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Short communication

# Clinical picture and outcome of the first identified case of human *Neoehrlichia mikurensis* infection in Finland

Ulla Hohenthal [a,1], Jessica Tikkala [b,c,1,*], Varpu Rinne [b,c], Riikka Österback [b], Anniina Keskitalo [b], Annukka Pietikäinen [b,c], Jukka Hytönen [b,c]

[a] Department of Infectious Diseases, University of Turku and Turku University Hospital, Turku, Finland
[b] TYKS Laboratories, Clinical Microbiology, Turku University Hospital, Turku, Finland
[c] Institute of Biomedicine, Faculty of Medicine, University of Turku, Turku, Finland

## 1. Introduction

*Neoehrlichia mikurensis* is a tick-borne bacterium causing the infectious disease neoehrlichiosis. The bacterium is maintained in an enzootic cycle involving ticks and wild rodents in Europe and northern Asia (Kawahara et al., 2004). In 2010, *N. mikurensis* was identified for the first time as a human pathogen in Sweden (Welinder-Olsson et al., 2010), and since then, neoehrlichiosis cases have been reported in several European countries (Fehr et al., 2010; Lenart et al., 2021; von Loewenich et al., 2010), as well as in China (Li et al., 2012). In South Africa, one patient infected by a species closely related to *N. mikurensis* has been described (Bamford et al., 2023). The described patients have mainly been immunosuppressed, splenectomised and/or with hematologic neoplasms. Fever of unknown origin is a common presentation of *N. mikurensis* infection in immunosuppressed patients, accompanied by vascular events, thrombophlebitis and/or deep vein thrombosis. However, *N. mikurensis* may also infect immunocompetent patients, resulting in an asymptomatic or mild infection (Boyer et al., 2021). This intracellular pathogen has proven to be difficult to culture, and diagnosis requires polymerase chain reaction (PCR) based methods (Wass et al., 2019). The presence of *N. mikurensis* in Finnish ticks has previously been reported (Laaksonen et al., 2018), while no human cases have been described in the country until now.

## 2. Methods

All laboratory analyses were performed at the laboratory of Clinical microbiology at Turku University Hospital, unless otherwise stated. For *N. mikurensis* PCR, approximately 2 ml of EDTA-plasma or CSF was concentrated by centrifugation (16 000 g x 5 min). Total DNA was extracted from 220 µl of concentrated sample with NUCLISENS® easyMAG® extraction platform (bioMérieux) using an elution volume of 55 µl. A real-time PCR specific for a 169-bp segment of the *groEL* gene of *N. mikurensis* was performed using Mic qPCR cycler (Bio molecular systems) (Table 1). Amplifications were performed in a 15-µL reaction mixture with 5 µL of DNA template. Reaction conditions were 95 °C for 5 min, followed by 45 cycles at 95 °C for 10 s, and a final cycle at 54 °C for 50 s. Ct values >40 were considered negative. 16S rRNA gene PCR and sequencing were performed as described before (Rantakokko-Jalava et al., 2000).

### 2.1. Ethics

A written, informed consent was obtained from the patient for the publication of the case.

### 2.2. Case report

In the beginning of October 2023, a 38-year-old man with follicular lymphoma, diagnosed a year earlier, presented with 39 °C fever and a slight cough, in Turku University Hospital (Turku, Finland). He had been splenectomised, as a part of a desmoid tumor operation, in March 2023. Lymphoma treatment with rituximab (800 mg)- bendamustine (180 mg) was initiated in June 2023. The patient had received his fifth rituximab-bendamustine infusion seven days before the onset of fever. He was in good general condition. The leukocyte count was normal and C-reactive protein (CRP) 19 mg/L (Fig. 1). A viral infection was suspected, and the patient was discharged. Two days later (day 2), he presented with persistent fever and swelling of the left arm with a striated erythema. An ultrasound revealed thrombophlebitis from the shoulder to the distal

* Correspondence author at: Kiinamyllynkatu 10/PL 52, 20 521 Turku, Finland.
*E-mail address:* jessica.tikkala@varha.fi (J. Tikkala).
[1] Equal contribution.

