Anita C. Schürch, Noël Tordo, Marco W.G. van de Bildt, Cornelis E. van Elk, Thijss Kuiken, Rogier Bodewes, and Albert D.M.E. Osterhaus

Author affiliations: Erasmus Medical Centre, Rotterdam, the Netherlands (J.Y. Siegers, M.W.G. van de Bildt, C.E. van Elk, A.C. Schürch, T. Kuiken, R. Bodewes, A.D.M.E. Osterhaus); Dolphinarium Harderwijk, Harderwijk, the Netherlands (C.E. van Elk); SOS Dolphin Foundation, Harderwijk, the Netherlands (C.E. van Elk); Institute Pasteur, Paris, France (N. Tordo); and Viroclinics Biosciences, Rotterdam, the Netherlands (A.D.M.E. Osterhaus)

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Address for correspondence: Rogier Bodewes, Department of Viroscience, Erasmus Medical Centre, Dr. Molewaterplein 50, 3015GE Rotterdam, the Netherlands; email: r.bodewes@erasmusmc.nl

Genetic and Ecologic Variability among Anaplasma phagocytophilum Strains, Northern Italy

To the Editor: The tick-borne pathogen Anaplasma phagocytophi-

lum is an increasing potential public health threat across Europe. Its intra-

specific genetic variability is associated with different reservoir host and vector tick species (1–4); however, the roles of various vertebrates as competent reservoirs of A. phago-

cytophilum in Europe need clarification (1). During March 2011–June 2013, we studied the prevalence and genetic variability of A. phagocyto-

philum in 821 questing Ixodes ricinus ticks (155 adults [A], 666 nymphs [N] collected by standard blanket dragging) and 284 engorged ixodid ticks (61A, 191N, 21 larvae [L]) collected from humans, dogs, sheep, hunted wild ungulates, live-trapped birds, and rodents. Blood samples from 1,295 rodents (yellow-necked mice [Apodemus flavicollis]), bank voles [Myodes glareolus], and harvest mice [Muscardinus avellanarius]) were also analyzed. All animal-handling procedures and ethical issues
were approved by the Provincial Wildlife Management Committee (authorization n. 595 issued on 04.05.2011). The study site, Valle dei Laghi (northeastern Italian Alps), is a well-studied focus of emerging tick-borne pathogens in northern Italy (4).

Tick species were identified morphologically and by molecular analyses by using 16SrRNA sequences. A. phagocytophilum was detected in questing and feeding I. ricinus ticks by using a nested PCR amplification of the partial 16S rRNA gene (546-bp fragment) as described (4,5) and in rodent blood by using a real-time PCR assay targeting the msp2 gene (77 bp) (6). All positive samples were confirmed by using Sanger sequencing.

Overall prevalence of A. phagocytophilum in questing I. ricinus ticks was 1.8% (6A, 9N of 821) (online Technical Appendix Table, http://wwwnc.cdc.gov/EID/article/20/6/13-1023-Techapp1.pdf). Among engorged ticks, only I. ricinus ticks were found positive for A. phagocytophilum, although tick species such as I. hexagonus (20 ticks from dogs and birds), I. trianguliceps (11 from rodents), and I. turdus (1 from a bird) were also analyzed. Infection prevalence in ticks from various hosts was: 4.3% (5N/115) in ticks from humans, 9.1% (1N/30) in ticks from dogs, 14.3% (4A, 1N, 2L/49) in ticks from wild ungulates, 7.7% (1A/30) in ticks from sheep, 10.7% (3N/28) in ticks from birds, and 6.1% (3N/49) in ticks from rodents (online Technical Appendix Table). Prevalence in rodent blood samples (A. flavicolis mice, M. avellanarius mice, M. glareolus bank voles) was 0.3% (4/1,295); only bank voles had positive results. None of the feeding I. ricinus larvae collected from rodents were infected with A. phagocytophilum.

We amplified and sequenced 2 genetic loci, groEL and msp4, from samples that were positive for A. phagocytophilum, which are known to be useful for phylogenetic studies (4,7,8). MrBayes v3.1.2 (http://sourceforge.net/projects/mrbayes/files/mrbayes/3.2.1/) was used to construct Bayesian phylogenetic trees for each gene (9). We deposited 54 new A. phagocytophilum sequences in GenBank with accession numbers KF031380–KF031433. Fourteen and 9 unique groEL and msp4 A. phagocytophilum genotypes, respectively, were found to circulate in this alpine valley.

Figure. Phylogenetic trees expressing 50% majority rule consensus constructed by using Bayesian analysis for Anaplasma phagocytophilum groEL gene partial sequences (1,119 bp) (A) and 73 msp4 gene partial sequences (300 bp) (B). Markov chains were run for 1,000,000 generations. The first 1,000 trees were discarded, and the remaining trees were used to construct the tree. Posterior probabilities of >0.60 are indicated above branches. New sequences are shown in boldface. Each A. phagocytophilum sequence is indicated with source: questing tick (e.g., Ixodes ricinus), engorged tick (e.g., I. ricinus-Human), or host blood (e.g., red deer). Two-letter country codes are given, and GenBank accession numbers are shown in parentheses. Scale bars indicate nucleotide substitutions per site.
The phylogenetic trees for groEL (Figure, panel A) and msp4 (Figure, panel B) loci have similar topologies with strong support for 2 main clades (Figure, panels A and B), each with different host and vector association. The first clade (clade 1) contained sequences from questing *I. ricinus* ticks and engorged ticks collected from humans, dogs, wild ungulates, rodents, sheep, and birds. Our findings suggest that humans are exposed to several *A. phagocytophilum* genotypes exclusively from clade 1 (Figure, panels A and B). Our 3 unique *A. phagocytophilum* sequences were from 3 *I. ricinus* nymphs that fed on the same human clustered within this clade, but no clinical symptoms were observed.

