

HAT data indicate seasonality of this disease; incidence is higher during January, February, and March ($p = 0.04$, by Mann-Whitney test). Seasonality of HAT incidence has been noted elsewhere and linked to seasonal influences on tsetse habitat suitability. We propose that seasonality of cattle trading may also play a role because cattle purchases increase before the Christmas season, which promote pathogen spread and increased transmission. This finding is consistent with research highlighting the role of livestock markets in the spread of *T. b. rhodesiense* in central Uganda and would further support a body of literature suggesting, as espoused by the SOS initiative, that control of animal reservoirs of the disease is a critical component of intervention measures (2,7–9). Implementation and enforcement of regulations for treatment of cattle before sale at markets would also contribute to limiting spread (9,10);

Interventions in districts in central Uganda in which convergence is predicted have been slow and incomplete. If convergence has occurred, this finding indicates that a specific region in Africa has had concurrent infection with both causes of HAT, with implications for prevention, treatment, and control. Since 2000, Uganda has had continued northward spread of *T. b. rhodesiense* infections, reducing the distance with TbG to <100 km, which we believe is a conservative estimate. Reinstatement of active surveillance of HAT and support for central data collection in Uganda are long overdue and warranted immediately.

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Rickettsia felis in *Aedes albopictus* Mosquitoes, Libreville, Gabon

To the Editor: *Rickettsia felis*, an emerging pathogen first identified in the cat flea (1), has been detected in other fleas, ticks, mites, and booklice (2). *R. felis* can be cultured in mosquito cell lines derived from *Anopheles gambiae* and *Aedes albopictus* (Asian tiger) mosquitoes (2), so its compatibility with mosquitoes in nature can be suspected. In sub-Saharan Africa, *R. felis* bacteremia in humans is common, especially during the rainy season, when mosquitoes proliferate. We tested anthropophilic mosquitoes for the presence of *R. felis* DNA (3–5).

During December 2008–January 2010, we randomly selected female *Ae. albopictus* and *Ae. aegypti* mosquitoes (96 each) from specimens obtained by human-landing collections from 4 sites in Libreville, Gabon (6). Specimens were collected during the rainy season (mid-January–end of May and end of September–mid-December); no parity data were available.

We extracted 192 DNA samples from homogenate (abdomen, wings, legs) of each nonengorged, host-seeking, adult mosquito by using the BioRobot 8000 (QIAGEN S.A.S., Courtaboeuf, France) and QIAamp Media MDx Kit (QIAGEN) according to the manufacturer's instructions. Samples were screened by quantitative real-time PCR (qPCR) targeting the biotin synthase (*bioB*) gene (4). Positive results were confirmed by qPCR-based molecular detection targeting the *orfB* gene, which codes for a transposition helper protein. This qPCR used a set of primers not previously used in our laboratory (R_fel.OrfB_F: 5'-CCCTTTTCGTAACGCTTTGCT-3' and R_fel.OrfB_R: 5'-GGGCTAAA CCAGGAAACCT-3') and the probe

R_fel.OrfB_P: 6-FAM-TGTTCCGGT TTTAACGGCAGATACCCA-TAMRA. Specificity of the qPCR was tested in silico and on the 31 *Rickettsia* spp. from our laboratory. The final qPCR reaction mixture contained extracted DNA (5 μ L) and mix (15 μ L) that contained master mix (10 μ L) from the QuantiTect Probe PCR Kit (QIAGEN, Hilden, Germany), each primer (0.5 μ L, 20 pmol), probe (0.5 μ L, 62.5 nmol), and RNase-free water (3.5 μ L). Amplification and sequence detection were performed in a CFX96 Touch thermocycler (Bio-Rad, Marnes-la-Coquette, France) as follows: 15 min at 95°C followed by 40 cycles of 1 s at 95°C, 40 s at 60°C, and 40 s at 45°C.

