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Experimental infection by capillary tube feeding of *Rhipicephalus sanguineus* with *Bartonella vinsonii* subspecies *berkhoffii*

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ABSTRACT

It has been speculated that ticks may serve as vectors of *Bartonella* species. Circumstantial, clinical, epidemiological and serological evidence suggest that *B. vinsonii* subspecies *berkhoffii* (*B. v. berkhoffii*) might be transmitted by *Rhipicephalus sanguineus*. The purpose of the present study was to determine whether adult *R. sanguineus* ticks can be infected with a *B. v. berkhoffii* genotype II isolate via capillary tube feeding and whether the infection can then be transmitted from adult females to their eggs via trans-ovarial transmission. Furthermore, tick fecal material was also collected and screened as a possible source of infectious inoculum for canine infections. *B. v. berkhoffii* DNA was detected in 50% (7 of 14) of females that did not oviposit and in 14.3% (2 of 14) of female ticks that laid eggs, but not detected in egg clutches (100 eggs/female). DNA was also detected in tick feces collected on days 2 through 6 post-capillary tube feeding, however, dogs ($n = 3$) did not become bacteremic or seroconvert when inoculated with tick fecal material. Therefore, trans-ovarial transmission of *B. v. berkhoffii* by *R. sanguineus* is unlikely, but further studies are needed to determine if tick fecal material can serve as a source of infection to canines.

1. Introduction

More than 30 *Bartonella* species and subspecies have been identified to date. Of those, six are known to be vector transmitted: *B. bacilliformis*, the cause of Oroya fever and verruga peruana, is transmitted by a sandfly, *Lutzomyia verrucarum*; *B. quintana*, the agent of trench fever, is transmitted by the human body louse, *Pediculus humanus humanus*; *B. henselae*, the agent of cat scratch disease, is transmitted by the cat flea, *Ctenocephalides felis*; *B. grahamii* and *B. taylorii* are both transmitted by another flea species, *Ctenophthalmus nobilis nobilis*; *Ixodes ricinus* ticks are competent vectors of *B. birtlesii* [1,2]. *Bartonella* DNA has also been detected in a variety of arthropods (fleas, lice, ticks, and biting flies); however, experimental studies have not been performed, in most cases, to verify the transmission capabilities of these potential vectors [1].

It was first suggested that ticks may serve as potential vectors of *Bartonella* species in 1992 [3]. Two male patients were hospitalized within weeks after tick attachment because of recurring fever, myalgia, arthralgias, headache, and sensitivity to light. *B. henselae* was detected by PCR analysis and bacteria was recovered from the blood of both patients. *B. henselae* is generally transmitted via inoculation of infectious flea fecal material by cat scratch or potentially through the bite of an infected flea or bacteremic cat [1,4]. However, one tick-exposed patient could not recall being bitten or scratched by a cat prior to the onset of

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clinical symptoms [3]. Since that initial report, *Bartonella* DNA has been detected in various tick species throughout the world and as reviewed by Billeter et al. [1], several case reports and seroepidemiological studies in dogs and human patients suggest that ticks may be involved in the transmission of *Bartonella* species. In 2008, Cotté et al. [5] demonstrated the potential trans-stadial transmission (infection maintained from one life stage to another) of *B. henselae* by *I. ricinus* ticks via feeding through an artificial membrane. Recently, Reis et al. [2] confirmed the vector competency of *I. ricinus* in the transmission of a rodent-associated *Bartonella* sp., *B. birtlesii*. In that study, *I. ricinus* larvae acquired *B. birtlesii* from a bacteremic mouse, maintained the bacteria through a molt, and successfully transmitted the bacteria to naïve mice [2].

B. v. berkhoffii, first isolated in a dog with aortic valve endocarditis in 1993, has been identified as a potential cause of cardiac arrhythmias, myocarditis, granulomatous lymphadenitis, uveitis, chorioditis, and sialometaplasia in domestic dogs [6–12] and as a cause of osteomyelitis in a cat [13]. *B. v. berkhoffii* has also been detected in gray foxes, coyotes, and humans and coyotes are believed to be the primary reservoirs of infection in California [11,14,15]. To date, four *B. v. berkhoffii* genotypes have been characterized based upon sequence differences within the 16S–23S intergenic spacer region (ITS) and bacteriophage associated heme-binding protein gene (Pap31): *B. v. berkhoffii* genotype I and II have been detected in a cat, dogs, coyotes, and humans and *B. v. berkhoffii* genotype III has also been described in gray foxes in the United States, dogs and a human with endocarditis from Europe, and a military working dog with endocarditis that originated in Germany [8,11,13,16–18]. *B. v. berkhoffii* genotype IV, which has yet to be isolated, was amplified from a postmortem aortic valve of a dog from Canada [11,19].

