Slovakia	2/68 (2.9)^{2}	Vegetation	[35]
		16/670 (2.39)^{3}		[78]
		4/378–14/121 (1.1–11.6)^{2}		[50]
		34/1375 (2.5)^{2}		[36]
		2/676 (0.3)^{2}	Rodents	
Ixodes trianguliceps		3/112 (2.7)^{2}		
		0/4 (0)^{2}		[27]
Ixodes ricinus		12/933 (1.3)^{2}		
Dermacentor reticulatus		0/1 (0)^{2}		
Haemaphysalis concinna		0/60 (0)^{2}		
		0/75 (0)^{2}	Vegetation	
Ixodes ricinus		62/3874 (1.6)^{2}		
Ixodes ricinus	Spain	2/200 (1)^{4}	Cattle	[79]
Ixodes ricinus	Sweden	57/949 (6)^{2}	Vegetation	[37••]
		18/1356 (1.3)^{5}		[80]
		24/1150 (2.1)^{2,3}	Birds	[45]
Ixodes ricinus	Switzerland	52/818(6.4)^{3}	Vegetation	[81]
		52/268 pools (3.5–8)^{5}		[8]
		15/575 (2.6)^{3}	Rodents	[26]
		7/215 (3.3)^{3}	Birds	[82]
		63/1078 (5.8)^{5}	Vegetation	[83]
Ixodes ricinus	The Netherlands	8/121 (6.6)^{3}	Roe deer	[84]
		160/2002 (8)^{2}	Vegetation	[19]
		26/409 (6.4)^{2}	Red deer	
		4/84 (8.3)^{2}	Wild boar	
		33/264 (12)^{2}	Sheep	
		10/233 (4.3)^{2}	Mouflon	
		300/5343 (5.6)^{2}	Vegetation	[85]
		21/180 (12)^{3}		[46]
		2350 pooled (MIR^{1}, 2.4–3.5)^{2}		[57]
		31/289 (11)^{3}	Humans	[86]
Ixodes hexagonus		0/25 (0)^{2}	Hedgehog	[87]
Ixodes ricinus		0/251 (0)^{2}		
		30/614 (4.9)^{2}	Songbirds	[88]
Ixodes frontalis		0/56 (0)^{2}		

[1] *MIR* minimum infection rate

[2] Detection method: qPCR real-time targeting the *groEL* gene (102 bp)

[3] Detection method conventional PCR targeting the *16S rRNA* gene (452–1426 bp)

[4] Detection method: nested PCR targeting the *groESL* operon (1320 bp)

[5] Detection method: real-time PCR targeting the *16S rRNA* gene (257 bp)

[6] Detection method: conventional PCR targeting the *groEL* gene (1024–1233 bp)

*Method not mentioned

negative [42, 43]. While Hornok et al. (2014) reported the absence of CNM in several bird species (*Luscinia* spp., *Turdus* spp., *Sylvia* spp., and others), a study from Sweden reported CNM in ticks collected from the common redpoll (*Carduelis flammea*), the thrush nightingale (*Luscinia luscinia*), and the dunnock (*Prunella modularis*) [44, 45]. All these results questions the role of other mammals or vertebrates as reservoir hosts for CNM, but they also suggest that more mammals than thought before might be susceptible at least to transient infection.

Clinical cases were reported in three dogs with immunosuppression that were suffering from Neoehrlichiosis suggesting dogs are not reservoir but dead-end hosts similar to humans [20, 42, 43]. The role and significance of CNM in domestic dogs or other domestic animals has thus far not received any major attention.

Occurrence of CNM in Potential Vectors

To date, CNM has been detected in several different tick species such as *Ixodes ricinus* [46], *Ixodes persulcatus* [47], *Ixodes hexagonus* [29], *Dermacentor reticulatus* [25], *Haemaphysalis concinna* [10], *Ixodes ovatus* [16], and *Ixodes frontalis* [48]. Positive ticks have occurred throughout Europe (Table 2). Most positive ticks belong to *Ixodes* spp. suggesting they might be the main vector genus of CNM. There is some evidence that ticks might be competent vectors as ticks collected from the vegetation revealed the same CNM sequence variants as people with Neoehrlichiosis from the same region [7, 8]. Thus far, transstadial transmission in ticks has been suggested but transovarial transmission seems questionable as several studies reported tick larvae as negative for CNM [24, 25]. However, a recent study reported four CNM-positive *I. ricinus* larvae, which were collected directly from vegetation in Austria [50]. This may be explained by an interrupted blood meal of those larvae, but may also be a hint for transovarial transmission in ticks.

Future Research Perspectives

Considering the multitude of aspects still unknown related to this tick-borne pathogen, there are also several needs for future research directions. The most important fact hampering the advancement of knowledge on CNM is the failure to cultivate CNM resulting in scarce information on the morphology and pathogenicity of this pathogen. Successful cultivation of the intracellular CNM would facilitate the development of serological diagnostic tests. The availability of the latter would lead to a significant advance in the knowledge on the epidemiology in terms of real prevalence data and on the clinical significance. This again would also aid clinicians in the diagnosis. Another important future research aspect is the search for the reservoir hosts, the competent vectors to shed light on the transmission cycle of CNM. The availability of an in vitro CNM strain would open the possibility for transmission experiments and pathogenicity studies in controlled laboratory settings. Furthermore, this would open the door to perform co-infection studies with other tick-borne pathogens. Several studies showed that ticks can be infected with more than just one pathogenic agent [25, 37••, 72, e.g.], and consequently, this may well influence the outcome and severity of clinical cases in humans.

Another gap of knowledge exists on potential direct transmission by rodents. According to recent studies, CNM may be more a rodent- than tick-associated agent, so the question rises if transmission would also be possible through contact to rodents or their excretions.

Conclusions

In conclusion, CNM is a highly interesting tick-borne and rodent-associated pathogen but many aspects of its significance in human and domestic animals are still unanswered. Under particular circumstances, it can cause severe disease in human beings. Finally, the cultivation of this intracellular bacterium of the order *Rickettsiales* seems to be the most pressing task to tackle on research on this pathogen.

Compliance with Ethical Standards

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

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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