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Authors: DeNatale, Christopher E., Burkot, Thomas R., Schneider, Bradley S., and Zeidner, Nordin S.

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Novel Potential Reservoirs for *Borrelia* sp. and the Agent of Human Granulocytic Ehrlichiosis in Colorado

Christopher E. DeNatale,[1] Thomas R. Burkot,[1] Bradley S. Schneider,[1] and Nordin S. Zeidner[1,2] [1] Division of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention, P.O. Box 2087, Rampart Road, Fort Collins, Colorado 80522, USA; [2] Corresponding author (email: naz2@cdc.gov).

ABSTRACT: Previous work demonstrated that *Ixodes spinipalpis* ticks maintained an enzootic cycle of *Borrelia bissettii* and the agent of human granulocytic ehrlichiosis (aoHGE) within woodrats (*Neotoma mexicana*) and deer mice (*Peromyscus maniculatus*) in northern Colorado (USA). Because *I. spinipalpis* is the only known vector of *B. bissettii* and aoHGE in Colorado, this study was designed to determine the reservoir status of other hosts of *I. spinipalpis* in five distinct ecological zones along the front range and foothills of Colorado. One hundred and twelve rodents of nine species were examined and 11 (10%) were polymerase chain reaction (PCR) positive for aoHGE; 37 (33%) were culture positive for *B. bissettii,* and five (4%) were coinfected with both organisms based on PCR and culture. Of these, three chipmunk species (*Tamias minimus, T. quadrivittatus,* and *T. umbrinus*) were culture positive for *B. bissettii,* with a single *T. minimus* coinfected with *B. bissettii* and aoHGE. In addition, one golden-mantled ground squirrel (*Spermophilus lateralis*) was positive for both *B. bissettii* and aoHGE. This is the first report of a golden-mantled ground squirrel harboring either *B. bissettii* or aoHGE and the initial observation that chipmunks may be a reservoir for *B. bissettii* in Colorado.

Key words: Borrelia bissettii, chipmunks, ground squirrels, HGE agent, *Spermophilus, Tamias.*

Human granulocytic ehrlichiosis (HGE) is a newly emerging tick-borne infectious disease in the United States (Chen et al., 1994). The highest incidence of HGE infections in people occurs in the northeastern coastal states and in the northern Midwest, areas coincident with the geographic distribution of the principal vector, *Ixodes scapularis* (Pancholi et al., 1995; McQuiston et al., 1999). In addition, a smaller focus of human infection has been reported in California associated with the bites of *Ixodes pacificus* ticks (Kramer et al., 1999).

Previous reports have indicated that white-footed mice (*Peromyscus leucopus*) are the main rodent reservoir for the agent of HGE (aoHGE) in endemic areas (Nicholson et al., 1998; Stafford et al., 1999). In the western United States, however, other rodents including several species of wood rats (*Neotoma fuscipes, N. lepida, N. albigula,* and *N. mexicana*) and deer or white-footed mice (*Peromyscus* spp. including *P. boylii, P. maniculatus,* and *P. gossypinus*) are seropositive for this infectious agent (Nicholson et al., 1999). In a recent survey of rodent species in northeastern Colorado, Mexican wood rats (*N. mexicana*), deer mice (*P. maniculatus*), and the prairie vole (*Microtus ochrogaster*), were shown to be coinfected with aoHGE and *Borrelia bissettii* (Zeidner et al., 2000). In this area of the western US, *Ixodes spinipalpis* was the principal arthropod vector infesting these rodent species and was found to be a competent transmitting vector of both *B. bissettii* and aoHGE (Maupin et al., 1994; Zeidner et al., 2000).

The recent discovery of the aoHGE in *I. spinipalpis* ticks and several rodent species inhabiting the foothills of the Rocky Mountains, led us to investigate the possibility that other rodent species might act as reservoir hosts of both *B. bissettii* and aoHGE. Our survey of rodent species within these areas of Colorado provides the first report of both least chipmunks (*Tamias minimus*) and golden-mantled ground squirrels (*Spermophilus lateralis*) being coinfected with aoHGE and *B. bissettii* as determined by culture and PCR.

