Detection of tick-borne ‘Candidatus Neoehrlichia mikurensis’ and *Anaplasma phagocytophilum* in Spain in 2013

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Abstract

**Background:** ‘*Candidatus Neoehrlichia mikurensis*’ is a tick-borne bacteria implicated in human health. To date, ‘*Ca. Neoehrlichia mikurensis*’ has been described in different countries from Africa, Asia and Europe, but never in Spain. However, according to the epidemiological features of the main vector in Europe, *Ixodes ricinus*, its circulation in our country was suspected.

**Methods:** A total of 200 *I. ricinus* ticks collected in the North of Spain were analyzed. DNAs were extracted and used as templates for PCRs targeting fragment genes for *Anaplasma/Ehrlichia* detection. The amplified products were sequenced and analyzed.

**Results:** ‘*Ca. Neoehrlichia mikurensis*’ was amplified in two specimens. Furthermore, *Anaplasma phagocytophilum* was detected in 61 samples analyzed.

**Conclusions:** The detection of ‘*Ca. Neoehrlichia mikurensis*’ in *I. ricinus* ticks from Spain indicates its circulation and the potential risk of contracting a human infection in this country.

**Keywords:** ‘*Candidatus Neoehrlichia mikurensis*’, *Anaplasma phagocytophilum*, *Ixodes ricinus*, Spain

Background

‘*Candidatus Neoehrlichia mikurensis*’ is an obligate intracellular bacterium member of the *Anaplasmataceae* family. It was first isolated from wild rats (*Rattus norvegicus*) and *Ixodes ovatus* ticks in the Mikura Island, Japan [1]. It was classified as a new genus (*Neoehrlichia*) added to those already known of the *Anaplasmataceae* family: *Ehrlichia*, *Anaplasma*, *Neorickettsia*, *Aegyptianella* and *Wolbachia* [1].

The presence of ‘*Ca. Neoehrlichia mikurensis*’ in rodents and ticks has been notified from different countries of Europe, Asia and Africa in the last decade [2,3]. In Europe, it has been mostly detected in *Ixodes ricinus*, although it has been associated to other tick species in other continents. *I. ricinus*, endemic in the North of Spain, is responsible for most human tick bites. It acts as vector of different human pathogens, such as *Borrelia burgdorferi* sensu lato (s.l.), *Anaplasma phagocytophilum* -formerly *Ehrlichia phagocytophila* - or different *Rickettsia* spp., protozoa and arboviruses. However, the risk of infections with ‘*Ca. Neoehrlichia mikurensis*’ to human health remains unclear in southern Europe.

The first implication of the bacterium in human pathology was reported in Sweden in 2010 [4]. Subsequently, seven new human cases severely affected by ‘*Ca. Neoehrlichia mikurensis*’ infections have been notified from Europe [5-8]. Several human cases have also been described in China [2].

‘*Ca. Neoehrlichia mikurensis*’ has not been previously described in Spain. However, according to the epidemiological features of the main vector, *I. ricinus*, in which the bacterium has been mostly detected in Europe, its circulation in our country was suspected.

**Methods**

In the routine analysis of tick-borne pathogens performed in the Center of Rickettsioses and Arthropod-borne Diseases (Logroño, Spain), 200 *I. ricinus* ticks collected from...
cows were tested. Samples were obtained in two different locations of La Rioja (Spain): Tobía (42°18' N; 2°48' W) and Jubera (42°18' N; 2°17' W) in April 2013. A total of 50 males and 50 engorged females from each location were processed. Ticks were kept at -80°C until DNA extraction with Qiagen DNeasy Blood & Tissue Kit, according to the manufacturer's instructions (Qiagen, Hilden, Germany).

All DNA extracts were used as templates for two nested PCRs targeting fragment genes for *Anaplasma*/*Ehrlichia* detection. Furthermore, a simple PCR was performed to confirm the amplification of species never detected in the area (Table 1) [9-11]. Two negative controls, one of them containing water instead of template DNA and the other with template DNA but without primers, as well as a positive control of *A. phagocytophilum* were included in all PCR assays. Amplification products were sequenced, and nucleotide sequences were compared with those available in GenBank by using a Basic Local Alignment Search Tool (BLAST) search (http://www.ncbi.nlm.nih.gov/blast).

