Emerging infectious diseases substantially affect public health. Analysis of a database of 335 emerging infectious disease events indicated that 60.0% were zoonotic diseases, most (71.8%) of which originated in wildlife, and that zoonoses are increasing over time (1). Half of these emerging infection events involved bacteria belonging, for the most part, to the proteobacterial order Rickettsiales (1).

In Western Europe, the widespread and abundant Ixodes ricinus tick is the most common vector for human and animal pathogens and is also a major vector of pathogens responsible for rodent-borne diseases (2). Lyme borreliosis (Lyme disease), which is caused by infection with the bacteria Borrelia burgdorferi sensu lato, is the most prevalent tick-borne and rodent-borne illness. However, persons bitten by ticks can also be infected by other bacteria belonging to 3 main genera: Anaplasma spp., Bartonella spp., and Rickettsiae spp. Over the past 10 years, these bacterial species have been associated with tick-borne infections in humans (2).

During the last decade, DNA of a new species of intracellular bacteria belonging to the family Anaplasmataceae was sequenced from isolates from ticks and rodents originating in Europe and Asia (3–5). The first isolate of this organism was obtained in 2004 from wild Rattus norvegicus rats and I. ovatus ticks from Japan (6). A comparison of the morphologic and molecular characterization of that isolate with earlier, closely related sequences in the GenBank database supported classification of the isolate in a novel genetic cluster within the family Anaplasmataceae; thus, the nomenclature "Candidatus Neoehrlichia mikurensis" was proposed for all current organisms in this group (6).

Since the 2004 discovery of Candidatus N. mikurensis, the bacterium has been identified in different tick species, including I. ricinus ticks in Europe, and in small rodents (other than R. norvegicus rats) suspected of being the main reservoir for the bacterium (7–10). In 2010, human infection with Candidatus N. mikurensis was reported in a Swedish patient (11). In that same year, infections were described in 5 persons in Germany, Switzerland, and the Czech Republic (12). More recently, Candidatus N. mikurensis infection was reported in a dog in Germany (13).

Signs and symptoms described in all cases were general and nonspecific (e.g., fever, cough, anemia, headache, pulmonary infiltrate, malaise, myalgia, joint pain, extreme fatigue, erythema), making diagnosis difficult, particularly in the absence of serologic tests. Thus, it is likely that the actual incidence of human Candidatus N. mikurensis infection in Europe is much higher than currently reported.

Previous studies of Candidatus N. mikurensis in Europe have advocated for an assessment of the geographic occurrence, possible vectors, and prevalence of this microorganism. In this study we demonstrate the presence of Candidatus N. mikurensis in France, specifically in the bank vole (Myodes glareolus), a suspected reservoir for the microorganism.

The Study

During the course of a 2008 study of the epidemiology of Puumala hantavirus, we trapped voles in the French Ardennes, a forested region on the border with Belgium, along a transect line of ≈80 km (14). Along this transect, we sampled 6 sites in forested areas and 4 sites in fragmented habitats (i.e., hedge networks). We euthanized the voles by cervical dislocation and determined their weight and sex. The animals were then dissected. Spleens were placed in RNAlater Storage Solution (Sigma-Aldrich, St Louis, MO, USA) and stored at −20°C for further analyses. We used the DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany) according to the manufacturer’s instructions to extract genomic DNA from a portion of the spleen into a final elution volume of 100 µL.

To test the spleen DNA for the presence of Candidatus N. mikurensis, we used PCR with specific primers targeting the Candidatus N. mikurensis groEL gene, as described (13): results were positive for 5 (1.8%) of 276 vole spleens tested. This prevalence lies within the range (0%–11.5%) found among small rodents trapped in other European countries (7,9). Rodents positive for Candida-
Candidatus N. mikurensis were trapped in forested patches in Woeries (49.903°N, 4.763°E), Elan (49.655°N, 4.770°E), and Croix-aux-Bois (49.419°N, 4.824°E), France, and also in a hedge network in Cliron (49.802°N; 4.619°E), France, where the I. ricinus tick is also abundantly present (E. Ferquel, pers. comm.). All Candidatus N. mikurensis–positive bank voles were trapped in close proximity (300 m–1 km) to human settlements and villages.

All PCR products (a 1,024-bp fragment of the groEL gene) were sequenced in forward and reverse directions by Eurofins MWG Operon (Ebersberg, Germany). Sequences were aligned and analyzed for phylogenetic purposes by using SeaView version 4 (15). All sequences were identical and matched those of the Candidatus N. mikurensis groEL gene. Sequences showed the highest identity with gene sequences of Candidatus N. mikurensis isolates in Europe. In particular, the sequences showed 99% nt identity with sequences obtained for isolates from 2 humans (GenBank accession nos. EU810407.1 and EU810406.1) and a dog (GenBank accession no. EU432375) residing in Germany. The lowest sequence identity was shared with isolates obtained outside of Europe: 95% nt identity with isolates from wild rodents in the People’s Republic of China (GenBank accession nos. JQ359066, JQ359067, and JQ359068); 94% identity with isolates from wild rodents and I. ovariatus ticks in Japan (GenBank accession nos. AB074461 and AB084583, respectively); and 91% identity with isolates from raccoons in the United States (GenBank accession no. EF633745).

We constructed the phylogenetic tree by using the neighbor-joining method and a Kimura 2-parameter distance. Bootstrap analysis was performed on 1,000 replicates.

Phylogenetic analysis of the comparatively long groEL gene sequence showed highly significant clustering of the samples into 3 groups supported by high bootstrap values (Figure), suggesting the existence of at least 3 types of Candidatus N. mikurensis sequence variants. Clustering was not related to the host or to the geographic origin of the sample. All sequences obtained from Candidatus N. mikurensis isolates from bank voles in France clustered together with sequences that had been amplified from samples from sick humans or animals in Europe.

Conclusions

Our results show that Candidatus N. mikurensis is present in bank voles in France; furthermore, the bacteria carried by the rodents in this study were of the same genotype as the Candidatus N. mikurensis that caused disease in humans and animals elsewhere in Europe. These findings have implications for public health because small rodents are the main source of blood meals for the larvae and nymphs of I. ricinus ticks and because the Candidatus N. mikurensis–positive rodents in our study were collected in close proximity to human dwellings. Thus, we are currently collecting I. ricinus ticks in the same region of France where we trapped the bank voles to determine whether ticks are a possible vector and a source of transmission of Candidatus N. mikurensis to humans.

In addition, nephropatia epidemica, a hemorrhagic fever caused by Puumala hantavirus, is endemic in the geographic region from which the bank voles in our study were collected. The symptoms for nephropatia epidemica (body aches, chills, sweats, fatigue, and high fever) are mild and could be confused with those caused by infection with Candidatus N. mikurensis. Diagnosis of Candidatus N. mikurensis infection relies only on PCR amplification of the bacterial DNA. The absence of serologic tests for Candidatus N. mikurensis, combined with the lack of knowledge relating to these bacteria among medical practitioners, makes diagnosis of Candidatus N. mikurensis infection particularly difficult. Thorough surveillance and improved diagnostic tools are required to gain more insight into the relevance of Candidatus N. mikurensis to public health.
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Dr Vayssier-Taussat is the head of a research team at Institut National de la Recherche Agronomique. Her area of interest is tick-borne bacteria, especially the development of molecular tools to identify tick and animal pathogens and to elucidate their mechanisms of infection.

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