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Amy J. Ullmann
Division of Vector-Borne Infectious Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, Colorado 80522

Robert S. Lane
Department of Environmental Science, Policy, and Management, Division of Insect Biology, University of California, Berkeley, California 94720

Klaus Kurtenbach
Department of Infectious Disease Epidemiology, Imperial College of Science Technology and Medicine, St. Mary's Campus, Norfolk Place, London

Michael Miller
Colorado Division of Wildlife, Fort Collins, Colorado

Martin E. Schriefer
Division of Vector-Borne Infectious Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, Colorado 80522

See next page for additional authors

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Authors

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Bacteriolytic Activity of Selected Vertebrate Sera for *Borrelia burgdorferi* Sensu Stricto and *Borrelia bissettii*

Amy J. Ullmann, Robert S. Lane*, Klaus Kurtenbach†, Michael Miller‡, Martin E. Schriefer, Nordin Zeidner, and Joseph Piesman, Division of Vector-Borne Infectious Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, Colorado 80522; *Department of Environmental Science, Policy, and Management, Division of Insect Biology, University of California, Berkeley, California 94720; †Department of Infectious Disease Epidemiology, Imperial College of Science Technology and Medicine, St. Mary's Campus, Norfolk Place, London W21PG, U.K.; ‡Colorado Division of Wildlife, Fort Collins, Colorado 80526; To whom correspondence should be addressed. *e-mail: aff1@cdc.gov*

ABSTRACT: An in vitro assay to evaluate the bacteriolytic activity of the complement pathway was applied to 2 strains of *Borrelia bissettii*, CO501 and DN127, and compared with that of *B. burgdorferi* sensu stricto B31. Sera from mule deer (*Odocoileus hemionus*) and the Western Fence lizard (*Sceloporus occidentalis*) were completely borreliacidal for *B. burgdorferi* and for both strains of *B. bissettii*. Serum from Bobwhite quail (*Colinus virginianus*) was nonlytic for *B. burgdorferi* and partially lytic for *B. bissettii* strains, CO-501 and DN127. Serum from a New Zealand White rabbit (*Oryctolagus cuniculus*) was partially lytic for all 3 strains of *Borrelia*, whereas serum from white-footed mice (*Peromyscus leucopus*) were nonlytic for all 3 *Borrelia* strains. The spectrum of complement sensitivity of *B. bissettii* appears to be similar to that of European *B. afzelii* in that tested rodent serum is not lytic to these 2 genospecies. Interestingly, both *B. bissettii* and *B. afzelii* have been found to be closely associated with rodents. Complement sensitivity demonstrated in these experiments may suggest and possibly predict specific reservoir–host associations.

Bacteria within the *Borrelia burgdorferi* sensu lato group must survive and proliferate in the arthropod vector as well as the host. Part of this survival involves the complement cascade of the host immune system. It has been theorized that sensitivity to serum complement of vertebrates can help define potential reservoirs of Lyme borreliosis spirochetes (Kurtenbach et al., 2002). Previous work has demonstrated that *Borrelia* genospecies (specifically *B. burgdorferi* sensu stricto, *B. afzelii, B. garinii, B. japonica*, and *B. valaisiana*) vary in their sensitivities to different host–serum complement including deer, rodent, avian, and reptilian sera (Kurtenbach et al., 1998; Kuo et al., 2000; Nelson et al., 2000). *Borrelia bissettii* belongs to the *B. burgdorferi* sensu lato complex and has been found throughout North America and in Europe (Picken et al., 1996; Lane et al., 1998; Postic et al., 1998; Wright et al., 1998; R.N. Picken and M.M. Picken, 2000; Schneider et al., 2000; Lin et al., 2001). However, the host range of reservoir-competent and -incompetent animals for *B. bissettii* has not been fully determined (Maupin et al., 1994; Schneider et al., 2000). The purpose of this study was to test the borreliacidal activity of deer (Cervidae), quail (Phasianidae), mouse (Muridae), rabbit (Leporidae), and lizard (Iguanidae) sera against *B. bissettii* and, for comparative purposes, *B. burgdorferi* sensu stricto to determine which of these vertebrate groups may be potential reservoirs for this genospecies of spirochetes.