### Results and discussion

Two nucleotide sequences of *groESL* fragment gene (1%) corresponding to 2 male tick specimens collected in Tobía showed 100% identity with *Ca. Neoehrlichia mikurensis*. They were identical to the one detected in two patients in Germany [5]. None of them yielded positive results when PCR tests for 16S rRNA were performed. For this reason, a different fragment of the 16S rRNA gene (EHR) was investigated to confirm our previous results. Nucleotide sequences of both samples were identical to each other and showed 100% identity with more than one sequence of *Ca. Neoehrlichia mikurensis* (Table 2). In our laboratory we had never worked with *Ca. Neoehrlichia mikurensis* before, so no contamination with this bacterium was possible.

### Table 1 PCR primer pairs used in this study

| Gene target | Primer name | Primer sequence 5'→3' | Amplified fragment (bp) | Annealing temperature (°C) | Reference |
|-------------|-------------|------------------------|-------------------------|-----------------------------|-----------|
| *groESL* heat shock operon of *Anaplasma* spp. (nested) | HS1a | AITGGGCTGGTAITGAAAT | 1350 | 48 | [9] |
| | HS6a | CICCIGGAIACCACTCTG | | | |
| | HS43 | ATATGCIGAAACAAGCATGTC | 1297 | 55 | |
| | HSVR | CTCAGACGTACTGAGC | | | |
| 16S rRNA (nested) | ge3a | CACATGCAAGTCGAACGGATTATTC | 932 | 55 | [10] |
| | ge10r | TCCGTTAAGAAGGATCTAATCTCC | | | |
| | ge9f | AAGGGATTACCTTATAACTTCTG | 546 | 55 | |
| | ge2 | GCGAGTATACAGACGCTACAGG | | | |
| 16S rRNA EHR | EHR 16SD | GGTACCTCATCCTGACG | 345 | 55 | [11] |
| | EHR 16SR | TAGCACTCATCCTGACG | | | |

### Table 2 *Anaplasmataceae* species detected in *Ixodes ricinus* removed from cows (N = 200) in La Rioja (North of Spain)

| Bacterium (no.) | *groESL* | *16S rRNA* | *16S rRNA-EHR* |
|-----------------|---------|------------|---------------|
|                 | Genbank accession no. | Identity (%) | Genbank accession no. | Identity (%) | Genbank accession no. | Identity (%) |
| 'Ca. N. mikurensis' (2) | 2 M | EU810407 | 100 | 2 M | JQ675350 | 100 |
| *A. phagocytophilum* (61) | Human pathogenic variant (8) | 1 M, 2 F | U72628 | 99.9-100 | 1 F* | U29251 | 100 |
| | Non-pathogenic variants (53) | 1 M | AF478558 | 100 | 1 M | JN181071 | 100 |
| | | 1 M | AF478563 | 100 | 1 F | EU839849 | 100 |
| | | 2 F | EU246959 | 99.8-100 | 1 M, 4 F | JN181071 | 100 |
| | | 5 F | AF548385 | 99.8-100 | 2 F | JN181071 | 99.9-100 |
| | | 1 M | JY81830 | 100 | 1 M | JN181071 | 100 |

No.: number; 'Ca. N. mikurensis': *Candidatus Neoehrlichia mikurensis*; *A. phagocytophilum*: *Anaplasma phagocytophilum*; M: Male; F: Female; *: only amplified with 16S rRNA fragment gene; †: Two of them only amplified with 16S rRNA fragment gene.
On the other hand, *A. phagocytophilum* was detected in 61 samples (30.5%) of this study. Specifically, 8 specimens (4%) showed maximum identity with the human pathogenic variant, and 53 (26.5%) with non-pathogenic variants (Table 2).

In this study, ‘*Ca. Neoehrlichia mikurensis*’ DNA was detected in two ticks from La Rioja (Spain) during 2013 but we do not know if this bacterium has been previously circulating in our area. Anyway, this infection may be underdiagnosed in our media. In addition, according to the recent finding of several human cases due to this bacterium, mainly in immunocompromised patients, physicians should be aware of the risk for those patients in the affected area. Moreover, infections and fever of unknown origin are common in immunocompromised patients and the responsible pathogen is not isolated in most cases [7]. The detection of ‘*Ca. Neoehrlichia mikurensis*’ and the features of the European human cases suggest that this microorganism is likely causing disease in our country too.

The prevalence of *A. phagocytophilum* in the studied area has been previously reported [12]. According to our results, the high prevalence of the bacterium in the engorged females collected in Jubera should be noted (40 out of 50 specimens, 80%). This could be due to the fact that all the female specimens processed were engorged on cows, hosts that are potential amplifiers of the bacterium [13].