Test results for all *Ae. aegypti* homogenates were negative for *R. felis* DNA. Of the 96 *Ae. albopictus* specimens, 3 (3.1%) had positive test results for the *R. felis* species-specific real-time qPCR and the confirmatory qPCR, with mean cycle thresholds \pm SDs of 37.34 ± 1.7 (*bioB* gene; mean copies/mosquito 5×10^2 [minimum 1.2×10^2 , maximum 1.4×10^3]) and 33.64 ± 1.4 (*orfB* gene; mean copies/mosquito 5×10^2 [minimum 1.5×10^2 , maximum 1×10^3]). One of the 3 samples was collected in January and 2 in March. The samples came from 3 different districts of Libreville (Akebe Poteau, Alibandeng, Camp des Boys) and were tested by nested PCR targeting the citrate synthase (*gltA*) gene (7). *Rickettsia montanensis* DNA was used as a positive control. Sequencing was performed as described (7), and ChromasPro version 1.34 (Technelysium Pty Ltd., Tewantin, Queensland, Australia) was used to analyze sequence data. Sequences of the *bioB* (120/120) and *gltA* (1,230/1,230) amplicons at the nucleotide level were 100% homologous to sequences for *R. felis* URRWXC12 (GenBank accession no. CP000053). The *gltA* fragment sequence was deposited in GenBank (accession no. JQ674484).

Mosquitoes were considered positive for *R. felis* when the qPCR result was ≤ 35 cycle thresholds for 1 target gene and the additional DNA sequence was successfully amplified. No sample in this study was positive for only 1 target gene or had a qPCR threshold ≥ 35 cycle thresholds for both genes.

Contamination is a critical problem for the PCR-based identification of microbes. However, the validity of the data we report is based on strict laboratory procedures and controls that are commonly used in the World Health Organization Reference Center for Rickettsial Diseases, including rigorous positive and negative controls to validate the test. Each positive qPCR result was confirmed by another *R. felis*-specific qPCR (*orfB*) not previously used in our laboratory (to avoid contamination with other amplicons).

Ae. albopictus mosquitoes are native to Southeast Asia, colonizing rural and peri-urban sites. In Gabon, *Ae. albopictus* was the vector for outbreaks of chikungunya and dengue virus infections (6). Our study indicates that mosquitoes can carry *R. felis*, and the prevalence and load (1.8%–70% and 1.3×10^3 – 1.6×10^7 , respectively) detected in mosquitoes in this study are consistent with the low-end range of those detected in cat fleas, the confirmed biological vector and reservoir (8,9).

We investigated the presence of *Rickettsia* spp. in mosquitoes neglected as possible vectors of rickettsial diseases (2). Other *Aedes* spp. and other genera of mosquitoes should be tested. The role of mosquitoes as *Rickettsia* spp. vectors remains to be demonstrated in additional studies that use the Mitchell criteria. These studies should include the use of cell culture to isolate or detect *R. felis* in salivary glands of specimens from wild-caught mosquitoes, PCR, immunofluorescence, and the fluorescence in situ hybridization technique; demonstration of infection

of a mosquito after experimental feeding on a bacteremic host or bacterial suspension; and demonstration of the transmission of bacteria to a vertebrate through the bite of a mosquito (10).

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Bartonella spp. Infection Rate and *B. grahamii* in Ticks

To the Editor: Bacteria of the genus *Bartonella* are transmitted by arthropods and are often implicated in human disease. Even though ticks are known to transmit a variety of pathogens, vector competences for transmission of *Bartonella* spp. by ticks were speculative (1) until recently, when in vivo transmission of *B. birtlesii* by *Ixodes ricinus* ticks was demonstrated in mice (2). This finding suggests that this tick species,

which is common in Europe, may also transmit zoonotic *Bartonella* spp. Evidence of possible tick transmission of bartonellae to humans under natural conditions was provided by Eskow et al. (3) and Angelakis et al. (4), who identified *Bartonella* spp. in tissue samples of patients who were recently bitten by ticks. We determined the prevalence of *Bartonella* spp. in questing *I. ricinus* ticks in the city of Hanover, Germany, which is nicknamed The Green Metropolis and was selected the German Capital of Biodiversity in 2011.

