

Molecular Evidence of Coinfection of Ticks with *Borrelia burgdorferi* Sensu Lato and the Human Granulocytic Ehrlichiosis Agent in Switzerland

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Adult *Ixodes ricinus* ticks were collected in Switzerland and tested for the presence of coinfection with *Borrelia burgdorferi* sensu lato and the human granulocytic ehrlichiosis (HGE) agent by real-time PCR. Of 100 ticks, 49% were positive for *B. burgdorferi* and 2% were positive for the HGE agent. The two HGE agent-positive ticks were also found to be positive for *B. burgdorferi*.

Lyme borreliosis and human granulocytic ehrlichiosis (HGE) are emerging infectious diseases of both humans and animals that are transmitted by ticks. In Switzerland, *Ehrlichia phagocytophila* has been identified in cattle (11) and an agent with 100% homology in its 16S rRNA gene to the agent of HGE has been detected in dogs (14) and horses (15). HGE is a recently discovered syndrome in humans (3) caused by an agent phylogenetically closely related to *E. phagocytophila* and *Ehrlichia equi* and was found in the United States and also in Europe (10). Coexistence of antibodies to tick-borne pathogens, such as *Babesia microti*, HGE agent, and *Borrelia burgdorferi*, have been reported (7), indicating that humans and animals may be infected simultaneously by these pathogens through tick bites.

In the present study, 100 DNA samples of adult female ticks were tested to find molecular evidence of coinfection with *B. burgdorferi* and the HGE agent in Switzerland.

Tick collection. A total of 100 adult *Ixodes ricinus* ticks were collected in Wangen, Canton of Zurich, Switzerland, a region with a sporadic occurrence of granulocytic ehrlichiosis in animals caused by an HGE-like agent. The ticks were collected with an umbrella that was covered with a terry cloth towel and repeatedly pushed through the low underbrush in forests. Ticks attached to the towel were removed, placed into tubes, and stored individually at -20°C until DNA extraction was performed.

DNA extraction. After thawing, ticks were placed in 200 μl of buffered phosphate solution in an Eppendorf tube and mechanically crushed with sterile scissors. DNA extraction was performed with a QIAamp tissue kit (Qiagen, Basel, Switzerland) according to the manufacturer's recommendations.

PCR and DNA sequencing. All DNA samples were examined for the presence of *B. burgdorferi* sensu lato by real-time TaqMan PCR and for the presence of *Ehrlichia* of the *E. phagocytophila* genogroup (13). The *Borrelia*-specific TaqMan PCR, designed for the inner part of the flagellin gene, was carried out with the following oligonucleotides ($5' \rightarrow 3'$): forward primer B.398f, GGAAGCAGATTTGTTTGACA; reverse primer B.484r, ATAGAGCAACTTACAGACGAAATTAATAGA,

and probe B.421p, ATGTGCATTTGGTTATATTGAGCTTGATCAGCAA. The agent of the *Borrelia* TaqMan PCR reacted with *Borrelia* species but not with 30 other bacterial species tested. This indicates the high analytical specificity for *Borrelia* species of the TaqMan PCR. The analytical sensitivity was 10 copies of a cloned PCR product (6a). Negative controls included DNAs from 50 noninfected, laboratory-reared adult ticks of the *I. ricinus* species that were purchased from the Institute of Zoology in Neuchâtel (Switzerland). *Borrelia*- and *Ehrlichia*-specific DNAs were verified by conventional nested-PCR amplification and sequencing of the amplified products. Visualization of the PCR amplification products was performed by gel electrophoresis on 1.8% agarose gels. Single bands were purified (QIAquick gel extraction kit; Qiagen), cloned with a TOPO TA cloning kit (Invitrogen, NV, Leek, The Netherlands), and sequenced with a fluorescence-based automated sequencing system (ABI 377 DNA sequencing; Microsynth, Balgach, Switzerland).

Of the 100 individually processed ticks, 49 were positive for *B. burgdorferi* and 2 were positive for the agent of HGE. The two HGE agent-positive ticks were also found to be positive for *B. burgdorferi*. The 50 control ticks were negative for the presence of both *Borrelia* and *Ehrlichia*.