The second clade (clade 2) includes sequences from rodents, specifically, bank voles (*M. glareolus*), other voles and shrews. Among tick species we found *I. persulcatus* to belong to this clade (Figure, panels A and B). We have found no evidence of circulation of this genotype in other hosts or in questing or engorged *I. ricinus* ticks in previously published data or in this study (Figure, panels A and B, clade 2). This finding suggests that the *A. phagocytophilum* genotype associated with mice, voles, and shrews in Europe may be maintained in enzootic cycles by another tick vector, such as *I. trianguliceps*, as observed in the UK for the field vole (*Microtus agrestis*) (8). This so-called ecologic strain probably does not represent an immediate threat to humans in northern Italy, unlike the rodent strain reported in the USA, since it occurs in very low prevalence, and because *I. trianguliceps* is an endophelic tick species that is unlikely to come into contact with humans.

In questing *I. ricinus* tick at the nymphal stage, we detected a groEL sequence (KF031399) identical to a sequence isolated from humans with human granulocytic anaplasmosis in Europe (AF033101). The msp4 sequence for the same sample (KF031406) belonged to clade 1, and contained sequences of a strain found in 96 infected persons in the United States. This suggests that ≥1 human pathogenic strain now circulates in the investigated area. However, we did not find this strain in any of the host-fed ticks analyzed, so the host responsible for maintaining the circulation of this pathogenic strain must be identified before any recommendation for preventive measures can be provided.

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H.C. Hauffe, A. Rizzoli (Institute of Zoology, Slovak Academy of Sciences, Bratislava, Slovak Republic (I. Baráková, M. Derdáklová); Institute of Parasitology SAS, Košice, Slovak Republic (M. Derdáklová); Yale School of Public Health, New Haven, CT, USA (G. Carpi); and Ospedale Santa Chiara, Trento (C. Ramponi)

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Zika Virus, French Polynesia, South Pacific, 2013

To the Editor: Isolated in 1947 from a rhesus monkey in Zika forest, Uganda, Zika virus (ZIKV) is a mosquito-borne flavivirus (1). For half a century, ZIKV was described only as causing sporadic human infections in Africa and Asia, which was mostly confirmed by serologic methods (2). In 2007, the first ZIKV outbreak reported outside Africa and Asia was retrospectively documented from biological samples of patients on Yap Island, Federated States of Micronesia, North Pacific, who had received an incorrect diagnosis of dengue virus (DENV) (3,4). We report here the early investigations that led to identification of ZIKV as the causative agent of an outbreak that started in October 2013 in French Polynesia.

French Polynesia is a French overseas territory located in the South Pacific. The ≈270,000 inhabitants live on 67 islands distributed into 5 archipelagoes (Society, Marquesas, Tuamotus, Gambier, and Austral Islands). Surveillance for acute febrile illnesses is coordinated by the Department of Health with the contribution of a sentinel network of public and private practitioners, the main public hospital (Centre Hospitalier du Taaone), and the public health and research institute (Institut Louis Malardé [ILM]). As part of this syndromic surveillance system, ILM has implemented protocols for detecting arboviruses that are known to cause outbreaks in French Polynesia, such as DENV, or that pose a risk for causing epidemics because of the presence of potential mosquito vectors. In addition, ILM provides DENV serotype identification for other Pacific island countries, including Yap State, as part of the regional surveillance of dengue (5). For that reason, a ZIKV reverse transcription PCR (RT-PCR) protocol by Lanciotti et al. (3) was implemented at ILM.

In October 2013 (week 41), a 53-year-old woman (patient 1) and 2 other members of the household—her 52-year-old husband (patient 2) and her 42-year-old son-in-law (patient 3)—experienced a mild dengue-like illness consisting of low fever (<38°C), asthenia, wrist and fingers arthralgia, headache, and rash. Patients 2 and 3 also had conjunctivitis. Patient 1 had swollen ankles and aphthous ulcers. For all 3 patients, results were negative for DENV by RT-PCR and nonstructural protein 1 (NS1) antigen tests (5), for West-Nile virus by RT-PCR, and for chikungunya virus by RT-PCR; results of RT-PCR for ZIKV were equivocal for patients 1 and 2. During week 43, a 57-year-old patient (patient 4) reported similar symptoms; results of RT-PCR for DENV were negative, but results of RT-PCR for ZIKV were positive. ZIKV infection was then confirmed by sequencing of the genomic position 858–1138 encompassing the prM/E protein coding regions of ZIKV.
Genetic and Ecologic Variability among Anaplasma phagocytophilum Strains, Northern Italy
Technical Appendix Figure. A) 50% majority rule consensus phylogenetic trees constructed by using Bayesian analysis of *Anasplasma phagocytophilum* groEL gene partial sequences (1,119 bp); B) 73 *msp4* gene partial sequences (300 bp). Markov chains were run for 1,000,000 generations. The first 1,000 trees were discarded and the remaining trees were used to construct the tree. Posterior probabilities of >0.60 are indicated above branches. New sequences are shown in boldface. Each *A. phagocytophilum* sequence is indicated with source: questing tick (e.g., *I. ricinus*), engorged tick (e.g., *I. ricinus*-Human), or host blood (e.g., red deer), international organization for standardization α-2 country codes and GenBank accession numbers are in parentheses. Scale bars indicate nucleotide substitutions per site.