During April–October 2010, we collected 2,100 questing ticks, consisting of 372 adults (177 female and 195 male), 1,698 nymphs, and 30 larvae, from 10 recreation areas in Hanover. Tick DNA was extracted by using the NucleoSpin 8 Blood kit (Macherey-Nagel, Düren, Germany). Plasmid DNA constructed from *B. henselae* reference strain ATCC49793 containing the 249-bp target sequence of the *gltA* gene was used as positive control. *Bartonella* spp. in ticks was detected by quantitative PCR (qPCR) by using the Mx3005 Multiplex Quantitative PCR System (Stratagene, Heidelberg, Germany) according to the protocol described by Mietze et al. (5), with minor modifications. Samples positive by qPCR were verified by gel electrophoresis. *Bartonella* species were differentiated by sequencing (Eurofins MWG Operon, Ebersberg, Germany), and obtained sequences underwent BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) comparison to published sequences.

On the basis of the amplicon-specific melting temperature and DNA bands representing the specific size of 249-bp after gel electrophoresis, results of qPCR showed 100 (4.76%) infected *I. ricinus* ticks (Table). Positive results did not vary by developmental tick stages; 4.84% (18/372) adult ticks (5.08% [9/177] female and 4.62% [9/195] male), 4.71% (80/1,698) nymphs, and 6.67% (2/30) larvae were infected (Table). Because *Bartonella* spp. do not seem to be transmitted transovarially (6), it is likely that larvae had interrupted blood meals and thus did not take up enough blood to develop into the nymphal stage.

Seasonal changes in *Bartonella* spp. infection rates resulted in a higher peak in May (38/300 [12.67%]) than in the other months (Table). For sampling locations, infection rates for grassy sampling location 6 (4/210 [1.90%] infected ticks) differed significantly (Bonferroni-Holm adjusted $p < 0.001$; $* < 0.0011$) from that of densely wooded sampling location 9 (22/210 [10.48%] infected ticks).

Sequencing of the *gltA* fragment resulted in *Bartonella* species identification for 56/100 positive samples; 52 of these samples (from 38 nymphs, 13 adults, and 1 larva) were identified as infected with *B. henselae*. In 51 samples (92.86%), maximum identity with the BLAST top hit sequence was 99% because of

Table. Seasonal distribution of *Ixodes ricinus* ticks infected with *Bartonella* spp., Hanover, Germany, 2010*

Ticks	April	May	June	July	August	September	October	Total
No. infected ticks/no. tested (%)	5/300 (1.67)	38/300 (12.67)	7/300 (2.33)	10/300 (3.33)	5/300 (1.67)	17/300 (5.67)	18/300 (6.00)	100/2,100 (4.76)
No. (%) adults positive	1/88 (1.14)	8/48 (16.67)	0/39	0/41	2/56 (3.57)	3/53 (5.66)	4/47 (8.51)	18/372 (4.84)
No. (%) females	1/32 (3.13)	3/19 (15.79)	0/20	0/17	1/32 (3.13)	2/28 (7.14)	2/29 (6.90)	9/177 (5.08)
No. (%) males	0/56	5/29 (17.24)	0/19	0/24	1/24 (4.17)	1/25 (4.00)	2/18 (11.11)	9/195 (4.62)
No. (%) nymphs	3/203 (1.48)	30/248 (12.10)	7/261 (2.68)	10/259 (3.86)	3/244 (1.23)	14/240 (5.83)	13/243 (5.35)	80/1,698 (4.71)
No. (%) larvae	1/9 (11.11)	0/4	ND	ND	ND	0/7	1/10 (10.00)	2/30 (6.67)

*ND, testing not done.