The nucleotide sequences obtained from DNAs purified from the two coinfecting ticks were identified as being part of the flagellin gene of *Borrelia* and of the 16S rRNA gene of *Ehrlichia* spp. One of the coinfecting ticks showed evidence of *Borrelia afzelii* infection (its sequence was comparable to GenBank accession no. X75202), and the other tick was infected with *B. burgdorferi* sensu stricto (its sequence was comparable to GenBank accession no. X75200). The nucleotide sequences of the 16S rRNA genes of both ticks showed 100% sequence homology to the agent of HGE from the United States (GenBank accession no. U02521) and to the agent of canine and equine granulocytic ehrlichiosis from Switzerland (accession no. AF057707).

To our knowledge, this is the first report showing ticks to be coinfecting with two human pathogens, *B. burgdorferi* sensu lato and the HGE agent, in Europe. Endemicity of Lyme borreliosis in or near tick-infested forests is well documented (8), and *B. afzelii* and *B. burgdorferi* sensu stricto are well-described human pathogens.

The *E. phagocytophila* genogroup consists of closely related

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species of *Ehrlichia*, including *E. phagocytophila*, the cause of tick-borne fever in sheep, goats, and cattle, *E. equi*, the cause of equine ehrlichiosis, and the HGE agent, a recently discovered species that infects humans (1, 3). Each of these three ehrlichial agents infects a different host species, has a different geographical distribution, and may cause different clinical signs. However, research indicates that they may be variants of the same species (1, 5). In Switzerland, prevalence of *Ehrlichia*-infected ticks was found to vary to some extent in different regions (8, 12). For this reason, a region with large tick populations and with a known occurrence of granulocytic ehrlichiosis in dogs and horses was chosen for the present study. Coinfection of ticks with *B. burgdorferi* and members of the *E. phagocytophila* genogroup has been reported in the United States with various levels prevalence of 1.9% (4) up to 29.6% (16).

Our molecular findings add further evidence that infections with *Ehrlichia* spp. may occur in tick-infested areas. Earlier studies suggest that coinfection with these agents may occur as a consequence of tick bites; coinfection may explain the variable clinical signs seen in humans and animals with Lyme borreliosis and ehrlichiosis (6, 9, 17). Patients who present with unexplained febrile illnesses (severe headache, arthralgias, and myalgias) and who have leukopenia, anemia, and/or thrombocytopenia together with elevated values of transaminases following tick bites (2) may have had exposure to multiple tick-borne pathogens, including ehrlichiae (17).

The results of this study emphasize that ticks coinfecting with *B. burgdorferi* and the agent of HGE are prevalent in the central part of Europe and, thus, that dual tick-borne infections may occur. Although the probability of dual infections appears low, differential diagnosis of dual infections and appropriate laboratory diagnosis is important because HGE agent does not respond to the beta-lactam antibiotics that are frequently used to treat Lyme borreliosis.

Nucleotide sequence accession numbers. The sequence of the flagellin gene of the *Borrelia*-positive ticks has been deposited in GenBank under the accession no. AF127531 (*B. burgdorferi* sensu stricto) and AF127532 (*B. afzelii*). The sequence of the 16S rRNA gene of the HGE agent-positive ticks has been deposited in GenBank under the accession no. AF084907.