Field survey studies were conducted at five undeveloped sites in eastern Colorado. These sites ranged in elevation from 1,750–2,340 m and the dominant flora included *Rhus trilobata, Cercocapus mon-*

tanus, and *Pinus ponderosa.* Rodents were collected with Sherman (H.B. Sherman Traps, Inc., Tallahassee, Florida, USA) and Tomahawk (Tomahawk Live Trap Company, Tomahawk, Wisconsin, USA) live traps baited with rolled oats and placed near rodent nests during the months of June, July, and August of 1999. A total of 445 trap nights were utilized in this study.

Animals were anesthetized with ketamine hydrochloride (Vedco, St. Joseph, Missouri, USA), weighed, and individual species were identified using the taxonomic keys of Fitzgerald et al. (1994). While under anesthesia ectoparasites were removed for analysis and characterization (Cooley and Kohls, 1944; Durden and Kierans, 1996), and the animals were then euthanized by cervical dislocation. Skin and splenic biopsies were harvested for culture of *Borrelia* sp. and DNA isolation as described previously for the detection of aoHGE (Zeidner et al., 2000). Cultures for *Borrelia* sp. were maintained at 34 C and monitored weekly by dark-field microscopy. Cultures were considered negative if no spirochete was detected by day 28 using dark-field microscopy at 500× magnification. Splenic DNA was tested for aoHGE using PCR to amplify regions of both the 16s rRNA gene and the p44 gene as described previously (Pancholi et al., 1995; Zeidner et al., 2000). *Borrelia* sp. was identified from positive cultures using a nested procedure to amplify a central portion of the flagellin gene as previously described (Johnson et al., 1992), and amplicons of appropriate size were sequenced using a dye terminator cycle sequencing reaction (Perkin-Elmer Applied Biosystems, Foster City, California, USA) and compared to known sequences in GenBank (National Center for Biotechnology Information, 2001). All PCR reactions were set up in dedicated PCR sites where aoHGE or *Borrelia* sp. had never been handled, and both templates and products were handled at clean second and third sites respectively. Negative PCR controls consisted of both water and DNA from uninfected nymphal *I. spinipalpis* ticks.

Ixodes spinipalpis were collected from rodents at three Colorado study sites: Carter Lake (40°19′40″N, 105°13′00″W), Colorado Road 27 (40°35′58″N, 105°20′25″W), and Pingree Park (40°40′12″N, 105°40′30″W). *Dermacentor andersoni* were found on rodents only at Pingree Park. A total of 27 *I. spinipalpis* larvae were collected from rodents. Six larvae were found on Mexican wood rats at Carter Lake while one and 13 *I. spinipalpis* larvae were found on deer mice at Colorado Road 27 and Pingree Park, respectively. Least chipmunks and golden-mantled ground squirrels at Pingree Park had three and four larvae, respectively. Nymphal *I. spinipalpis* were found only on deer mice ($n = 1$), least chipmunks ($n = 7$), and golden-mantled ground squirrels ($n = 4$) at Pingree Park.

Dermacentor andersoni was found only at Pingree Park. One nymphal *D. andersoni* was found on a deer mouse and one on a least chipmunk while two nymphs were found on golden-mantled ground squirrels. Three larval *D. andersoni* were collected from golden-mantled ground squirrels.

A total of 112 animals from five study sites in Colorado (Carter Lake, Colorado Road 27, Pingree Park, Canon City [38°34′50″N, 105°16′00″W], and Glen Haven [40°27′14″N, 105°26′55″W]) were examined for *Borrelia* sp. and aoHGE. Eleven animals (10%) were PCR positive for aoHGE, 37 (33%) were positive for *B. bissettii,* and five (4%) were co-infected with aoHGE and *B. bissettii* (Table 1). The highest percentage of animals infected with *B. bissettii* were wood rats with prevalence ranging from 63% (12 of 19) at Carter Lake to 100% (3 of 3) along Colorado Road 27 (Table 1). Likewise, prevalence of *B. bissettii* infection in deer mice ranged from 6% (1 of 17) in the Canon City area to 70% (7 of 10) along Colorado Road 27 (Table 1). The highest percentage of aoHGE infection was in least chipmunks near Pingree Park, where 40% (2

TABLE 1. Prevalence of the HGE agent and *Borrelia bissettii* as determined by PCR and culture in rodents from Colorado, 1999.

