

“*Candidatus Neoehrlichia mikurensis*”- a new agent of tick-borne infectious disease

Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademien, Göteborgs universitet kommer att offentligen försvaras i föreläsningssalen, Klinisk Mikrobiologi, Guldhedsgatan 13A, Göteborg,

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Av

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Fakultetsopponent: Professor Anders Sjöstedt Institutionen för klinisk mikrobiologi, Umeå Universitet

Avhandlingen baseras på följande delarbeten

- I. Grankvist A, Andersson PO, Mattsson M, Sender M, Vaht K, Hoper L, Sakiniene E, Trysberg E, Stenson M, Fehr J, Pekova S, Bogdan C, Bloemberg G, Wenneras C. **Infections with the tick-borne bacterium “*Candidatus Neoehrlichia mikurensis*” mimic non-infectious conditions in patients with B cell malignancies or autoimmune disease.** *Clin. Infect. Dis* 2014; 58: 1716–1722.
- II. Grankvist A*, Sandelin LL*, Andersson J, Fryland L, Wilhelmsson P, Lindgren PE, Forsberg P, Wenneras C. **Infections with *Candidatus Neoehrlichia mikurensis* and Cytokine Responses in 2 persons bitten by ticks, Sweden.** *Emerg. Infect. Dis* 2015; 21: 1462-1465
- III. Grankvist A, Moore ER, Svensson Stadler L, Pekova S, Bogdan C, Geissdorfer W, Grip-Linden J, Brandstrom K, Marsal J, Andreasson K, Lewerin C, Welinder-Olsson C, Wenneras C. **Multilocus Sequence analysis of clinical “*Candidatus Neoehrlichia mikurensis*” strains from Europe.** *J. Clin. Microbiol* 2015; 53: 3126-3132
- IV. Grankvist A, Sikora P, Wennerås C. **Complete reference genome of a clinical isolate of *Candidatus Neoehrlichia mikurensis* and comparison with two additional Swedish isolates**
In manuscript

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Abstract

“*Candidatus Neoehrlichia mikurensis*” (*Ca. N. mikurensis*) is a tick-borne bacterial pathogen that can cause disease particularly among immune compromised persons. This new infectious disease is called neoehrlichiosis. The clinical picture of neoehrlichiosis is characterized by fever, migrating pain, and vascular/thromboembolic complications. The bacterium received its name in 2004, after its discovery in ticks and rodents on the Japanese island of Mikura. This thesis has four main aims 1) Map this new infectious disease with respect to what types of patients that are afflicted, the clinical picture displayed by the patient categories, and the pattern of laboratory findings seen in infected patients. This is described in paper I; where clinical data of six patients participating in the “NEO-VÄST study” are described together with additional cases from Europe. 2) Determine if *Ca. N. mikurensis* is an opportunist that only afflicts immune compromised patients? In paper II we describe two immune competent patients who had raised levels of *Ca. N. mikurensis* DNA in the blood accompanied by a cytokine response for several months. The patients were diagnosed after PCR screening of plasma samples from 102 tick-bitten persons in Sweden who participated in the Tick-Borne Disease Study called STING. A PCR assay for clinical use was developed in this study and the cytokine levels were measured with multiplex technology. 3) Establish if *Ca. N. mikurensis* strains in Europe vary genetically. Paper III describes the development and use of a multilocus sequence analysis (MLSA) protocol to investigate the genetic diversity of clinical *Ca. N. mikurensis* strains in Europe. A low genetic diversity was seen among the strains, all of which were derived from immune compromised patients. Unexpectedly, *Ehrlichia ruminantium* was found to be the closest relative of *Ca. N. mikurensis* within the family of *Anaplasmataceae*. 4) Perform *de novo* whole-genome sequencing of *Ca. N. mikurensis* to characterize the bacterium. In paper IV we determined the complete reference genome sequence of *Ca. N. mikurensis*, sequenced directly from the blood of three immune suppressed patients. We also compared these sequences with those of other whole-genome sequenced relatives of *Ca. N. mikurensis*. The sequencing strategy relied on library preparation using a new type of technology called 10X Chromium followed by HiSeq Illumina sequencing, sequence assembly and *de novo* annotation. Our studies have yielded more knowledge about this anonymous emerging pathogen but much remains to be resolved, the work continues!

Keywords: Tick-borne, *Candidatus Neoehrlichia mikurensis*, infectious disease, human, neoehrlichiosis