Bartonella spp. DNA Associated with Biting Flies from California

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Bartonella DNA was investigated in 104 horn flies (Haematobia spp.), 60 stable flies (Stomoxys spp.), 11 deer flies (Chrysops spp.), and 11 horse flies (Tabanus spp.) collected on cattle in California. Partial sequencing indicated BartonellaDNA in one stable fly and Bartonella henselae type M DNA in one stable fly.

Bartonella spp. are vector-borne bacteria associated with numerous emerging infections in humans and animals (1). Four Bartonella species have been isolated from wild and domestic ruminants. Bartonella schoenbuchensis and Bartonella capreoli were recovered from wild roe deer (Capreolus capreolus) (2,3) in Europe, whereas Bartonella bovis (formerly Bartonella weissii) was recovered from domestic cattle in the United States and Europe (3–5). Strains similar to Bartonella bovis and Bartonella capreoli were also isolated from mule deer (Odocoileus hemionus) and elk (Cervus elaphus) from California (3,4). Recently, Bartonella chomelii was recovered from bactereic cows in France (6). A high prevalence of infection with various Bartonella species has been reported in domestic and wild ruminants in North America and Europe (2–4). Of the herds investigated in California, 95% of beef cattle and 17% of dairy cattle were bacteremic for Bartonella bovis and 90% of the mule deer were bacteremic for Bartonella spp. (4). The main vector of these ruminant-infecting Bartonella spp. has not been identified.

The role of ticks as potential vectors for Bartonella in cattle was investigated (7,8). In Europe, >70% of 121 Ixodes ricinus ticks collected from roe deer had 16S rRNA gene sequences for Bartonella or other closely related species (7). In California, Bartonella DNA was detected in approximately 19% of 151 questing adult I. pacificus ticks (8), but the direct role of ticks in Bartonella transmission among ruminants has never been established. In a search for an efficient Bartonella vector, which could explain such high prevalence of infection in wild and domestic ruminants, we tested biting flies for Bartonella spp. DNA to establish the potential role of biting flies as vectors of Bartonella in cattle.

The Study
Flies were collected by hand, with a bug net, at various locations on the University of California campus, mainly the dairy barn, beef barn, and feedlot, from early July to mid-August 2003. Flies were identified on the basis of morphologic characteristics visually or under binocular lenses for the smaller flies by an experienced entomologist. Of the 370 biting flies collected, 104 (62%) of the horn flies (Haematobia spp.), 60 (33%) of the stable flies (Stomoxys spp.), 11 (92%) of the deer flies (Chrysops spp.), and 10 (91%) of the horse flies (Tabanus spp.) were tested for Bartonella DNA. The stable flies were collected from the dairy and the feedlot barns. The horn flies, deer flies, and horse flies were collected from the beef barn.

Before DNA extraction, the flies were placed in a sterile 1.5-mL microtube, washed with 70% ethanol, and rinsed with sterile water. Because of size differences among the flies, 2–3 horn flies were grouped together in a single microtube, while each stable fly was placed in an individual vial. The abdomen of deer flies and horse flies was first removed and then placed in individual vials. DNA extraction was performed by using the DNeasy Tissue Kit (Qiagen, Valencia, CA) according to the manufacturer’s instructions, with some minor adjustments. The amount of reagents for the deer and horse flies were doubled, and the flies were incubated in a waterbath overnight at 55°C.

Bartonella DNA was detected by polymerase chain reaction (PCR) using primers for the citrate synthase (gltA) gene, as previously published (9). Undiluted DNA extracted from the flies was used as the DNA template. As a positive control, a low concentration of Bartonella henselae was added to a separate set of the same DNA template. A negative control was made by using sterile water instead of the DNA template. Using gel electrophoresis, we analyzed PCR products for the appearance of an ~380-bp fragment. Any evidence of a 380-bp fragment was further analyzed by restriction fragment length polymorphism (RFLP) procedures, by using TaqI (Promega Corp., Madison, WI), HhaI, AcI, and MseI endonucleases (New England Biolabs, Beverly, MA), and DNA sequence analysis (Davis Sequencing, Davis, CA).

Four of the 60 stable flies and one pool (2 flies) of the 45 horn fly pools showed a 380-bp fragment. PCR/RFLP analysis confirmed Bartonella DNA in one of the four stable flies and in the horn fly pool. However, for the three other stable flies, the PCR/RFLP profiles did not match any known Bartonella digestion profile. The sequence obtained from the horn fly pool (Haematobia spp.) collected in the beef cattle barn was identical to that for Bartonella bovis.
(Figure 1). The sequence obtained from a stable fly (Stomoxys spp.) collected in the dairy cattle barn was identical to that for B. henselae type M (Marseille) (Figure 2). The highlighted area indicates the divergence between B. henselae type H (Houston I) and B. henselae type M, as previously described (10).

Conclusions

This identification of Bartonella DNA is the first associated with horn and stable flies and the first identification of B. henselae from a biting fly. It is also the first report of identification of Bartonella DNA from flies from North America. This finding demonstrates, as for ticks, that Bartonella DNA is present in various biting insects. We found a very low percentage of Bartonella DNA–positive flies, in contrast to the very high prevalence (57 [88%] of 65 observed in Hippoboscidae adult flies (Lipoptena cervi and Hippobosca equina) collected from domestic cattle and wild roe deer in France (H.J. Boulouis, pers. comm.). This low prevalence may be related to the fact that different fly species were tested but more likely could be associated with a low level of Bartonella bacteremia in our herds. In a previous study, only 17% of cows in a dairy herd were bacteremic (4), and prevalence was even lower in another dairy herd from Tulare, in the central valley of California (B.B. Chomel et al., unpub. data). A follow-up for this study would be to collect blood from herds at the University of California, Davis, and establish the status of Bartonella bacteremia. Future research should include collecting flies in different locations and herds in which high levels of bacteremia were previously detected. Inhibitory factors were unlikely to be associated with such a low prevalence because spiked controls were systematically detected.

Identification of B. henselae DNA in a stable fly indicates the wide range of blood-sucking arthropods that can harbor this human pathogen. The partial gltA sequence was identical to that for B. henselae type Marseille, the most common type found in cats and humans in California (11). Fleas have been shown to be an efficient vector of B. henselae (12–14). More recently, B. henselae DNA was identified in adult questing I. pacificus ticks from California and from I. ricinus ticks collected on humans in Italy (8,15). The role of ticks as potential vectors of B. henselae in humans has also been suggested (16–18).

Since Bartonella are likely to be present in biting flies, investigating the potential of biting flies as either mechanical or biologic vectors of Bartonella in cattle and possibly humans should be pursued.

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