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**Table 1**
N. mikurensis PCR.

| Reagent | | Conc. per reaction | Ref. |
|---|---|---|---|
| 2x SensiFAST Probe No-ROX Mix | | 1x | |
| groEL_F | 5′- CGG AAA TAA CAA AAG ATG GA −3′ | 0,4 μM | (Grankvist et al., 2015) |
| groEL_R | 5′- ACC TCC TCG ATT ACT TTA G −3′ | 0,4 μM | |
| groEL probe | 5′- 6-FAM- TTG GTG ATG GAA CTA CA -MGB-Eclipse −3′ | 0,1 μM | |
| GH1_fwd[#] | 5′- GCC TTC CCA ACC ATT CCC TTA −3′ | 0,5 μM | this study |
| GH1_rev[#] | 5′- TCA CGG ATT TCT GTT GTG TTT C −3′ | 0,5 μM | |
| GH1_probe[#] | 5′- ROX- TGC AGG CAG ATG AGC ACA CGC TGA -BHQ2 −3′ | 0,25 μM | |

[#] Internal control primers and probe amplifying human growth hormone gene.

forearm. Treatment with oral cephalexin (500 mg every 8 h) and subcutaneous enoxaparin (40 mg/day) was commenced. In telephone contact on days eight and 14, the patient reported ongoing fever at 38 °C, a slight cough, and an episode of vomiting. CRP was 52 mg/L. Blood cultures, and cytomegalovirus and Epstein-Barr virus PCR test results were negative. A computer tomography of the thorax was free from pathological findings. Antibiotic treatment was changed to oral amoxicillin-clavulanate (500/125 mg every 8 h).

The fever persisted, and the patient was admitted to the hospital on day 27. The CRP level was 73 mg/L, procalcitonin (PCT) 1.11 μg/L and leucocyte count was slightly elevated ($10.4 \times 10^9$/L). Treatment with i. v. piperacillin/tazobactam (4 g every 8 h) was initiated. At this point, a clinical suspicion of thrombophlebitis in the right forearm was confirmed by ultrasonography. A positron emission tomography/computed tomography ($^{18}$F-FDG PET/CT) showed no signs of lymphoma or infectious foci. Due to persistent fever and increasing CRP (132 mg/L), levofloxacin treatment (750 mg daily) was added on day 31. Four days later, the patient was fever-free with CRP at 17 mg/L and PCT at 0.25 μg/L, and discharged with levofloxacin treatment on day 37. The fever returned, and the patient was admitted to the hospital on day 43. The patient reported, in addition to fever, headache, neck stiffness and night sweats. The patient's medical history revealed that he had spent time in a tick-endemic area in the southwestern archipelago of Finland, and had been exposed to a tick-bite in the abdomen in September 2023. A tick-borne infection was suspected, and treatment with doxycycline 100 mg twice daily was initiated on day 45. Due to a suspicion of a peripheral catheter infection the patient received also treatment with linetzolid (600 mg twice daily for four days). The CSF sample taken showed no signs of infection. Antibodies against *Borrelia burgdorferi* s.l. and TBEV in serum and CSF samples were negative. Results of all microbiological laboratory assays performed are provided in Supplementary Table 1. The patient responded quickly to doxycycline treatment and was discharged from the hospital. Doxycycline treatment was continued for a total of 21 days.

The plasma sample collected on day 48 (three days after commencement of doxycycline treatment), as well as the retrospectively analysed plasma taken on day 30 (15 days before treatment), both yielded a positive result in *N. mikurensis* PCR with Ct values 23.23 and 25.36, respectively. The CSF sample from Nov 25th (day 45) remained PCR negative. The positive PCR results of the serum samples were verified in the laboratory of Clinical microbiology at Sahlgrenska University Hospital (Gothenburg, Sweden). In addition, 16S rRNA gene sequencing from the plasma samples, confirmed *N. mikurensis* identification. The detected 16S rRNA sequence (submitted to GeneBank, accession number PQ253042) was 100 % identical to GenBank entry CP089285.1 (Azagi et al., 2022).

Two weeks after the commencement of doxycycline treatment, the *N. mikurensis* PCR was still positive (Ct 29.04), but the patient had no signs or symptoms of infection. The control sample, taken two weeks after completed treatment was negative for *N. mikurensis* DNA.

## 3. Discussion

We report the first confirmed case of *N. mikurensis* infection in Finland, detected in the patient's plasma, using real-time PCR specific for the *groEL* gene of *N. mikurensis*.

