Infections with Candidatus Neoehrlichia mikurensis and Cytokine Responses in 2 Persons Bitten by Ticks, Sweden

Anna Grankvist,1 Lisa Labbé Sandelin,1 Jennie Andersson, Linda Fryland, Peter Wilhelmsson, Per-Eric Lindgren, Pia Forsberg, Christine Wennerås

The prevalence of Candidatus Neoehrlichia mikurensis infection was determined in 102 persons bitten by ticks in Sweden. Two infected women had erythematous rash(es); 1 was co-infected with a Borrelia sp., and the other showed seroconversion for Anaplasma phagocytophilum. Both patients had increased levels of Neoehrlichia DNA and serum cytokines for several months.

Candidatus Neoehrlichia mikurensis is a tick-borne pathogen found in Europe and Asia (1). It causes an infectious disease in immunocompromised persons that is characterized by fever and thromboembolic events (2). In contrast, Candidatus N. mikurensis infection in immunocompetent hosts has been linked to asymptomatic infection (3), systemic inflammation with various symptoms (4,5), and possibly lethal infection (6). Knowledge regarding the capacity of Candidatus N. mikurensis to cause disease in immunocompetent persons is still limited. The purpose of this study was to investigate the prevalence, rate of co-infections, clinical picture, and cytokine response to Candidatus N. mikurensis infection in immunocompetent patients participating in the Tick-Borne Diseases Study (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/21/8/15-0060-Techapp1.pdf).

The Study

The study was approved by the Ethics Committees of Linköping University (M132-06), and Åland Health Care (2008-05-23). DNA was robot-extracted (MagNA Pure Compact Extraction Robot; Roche, Basel, Switzerland) from 400 μL of EDTA-plasma (Nucleic Acid Isolation Kit I; Roche) and analyzed by using a real-time PCR specific for a 169-bp segment of the groEL gene of Candidatus N. mikurensis. Amplifications were performed in a 20-μL reaction mixture containing 1× FastStart Taqman Probe Master (Roche), 1 μmol/L of each primer (5′-CGG AAA TAA CAA AAG ATG GA-3′; 5′- ACC TTC TCG ATT ACT TTA G-3′), 100 nmol/L of probe (5′-6FAM-TTG GTG ATG GAA CTA CA-A-MGB-3′), and 4 μL of DNA template. Real-time PCR was performed by using Rotorgene 6000 (QIAGEN, Hilden, Germany). Reaction conditions were 95°C for 10 min, followed by 45 cycles at 95°C for 15 s, and a final cycle at 54°C for 1 min. A synthetic plasmid containing the 169-bp sequence cloned into a pUC57 vector (Genscript, Piscataway, NJ, USA) was used to estimate bacterial gene copy numbers. Positive samples were verified by using a pan-bacterial PCR specific for the 16S rRNA gene (online Technical Appendix). All PCR products were sequenced after electrophoresis on 2% agarose gels and analyzed by using an ABI PRISM 3130 Genetic Analyzer (Life Technologies Europe BV, Bleiswijk, the Netherlands). Obtained DNA sequences were edited and further analyzed by using the GenBank BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and Ripsseq mixed software (Isentio, Palo Alto, CA, USA).

Patient serum samples were analyzed for antibodies against Borrelia burgdorferi sensu lato by using the RecomBead Borrelia IgM and IgG Kit (Mikroagnostik, Neuried, Germany). Samples were analyzed for IgG against Anaplasma phagocytophilum by using the A. phagocytophilum IFA IgG Kit (Focus Diagnostics, Cypress, CA, USA) and for 20 cytokines by using the Bio-Plex 200 System (Bio-Rad, Hercules, CA, USA).

A total of 102/3,248 study participants sought medical care during the 3-month study period and were further investigated. Their median age was 63 years (range 28–79 years) and 73 (72%) were women. All but 3 participants were immunocompetent (2 had cancer; 1 of them used methotrexate). Candidatus N. mikurensis DNA was detected in 2 (2.0%) of 102 patients, which is consistent with prevalences of 1.1% in China (5) and 1.6% in Poland (3).

Patient 1 was a healthy 68-year-old woman who lived on the island of Tjurkö, southeast of Sweden. She sought medical care on day 77 of the study because of a rash on her right breast. She reported being bitten by a tick in the same

1These authors contributed equally to this article.
The rash of patient 1 may have been caused by co-infection with A. phagocytophilum. The patient was given a diagnosis of erythema migrans and received phenoxymethylpenicillin (1 g, 3×/d for 10 days), and the rash disappeared.