Site	Species	Number collected	aoHGE number pos (%)	*B. Bisssettii* number pos (%)	Co-infection number pos (%)
Carter Lake	*N. mexicana*	19	2 (11)	12 (63)	0
	P. maniculatus	11	1 (9)	2 (18)	0
County Rd 27	*N. mexicana*	3	0	3 (100)	0
	P. maniculatus	10	2 (20)	7 (70)	2 (20)
Pingree Park	*P. maniculatus*	13	3 (23)	4 (31)	1 (8)
	T. minimus	5	2 (40)	1 (20)	1 (20)
	S. lateralis	8	1 (13)	3 (38)	1 (13)
Canon City	*N. mexicana*	8	0	1 (13)	0
	P. maniculatus	17	0	1 (6)	0
	P. boylii	3	0	0	0
	P. truei	1	0	0	0
	T. quadrivittatus	2	0	1 (50)	0
	S. variegatus	3	0	0	0
Glen Haven	*P. maniculatus*	4	0	1 (25)	0
	T. umbrinus	5	0	1 (20)	0
	Totals	112	11 (10%)	37 (33%)	5 (4%)

of 5) were PCR positive, followed by deer mice (23% [3 of 13] in the Pingree Park area, 20% [2 of 10] along Colorado Road 27) (Table 1). Coinfection with aoHGE and *B. bissettii* was noted in deer mice (3/23), chipmunks (1/5), and golden-mantled ground squirrels (1/8), all captured near Pingree Park or along Colorado Road 27 (Table 1). Sequence analysis for both the 16s rRNA and p44 gene of aoHGE indicated >98% homology to reported sequences of reference cases within the midwestern United States (Chen et al., 1994).

This is the first report of the golden-mantled ground squirrel harboring both the aoHGE and *B. bissettii*. Previous attempts to infect golden-mantled ground squirrels in the laboratory with *B. burgdorferi* sensu stricto were unsuccessful (Ubico et al., 1996). Whether this was due to the strain of *Borrelia* sp. used, a difference in susceptibility of the golden-mantled ground squirrel for *B. bissettii* and *B. burgdorferi*, or the fact that these animals were inoculated by syringe and not the natural tick vector remain to be investigated. To our knowledge this is also the first report of a chipmunk species as a possible reservoir host for both aoHGE and *B. bissettii*. Previous studies have demonstrated that chipmunks were important hosts for *Ixodes* sp. nymphal ticks (Slajchert et al., 1997), were susceptible to infection with *B. burgdorferi* sensu stricto (Mclean et al., 1993), and may be the primary source of *B. burgdorferi* infection of *I. scapularis* nymphs within specific geographic zones of the midwestern United States (Slajchert et al., 1997). Whether chipmunks play a major role in supporting the enzootic cycle of aoHGE and *B. bissettii* infection in the southwestern United States needs to be formally investigated.

The only tick species collected from both chipmunks and ground squirrels in our study were *I. spinipalpis* and *D. andersoni*. Given that *D. andersoni* ticks were incompetent vectors of *Borrelia* sp. (Dolan et al., 1997) and that *Dermacentor* sp. nymphal ticks were incapable of transmitting aoHGE in the laboratory (Des Vignes et al., 1999), it appears that *I. spinipalpis* ticks may be responsible for maintaining both aoHGE and *Borrelia* sp. infection within ground squirrels and chipmunks. Because the incidence of hu-

man infection with aoHGE and *Borrelia* sp. in the southwestern United States is minimal, the significance of these studies to human health is unclear. Recent studies have indicated that *I. spinipalpis* ticks actively feed and transmit *B. bissettii* and aoHGE to sentinel mice away from rodent nests (Burkot et al., 2001). Risk assessment studies may be able to determine the probability that *I. spinipalpis* may act as a bridging vector from these novel animal reservoirs to humans.

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