surveillance. Second, health workers at all levels should be trained to recognize the disease. Third, a detailed assessment of the extent of Buruli ulcer in the 3 counties visited as well as in other counties should be prepared. Fourth, partner/donor support for Buruli ulcer activities should be enhanced. Fifth, capacity of the National Reference Laboratory to be able to perform PCR for confirmation of Buruli ulcer cases should be expanded. Last, Buruli ulcer should be incorporated into the national surveillance system to enable better data collection.

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Candidatus Neoehrlichia mikurensis and *Anaplasma phagocytophilum* in Urban Hedgehogs

To the Editor: *Candidatus* Neoehrlichia mikurensis is a member of the order Rickettsiales, family *Anaplasmataceae* (*1*). Manifestations of infection with these bacteria are atypical and severe and include cough, nausea, vomiting, anemia, headache, pulmonary infiltration, malaise, myalgia, arthralgia, fatigue, recurrent fever for ≤8 months, and/or death (*2–5*). *Candidatus* N. mikurensis has been detected in *Ixodes ovatus*, *I. persulcatus*, and *Haemaphysalis concinna* ticks in Asia (*1,5*).

Candidatus N. mikurensis has been identified as one of the most prevalent pathogenic agents in *I. ricinus* ticks throughout Europe (*2,3,6*). Rodents of diverse species and geographic origins have been shown to carry these bacteria, but transmission experiments have not been conducted to unambiguously identify natural vertebrate reservoirs (*1–3,5–7*). This emerging tickborne pathogen has been detected mainly in immunocompromised patients in Sweden (n = 1), Switzerland (n = 3), Germany (n = 2), and the Czech Republic (n = 2) and in immunocompetent patients in China (n = 7) (*2–5*).

Anaplasma phagocytophilum is an obligate, intracellular, tickborne bacterium of the family *Anaplasmataceae* and causes granulocytic anaplasmosis in humans and domestic animals. In Europe, *I. ricinus* ticks are its major vector, and red deer, roe deer, rodents, and European hedgehogs (*Erinaceus europaeus*) are suspected reservoir hosts (*8*).

Northern white-breasted hedgehogs (*Erinaceus roumanicus*) are urban-dwelling mammals (order Eulipotyphla, family Erinaceidae) that serve as major maintenance hosts for the 3 stages of

I. ricinus ticks (*9*). However, *E. roumanicus* hedgehogs have not been studied for their ability to carry *A. phagocytophilum*. In addition, no suspected reservoirs other than rodents have been investigated for *Candidatus* N. mikurensis. The purpose of this study was to determine whether this hedgehog is a reservoir of these 2 bacteria.

We conducted an ecoepidemiologic study during 2009–2011 to obtain information about ticks and tickborne pathogens of urban hedgehogs in a park on Margaret Island in central Budapest, Hungary (*9*). Ear tissue samples were obtained from hedgehogs anesthetized with intramuscular ketamine (5 mg/kg) and dexmedetomidine (50 μg/kg).

DNA was extracted from samples by using the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) or the Miniprep Express Matrix protocol (MP Biomedicals, Santa Ana, CA, USA). We used quantitative real-time PCRs that partially amplify the heat shock protein gene (*groEL*) of *Candidatus* N. mikurensis and the merozoite surface protein 2 gene (*msp2*) of *A. phagocytophilum* (*3*). PCR was performed in a 20-μL volume containing iQ Multiplex Powermix (Bio-Rad Laboratories, Hercules, CA, USA) in a LightCycler 480 Real-Time PCR System (F. Hoffmann-La Roche, Basel, Switzerland). Final PCR concentrations were 1× iQ Powermix, 250 nmol/L of primers ApMSP2F and ApMSP2R, 125 nmol/L of probe ApMSP2P-FAM, 250 nmol/L of primers NMikGroEL-F2a and NMikGroEL-R2b, 250 nmol/L of probe NMikGroEL-P2a-RED, and 3 μL of template DNA.

To confirm quantitative PCR results, we performed conventional PCRs in a Px2 Thermal Cycler (Thermo Electron Corporation, Waltham, MA, USA) on selected PCR- positive samples for both pathogens (*3*). Sequences obtained were submitted to GenBank under accession nos. KF803997 (*groEL* gene of *Candidatus* N. mikurensis) and KF803998 (*msp2* gene of *A. phagocytophilum*).

Candidatus N. mikurensis was detected in 2 (2.3%) of 88 hedgehog tissue samples. Rodents were the only wild mammals found to serve as reservoirs for this pathogen. Results of studies that attempted to detect these bacteria in common shrews (*Sorex araneus*), greater white-toothed shrews (*Crocidura russula*) (*2,3*), or common moles (*Talpa europaea*) (*2*) were negative. However, our results indicate that northern white-breasted hedgehogs might be a non-rodent reservoir for *Candidatus* N. mikurensis.