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REFERENCES

- Anderson, B. E., J. E. Dawson, D. C. Jones, and K. H. Wilson. 1991. *Ehrlichia chaffeensis*, a new species associated with human ehrlichiosis. *J. Clin. Microbiol.* **29**:2838-2842.
- Bakken, J. S., J. Krueth, C. Wilson-Nordskog, R. L. Tilden, K. Asanovich, and J. S. Dumler. 1996. Clinical and laboratory characteristics of human granulocytic ehrlichiosis. *JAMA* **275**:199-205.
- Chen, S. M., J. S. Dumler, J. S. Bakken, and D. H. Walker. 1994. Identification of a granulocytotropic *Ehrlichia* species as the etiologic agent of human disease. *J. Clin. Microbiol.* **32**:589-595.
- Daniels, T. J., T. M. Boccia, S. Varde, J. Marcus, J. Le, D. J. Bucher, R. C. Falco, and I. Schwartz. 1998. Geographic risk for Lyme disease and human granulocytic ehrlichiosis in southern New York state. *J. Clin. Microbiol.* **64**:4663-4669.
- Dumler, J. S., K. M. Asanovich, J. S. Bakken, P. Richter, R. Kimsey, and J. E. Madigan. 1995. Serologic cross-reactions among *Ehrlichia equi*, *Ehrlichia phagocytophila*, and human granulocytic ehrlichia. *J. Clin. Microbiol.* **33**:1098-1103.
- Larsen, H. J., G. Overnes, H. Waldeland, and G. M. Johansen. 1994. Immunosuppression in sheep experimentally infected with *Ehrlichia phagocytophila*. *Res. Vet. Sci.* **56**:216-224.
- Leutenegger, C. M. Submitted for publication.
- Magnarelli, L. A., J. S. Dumler, J. F. Anderson, R. C. Johnson, and E. Fikrig. 1995. Coexistence of antibodies to tick-borne pathogens of babesiosis, ehrlichiosis, and Lyme borreliosis in human sera. *J. Clin. Microbiol.* **33**:3054-3057.
- Maiwald, M., R. Oehme, O. March, T. N. Petney, P. Kimmig, K. Naser, H. Zappe, D. Hassler, and M. von Knebel Doeberitz. 1998. Transmission risk of *Borrelia burgdorferi* sensu lato from *Ixodes ricinus* ticks to humans in southwest Germany. *Epidemiol. Infect.* **121**:103-108.
- Nadelman, R. B., F. Strle, H. W. Horowitz, W. A. Agger, and G. P. Wormser. 1997. Leukopenia, thrombocytopenia, and Lyme borreliosis: is there an association? *Clin. Infect. Dis.* **24**:1027-1029.
- Petrovec, M., S. L. Furlan, T. A. Zupanc, F. Strle, P. Brouqui, V. Roux, and J. S. Dumler. 1997. Human disease in Europe caused by a granulocytic *Ehrlichia* species. *J. Clin. Microbiol.* **35**:1556-1559.
- Pfister, K., P. H. Boss, and B. Balsiger. 1987. *Ehrlichia phagocytophila* als Erreger des Weidediebers im Berner Oberland. Schweiz. Arch. Tierheilkd. **129**:343-347.
- Pusterla, N., J. Huder, C. Wolfensberger, B. Litschi, A. Parvis, and H. Lutz. 1997. Granulocytic ehrlichiosis in two dogs in Switzerland. *J. Clin. Microbiol.* **35**:2307-2309.
- Pusterla, N., J. B. Huder, K. Feige, and H. Lutz. 1998. Identification of a granulocytic *Ehrlichia* strain isolated from a horse in Switzerland and comparison with other rickettsiae of the *Ehrlichia phagocytophila* genogroup. *J. Clin. Microbiol.* **36**:2035-2037.
- Pusterla, N., C. M. Leutenegger, J. B. Huder, R. Weber, U. Braun, and H. Lutz. 1999. Evidence of the human granulocytic ehrlichiosis agent in *Ixodes ricinus* ticks in Switzerland. *J. Clin. Microbiol.* **37**:1329-1331.
- Pusterla, N., J. B. Huder, C. M. Leutenegger, U. Braun, J. E. Madigan, and H. Lutz. 1999. Quantitative real-time PCR for the detection of members of the *Ehrlichia phagocytophila* genogroup in host animals and *Ixodes ricinus* ticks. *J. Clin. Microbiol.* **37**:1332-1334.
- Schauber, E. M., S. J. Gertz, W. T. Maple, and R. S. Ostfeld. 1998. Coinfection of blacklegged ticks (*Acar: Ixodidae*) in Dutchess County, New York, with the agents of Lyme disease and human granulocytic ehrlichiosis. *J. Med. Entomol.* **35**:901-903.
- Weber, R., N. Pusterla, M. Loy, and H. Lutz. 1998. Fever, leukopenia, and thrombocytopenia in a patient with acute Lyme borreliosis were due to human granulocytic ehrlichiosis. *Clin. Infect. Dis.* **26**:253-254.