As with most *Bartonella* species, it remains unclear which vector, if any, is responsible for the transmission of *B. v. berkhoffii*. It has been speculated, however, that the brown dog tick, *R. sanguineus*, may play a role in the transmission of *B. v. berkhoffii* [1]. In 1999, Kordick et al. [20] published a study detailing tick-borne diseases outbreak in ill Walker Hounds and their owners in North Carolina. Of 27 dogs screened, 25 were *B. v. berkhoffii* seroreactive and all but one dog was seroreactive to *Ehrlichia canis*, a bacterium transmitted by *R. sanguineus*. Furthermore, *R. sanguineus* ticks were removed from these dogs on three separate kennel visits [20]. In 12 dogs diagnosed with ehrlichiosis, at the North Carolina State University (NCSU) Veterinary Teaching Hospital, 42% were shown to harbor *B. v. berkhoffii* antibodies [21]. In another study, 8.7% of 1,875 U.S. military working dogs were *B. v. berkhoffii* seroreactive and of those, 43% (13 out of 30) were concurrently *E. canis* and *B. v. berkhoffii* seroreactive [22]. Dogs from Reunion Island, located in the Indian Ocean, were also screened for the presence of *B. v. berkhoffii* and *E. canis* antibodies: 26% of 165 dogs were *E. canis* seroreactive, 8.85% were *B. v. berkhoffii* seroreactive, and 5% harbored antibodies to both organisms [23]. A more recent publication also described the detection of *E. canis* and *B. v. berkhoffii* antibodies in a dog residing on a Hopi Indian Reservation in Arizona [24]. Though contact with other vectors in these studies was likely, historical data has provided circumstantial evidence that *R. sanguineus* may be involved in the transmission of *B. v. berkhoffii*.

Clinically, and similar to the initial human cases supporting tick transmission of *B. henselae*, three dog case studies have also been published suggesting that *R. sanguineus* may be a competent vector of *B. v. berkhoffii*. In 1996, a dog was seen at the NCSU Veterinary Teaching Hospital because of progressive left submandibular swelling accompanied by recurrent fever [8]. Seven days prior, an engorged tick had been removed from the dog's left ear. A biopsy of the left lymph node demonstrated the presence of *Bartonella* DNA when screened by PCR and the dog was *B. v. berkhoffii* seroreactive. The tick species was not identified as the specimen was discarded. It was speculated, however, that the tick was likely *R. sanguineus* as this species is often found within homes or shelters during the winter and the dog was kept mainly indoors during this time [8]. In 2003, a dog was brought into the NCSU Veterinary Teaching Hospital due to collapsing episodes (syncope) of 1 year duration and it was determined that the dog harbored *B. v. berkhoffii* antibodies [25]. Despite clinical and hematological improvement following treatment with antibiotics, thrombocytopenia persisted. Based upon blood smear and PCR results, the dog was later found to be co-infected with *Babesia vogeli*, a protozoan transmitted by *R. sanguineus* [25]. In another case study, a Labrador retriever was referred to the NCSU Veterinary Teaching Hospital due to lethargy, intermittent inappetance, and historical evidence of polyarthritis [26]. Two weeks later, the dog developed a grand mal seizure, accompanied by spontaneous urination. Episodes of listlessness and epistaxis occurred in subsequent months and the dog was ultimately diagnosed with vegetative valvular endocarditis. The dog was seroreactive to *E. canis* antibodies both by an immunofluorescence assay and Western immunoblot analysis. *B. v. berkhoffii* genotype I was isolated from the blood of this dog, suggesting that the dog was exposed to both *B. v. berkhoffii* and *E. canis* [26]. Again, these case reports are suggestive, but do not confirm transmission of *B. v. berkhoffii* by *R. sanguineus*, the known vector of *E. canis* and *B. vogeli* infections in dogs.

The purpose of the present study was to determine whether adult *R. sanguineus* ticks can be infected with a *B. v. berkhoffii* genotype II isolate via capillary tube feeding and whether the infection can then be transmitted from adult females to their eggs via trans-ovarial transmission. Furthermore, tick fecal material was also collected and screened as a possible source of infectious inoculum for canine infections.

2. Materials and methods

2.1. Bacteria

A first passage isolate of *B. v. berkhoffii* genotype II, initially obtained from a bacteremic coyote at the University of California, Davis, was grown on brain heart infusion (BHI) plates containing 5% rabbit blood (Centers of Disease Control and Prevention, Atlanta, GA) at 35 °C, 5% CO_2. After 7 days, bacteria were collected using a sterile loop and suspended in defibrinated rabbit blood