Lyme borreliosis and tick-borne encephalitis are the most common tick-borne infections in Finland. Ticks of the *Ixodes* species may also carry less well-known, so-called emerging tick-borne disease pathogens, including *Neoehrlichia mikurensis, Anaplasma phagocytophilum, Borrelia miyamotoi, Rickettsia helvetica*, and *Babesia* species. Human disease caused by these pathogens are increasingly documented in Europe (Quarsten et al., 2023).

*Neoehrlichia mikurensis* bacteria have been detected *in I. ricinus* ticks in Finland, with a prevalence of a few percentages (0.5 – 5.8 %) (Laaksonen et al., 2018; Sormunen et al., 2020) Interestingly, it has been

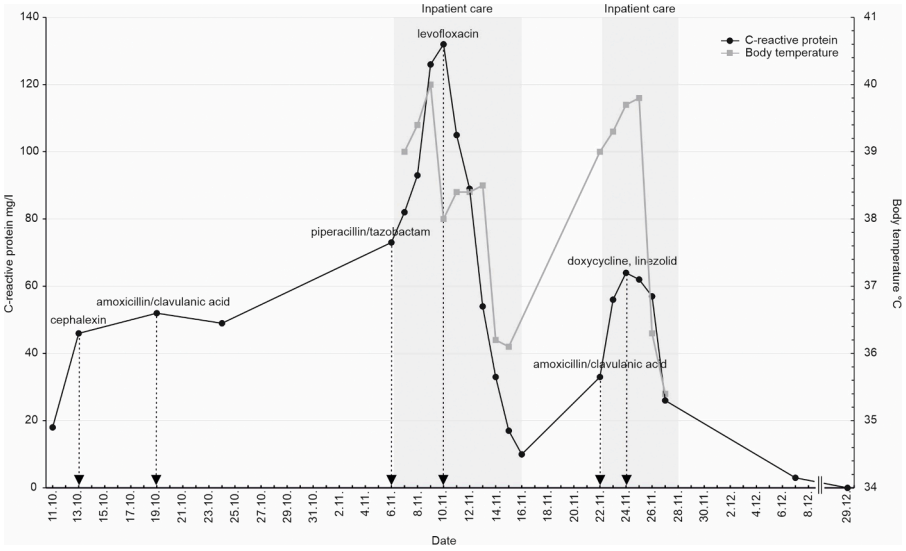


**Fig. 1.** Antibiotic treatments, C-reactive protein levels and body temperature ( °C) during the infection. Body temperatures readings were available only for periods of inpatient care (marked by grey background). The dates of initiation of antibiotics are marked with arrows.

suggested that *N. mikurensis* often co-infects ticks together with *Borrelia afzelii*, supporting the theory of a common rodent reservoir (Sormunen et al., 2020). This in turn, leads to an increased probability of co-infection with both pathogens in a human host. However, in our patient, antibodies against borrelia (and TBEV) were negative.

Infection with *N. mikurensis* should be considered a differential diagnosis in tick-exposed patients with persistent fever, venous thrombosis or thrombophlebitis and a concurrent hematologic malignancy and/or immunosuppression. Splenectomy and rituximab treatment are also previously documented predisposing factors. In addition, *N. mikurensis* infections should also be considered in immunocompetent patients with persisting fever, especially in the presence of recurrent arteritic vascular events (Höper et al., 2021).

Since *N. mikurensis* does not grow in routine blood cultures, but instead requires a specific PCR-based method for detection, cases of neoehrlichiosis may be missed, or the diagnoses may be delayed. This highlights the need of education to clinicians and clinical microbiology laboratories regarding novel tick-borne pathogens.

## CRediT authorship contribution statement

**Ulla Hohenthal:** Writing – original draft, Investigation, Data curation, Conceptualization. **Jessica Tikkala:** Writing – original draft, Data curation, Conceptualization. **Varpu Rinne:** Writing – original draft, Methodology. **Riikka Österback:** Writing – original draft, Methodology. **Anniina Keskitalo:** Writing – original draft, Methodology. **Annukka Pietikäinen:** Writing – original draft, Visualization, Conceptualization. **Jukka Hytönen:** Writing – original draft, Conceptualization.

## Declaration of competing interest

The authors have no conflict of interest regarding the topic of the study. No external funding was received for this study.

## Data availability

No data was used for the research described in the article.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ttbdis.2024.102395.

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