Patient 2 was a 57-year-old woman who lived in Kalmar, Sweden. She had a history of allergy and was regularly taking aspirin. She had received treatment for Lyme borreliosis 8 years earlier. On day 65 of the study, she sought medical care because of a rash on her left breast. She reported being bitten by a tick in the same location 1.5 months earlier. The patient was also given a diagnosis of erythema migrans and received phenoxymethylpenicillin (1 g, 3×/d for 10 days).

Patient 1 had IgM against Borrelia outer surface protein C and pre-existing Borrelia-specific IgG titers that increased during the study (Table 1). Patient 2 was seronegative for Borrelia antigens throughout the study (Table 2). The rash of patient 1 may have been caused by co-infection with a Borrelia spp. Although there was no evidence of a Borrelia infection in patient 2, only 50% of Borrelia culture-positive patients with erythema migrans show development of specific antibodies (7). Moreover, early treatment for erythema migrans might abrogate the IgG response (8), although not always (9). Nevertheless, 20% of patients with erythema migrans show negative results for Borrelia DNA in the skin, which indicates that these rashes might be caused by other infectious agents (10). Our study indicates that an erythematous rash in persons bitten by ticks might not be caused by Borrelia spp. and might require treatment with doxycycline instead of penicillin.

Patient 1 had pre-existing IgG against A. phagocytophilum that remained unchanged (Table 1). Patient 2 had borderline levels of IgG against A. phagocytophilum on day 0, which increased successively on days 65 and 98 (Table 2). This seroconversion may have resulted from cross-reactivity with Candidatus N. mikurensis, which was previously reported for an immunocompetent patient from Switzerland (4). Relatively high rates of seropositivity to A. phagocytophilum in Sweden (11,12) might be caused by cross-reactive antibodies because Candidatus N. mikurensis is common in ticks in Sweden, in contrast to A. phagocytophilum (13).

Both patients showed increased serum levels of cytokines, which appeared to mirror the numbers of Candidatus N. mikurensis gene copies (Figures 1, 2; online Technical Materials).
Appendix Figure). Cytokine levels for patient 1 were maximum on day 77 and returned to reference levels on day 167. All cytokines, except for interferon-γ-induced protein 10, reached maximum levels on day 98 for patient 2. The cytokines were selected because systemic inflammation (Figure 1) with neutrophilia (online Technical Appendix) is typical of neoehrlichiosis in immunocompromised patients (2). In addition, a Th1-like immune response (Figure 2) is presumably required to eliminate an intracellular pathogen, such as Candidatus N. mikurensis. However, the cytokine response of patient 1 may in part have been caused by Borrelia spp. (14).

Conclusions
Candidatus N. mikurensis DNA was detected in the blood of both patients for >1 and 3 months, respectively. Similarly, a healthy person in Poland showed a positive result for Candidatus N. mikurensis twice in a 4-month period (3). This finding suggests that Candidatus N. mikurensis infections persist for a long time or that frequent reinfections occur. Prolonged carriage seems more probable in view of the common occurrence of neoehrlichiosis during winter among immunocompromised patients (2); immunosuppressive therapy might reactivate such infections. An analogous finding was reported in a dog, which was believed to have been a chronic carrier of Candidatus N. mikurensis; infection became symptomatic when immune defenses were compromised by surgery (15).

In conclusion, an erythematous rash in a person bitten by a tick can be caused by Candidatus N. mikurensis, rather than by Borrelia spp. Moreover, immunocompetent persons may be infected by Candidatus N. mikurensis for...
unexpectedly long periods, even after symptoms have disappeared. Patients scheduled to receive immunosuppressive treatment, and who live in *Candidatus* N. mikurensis-endemic areas should be screened for this pathogen before beginning therapy.

This study was supported by ALF-Göteborg (71580); the Cancer and Allergy Foundation (149781); Västra Götaland Region Research and Development (94510); Laboratory Medicine at Sahlgrenska University Hospital (6333); the Medical Research Council of South-East Sweden (FORSS-297311, -307591, and -87231); The Swedish Research Council/Medicine (2011-345); and ALF-Östergötland.

Dr. Granqvist is a molecular biologist in the Department of Clinical Microbiology at Sahlgrenska University Hospital, Göteborg, Sweden. Her research interests are noncultivatable infectious agents, with a focus on *Candidatus* N. mikurensis.

References


Address for correspondence: Christine Wennerås, Department of Clinical Microbiology, Gothenburg University, Guldhedsgatan 10, S-413 46 Göteborg, Sweden; email: christine.wenneras@microbio.gu.se