The low pathogen prevalence observed in this urban hedgehog population compared with that in rodents in other locations (*2,3*) might be caused by use of skin samples. Skin samples from rodents showed only 1.1% positivity in a study in Germany; however, average prevalence of *Candidatus* N. mikurensis in transudate, spleen, kidney, and liver samples from the same animals was 37.8%–51.1% (*2*). Although we did not test other organs, we hypothesize that prevalence of *Candidatus* N. mikurensis infection urban hedgehogs is probably >2.3%.

We detected *A. phagocytophilum* in 67 (76.1%) of 88 urban hedgehogs. This prevalence was similar to that found among European hedgehogs in Germany (*8*). *I. ricinus* ticks are more common than *I. hexagonus* ticks in this urban hedgehog population (*9*). Thus, *I. ricinus* ticks can acquire these bacteria when feeding on hedgehogs and the risk for human infection with *A. phagocytophilum* in this park in Budapest is high.

Neoehrlichiosis and granulocytic anaplasmosis have not been diagnosed in humans in Hungary. This finding is probably caused by diagnostic difficulties rather than absence of these pathogens in the environment. Infection with *Candidatus* N. mikurensis and *A. phagocytophilum* cause predominantly noncharacteristic symptoms. Laboratory cultivation and serologic detection of *Candidatus* N. mikurensis has not been successful, and this pathogen has not been identified in blood smears. Thus, accurate diagnosis of suspected cases requires suitable molecular methods.

Parks can be considered points of contact for reservoir animals, pathogens, ticks, and humans. Our results indicate that *E. roumanicus* hedgehogs play a role in urban ecoepidemiology of ≥2 emerging human pathogens. To better understand the urban cycle of these pathogens, potential reservoir hosts, ticks collected from these hosts, and vegetation in parks should be investigated.

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Rickettsia and Vector Biodiversity of Spotted Fever Focus, Atlantic Rain Forest Biome, Brazil

To the Editor: *Rickettsia rickettsii*, *R. felis*, and *R. parkeri*,strain Atlantic rainforest, have been characterized in areas to which Brazilian spotted fever (BSF) is endemic (*1*,*2*), which indicates the complexity of their epidemic and enzootic cycles. The Atlantic rain forest is one of the largest and richest biomes of Brazil, and antropic action has intensely influenced its transformation. Most BSF cases and all BSF-related deaths are recorded in this biome area.

Many BSF cases were recorded in Paraíba do Sul river basin, one of the most urbanized and industrialized areas of Brazil. To better understand arthropod and *Rickettsia* diversity in this area,, we analyzed 2,076 arthropods from Rio de Janeiro state, Atlantic rain forest biome.

During October 2008–November 2009, we collected ticks and fleas from hosts and environments in 7 cities where high numbers of BSF cases were recorded (Rio de Janeiro State Health Secretary, unpub. data) and where fisiogeographic characteristics differed. After morphologic classification (*3*), the arthropods were individually separated or grouped by sex, developmental stage, and host for total DNA extraction (*4*).

We used 2 *Rickettsi*a-specific primer sets (CS2–78/CS2–323 and CS4–239/CS4–1069) to amplify 401 bp and 834 bp, respectively, of the citrate synthase gene (*gltA*) (*5*,*6*). Presumptive *Rickettsia*-positive samples were tested for spotted fever group (SFG)–specific primer set *Rr*190.70p/*Rr*190.602n for 532 bp from the *ompA* gene (*7*). *R. rickettsii* DNA and bi-distilled water were used as positive and negative controls, respectively. PCR products were purified (NucleoSpin Extract II kit; Macherey-Nagel, Düren, Germany), cloned (pTZ57R/T; Fermentas-Thermo Fisher Scientific, Waltham, MA, USA), and sequenced by using specific vector primer sets (BigDye Reaction kit, Applied Biosystems, Foster City, CA, USA). Sequences were edited by using SeqMan program (Lasergene 10.1; DNASTAR Inc., Madison, WI, USA), and similarities were obtained by BLAST analysis (http://blast.ncbi.nlm.nih.gov). The phylogenies were assessed by applying neighbor-joining and maximum-parsimony methods, with the Kimura 2-parameter correction model. We used ClustalW 2.1 (www.clustal.org) to align sequences and produced phylogenetic trees by using 1,000 replicates bootstrap in MEGA 5.0 software (www.megasoftware.net).

We collected and analyzed ticks of the following species: *Amblyomma cajennense* (1,723 ticks), *Rhipicephalus sanguineus* (109), *Anocentor nitens* (63), *Boophilus microplus* (33), *Amblyomma aureolatum* (2), and *Amblyomma dubitatum* (2). We collected and analyzed *Ctenocephalides felis* (143 fleas) and *C. canis* (1) fleas.

PCR analysis showed Rickettsia DNA in 11 individual or pooled samples. This finding indicated minimal infection rates of 0.2% (4/1,723) for A. cajennense ticks, 50% (2/4) for A. dubitatum ticks, 3.0% (1/33) for B. microplus ticks, 100% (1/1) for C. canis fleas, and 2.8% (4/143) for C. felis fleas. Expected amplicon size, determined by using the gltA 401-bp primer set, was observed for all positive samples. Two were also positive by PCR