**Conclusions**

‘*Ca. Neoehrlichia mikurensis*’ has been detected in *I. ricinus* ticks removed from cows in Spain. *A. phagocytophilum* was amplified in 61 out of 200 samples (8 of them corresponding to the human pathogenic variant). Our results suggest that human infections by ‘*Ca. Neoehrlichia mikurensis*’ might be undiagnosed in this country. Further research should be carried out to study the epidemiology of the bacterium as well as to be aware of possible human cases.

**Competing interests**

The authors declare they have no competing interests.

**References**

1. Kawahara M, Rikihisa Y, Isogai E, Takahashi M, Misumi H, Sato C, Shibata S, Zhang C, Tsuji M. Ultrastructure and phylogenetic analysis of * Candidatus Neoehrlichia mikurensis* in the family *Anaplasmataceae*, isolated from wild rats and found in Ixodes ovais ticks. *Int J Syst Evol Microbiol* 2004, 54:1837–1843.

2. Li H, Jiang JF, Liu W, Zheng YC, Huo QO, Tang K, Zuo SY, Liu K, Jiang BG, Yang H, Gao WC. Human infection with * Candidatus neoehrlichia mikurensis*. *China, Emerg Infect Dis* 2012, 18:1636–1639.

3. Kamani J, Baneth G, McMuguidh KY, Waziri NE, Eyal O, Guthmann Y, Hanus S. Molecular detection and characterization of tick-borne pathogens in dogs and ticks from Nigeria. *PLoS Negl Trop Dis* 2013, 7:e2108.

4. Weidner-olson C, Jekel F, Voehr K, Jacobson S, Werner R, C. First case of human ‘*Candidatus Neoehrlichia mikurensis*’ infection in a febrile patient with chronic lymphocytic leukemia. *J Clin Microbiol* 2010, 48:1596–1599.

5. von Loewenich FD, Geissdörfer W, Frankova S, Haugvicova R, Mather TN, Solberg VB, Harrus S. Detection of *Candidatus Neoehrlichia mikurensis* in two patients with severe febrile illnesses: evidence for a European sequence variant. *J Clin Microbiol* 2010, 48:2630–2635.

6. Fehr JS, Bloemberg GV, Ritter C, Hombach M, Lüscher TF, Weber R, Keller PM. Septicemia caused by tick-borne bacterial pathogen ‘*Candidatus Neoehrlichia mikurensis*’. *Emerg Infect Dis* 2010, 16:1127–1129.

7. Pekova S, Vydra J, Kabickova H, Frankova S, Haugvicova R, Mazal O, Cmijila R, Harderkopf DW, Jancuskova T, Kazak T. ‘*Candidatus Neoehrlichia mikurensis*’ infection identified in 2 hematolonic patients: benefit of molecular techniques for rare pathogen detection. *Diagn Microbiol Infect Dis* 2011, 69:266–270.

8. Mauer FP, Keller PM, Beute C, Joha C, Achermann Y, Gubler J, Bircher D, Karer U, Fehr J, Zimmerman L, Bloemberg GV. Close geographic association of human neoehrlichiosis and tick populations carrying ‘*Candidatus Neoehrlichia mikurensis*’ in eastern Switzerland. *J Clin Microbiol* 2013, 51:1669–176.

9. Liz JS, Sumner JW, Pfister K, Brossard M. PCR detection and serological evidence of granulocytic ehrlichial infection in roe deer (Capreolus capreolus) and chamois (Rupicapra rupicapra). *J Clin Microbiol* 2002, 40:892–897.

10. Massung RF, Slater K, Joha C, Mathon NN, Solberg VB, Othon JG. Nested PCR assay for detection of granulocytic ehrlichiae. *J Clin Microbiol* 1998, 36:1090–1095.

11. Inokuma H, Raoul D, Brouqui P. Detection of *Ehrlichia plpatibility* DNA in brown dog ticks (*Rhipicephalus sanguineus*) in Okinawa Island, Japan. *J Clin Microbiol* 2000, 38:4219–4221.

12. Portillo A, Perez-Martinez L, Santibañez S, Santibañez P, Palomar AM, Oteo JA. *Anaplasmata* spp. in wild mammals and *Ixodes ricinus* from the North of Spain. *Vector Borne Zoonotic Dis* 2011, 11:113–8.

13. Stuen S, Granquist EG, Slagh C. *Anaplasm phagocytophilum* - a widespread multi-host pathogen with highly adaptive strategies. *Front Cell Infect Microbiol* 2013, 3:331.

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