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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.

nucleotide substitutions in position 198 (T→C) and in position 136 (A→G) of the 249-bp fragment. The remaining sample showed 100% identity with *B. henselae* strains Brazil-1 and 45-00249 (GenBank accession nos. HQ012580 and GQ225709).

Four of the 56 successfully sequenced samples (7.14%; all samples from nymphs) showed the sequence pattern of *B. grahamii*. One sample revealed 100% identity with *B. grahamii* (GenBank accession no. EU014266); the remaining 3 samples showed an identity of 98% with the *B. grahamii* strain Hokkaido-1 (GenBank accession no. AB426652) and 99% (T→C in position 93) with a sequence described as *B. grahamii*-like (GenBank accession no. AY435122). Sequences obtained in this study (deposited in GenBank under accession nos. JQ770304 and JK758018) support the genetic variability of *Bartonella* spp., as demonstrated by others (5,7,8).

It remains unclear whether ticks are involved in transmission of pathogenic *Bartonella* spp. to humans under natural conditions. However, the total prevalence rate of 4.76% (100/2,100) questing *I. ricinus* ticks infected with *B. henselae* and *B. grahamii* highlights the need for public awareness and draws attention to the possibility of an infection with zoonotic *Bartonella* spp. after a tick bite (3,4). *B. henselae*, the predominantly identified species, has been associated with cat scratch disease, peliosis hepatis, and bacillary angiomatosis in humans. Eskow et al. (3) also connected chronic symptoms of Lyme disease to co-infections with *Borrelia burgdorferi* and *B. henselae*. *B. grahamii* has been associated with neuroretinitis and ocular artery thrombosis in humans (9,10). The potential risk for zoonotic *Bartonella* spp. infection in urban recreation areas should not be underestimated.

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Human Parvovirus 4 Viremia in Young Children, Ghana

To the Editor: Establishment of viremia is a characteristic feature of infection with human parvovirus 4 (PARV4). In northern Europe, PARV4 (human parvovirus 4) is primarily transmitted by blood-borne routes (1,2). In other areas (southern Europe, western Africa, South Africa, Asia) infection seems to be more widespread, suggesting alternative modes of virus acquisition (3–6).

We reported PARV4 genotype 3 viremia in young children with unknown parenteral blood exposure from the rural Ashanti region of Ghana (7). In that study, 2 (2.1%) of 94 children (median age 14.9 months) and 22 (11.9%) of 185 children (median age 24.0 months) were virus positive (ages of the 2 virus-positive children from the younger cohort 14.9 and 15.6 months). Because the number of infants was small in that study, we extended our investigations on PARV4 viremia to an additional

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