Borrelia burgdorferi sensu stricto B31 (Shelter Islands, New York), *B. bissettii* CO-501 (Larimer County, Colorado), and *B. bissettii* DN127 (Del Norte, California) were grown in complete BSK-H medium (Sigma Chemical Co., St. Louis, Missouri) (Sinsky and Piesman, 1989) at 33 C until a density of 10^7 cells per ml was achieved (Piesman et al., 1990). For borreliacidal assays, 1 ml of this culture was placed in BSK-H medium alone with no serum or antibiotics and incubated at 33 C for 24 hr. Only low-passage isolates were used in these studies (passaged fewer than 10 times in vitro).

Sera were collected from 5 individual mule deer (*Odocoileus hemionus*) (collected from a captive deer herd at the College of Veterinary Medicine, Colorado State University), 5 individual Western Fence lizards (*Sceloporus occidentalis*) (collected from a salt marsh area adjacent to San Francisco Bay, where *Ixodes pacificus* are not found), 6 individual Bobwhite quail (*Colinus virginianus*) (collected from animals bred at Genesis Laboratories, Wellington Colorado), 7 individual white-footed mice (*Peromyscus leucopus*) (obtained from the University of South Carolina *Peromyscus* Genetic Stock Center laboratory), and a single New Zealand White rabbit (*Oryctolagus cuniculus*). All sera, except for that of the Western Fence lizard, were obtained from captive animals previously unexposed to ticks and were pooled before use. Sera of *C. virginianus*, *P. leucopus*, *O. cuniculus*, and *O. hemionus* were all examined by ELISA and Western Blot assay and were found to be negative for antibodies to *Borrelia* spp., as previously described (data not shown) (Schwan et al., 1996). The Western Fence lizard sera were not tested for anti-*Borrelia* antibodies because sera taken from this area were previously tested and were found to be negative (Lane and Quistad, 1998). All sera were used without earlier freezing, except for sera from Western Fence lizards, which were shipped frozen and subsequently thawed once on ice before use.

Fifty microliters of *B. burgdorferi* cultures grown in serum-free BSK-H was added to 50 μl of test serum to give final volumes of *B. burgdorferi* serum suspensions of 100 μl per well in 96-well microtiter plates (Nunc, Rochester, New York). All tests were run in triplicate with serum samples pooled from at least 5 individual animals, with the exception of New Zealand White rabbit serum as noted above. Some aliquots of sera were depleted of functional complement, or components thereof, by heat treatment (0.5 hr of incubation at 56 C), whereas other aliquots were left untreated. Controls consisted of *Borrelia* cultures diluted 1:1 in saline and incubated in serum-free media. The plates were sealed with sealing tape (Corning, Acton, Massachusetts) and incubated at 33 C (Kurtenbach et al., 1998). Each well was read at 0, 2, 4, and 22 hr by collecting 10 μl of culture suspension and then quantifying mobile spirochetes by dark-field microscopy (×400 magnification). Immobilization and bacteriolysis were used as criteria for assessing borreliacidal activity.

All bacterial-serum wells were run in triplicate and the mean values of motile spirochetes calculated using the following equation: % mortality = ([no. spirochetes in control − no. spirochetes in serum sample]/[no. spirochetes in control]) × 100, as described by Kuo et al. (2000) Statistically significant differences in percentage of bacteriolysis were established using analysis of variance, with $P < 0.05$ considered statistically significant.

Immobilization or lysis of spirochetes was apparent within 2 hr of incubation and increased over time (Table I). Deer and lizard sera were completely borreliacidal for *B. burgdorferi*, *B. bissettii* CO-501, and *B. bissettii* DN127 ($P < 0.01$, Table I). Serum collected from quail was nonlytic for *B. burgdorferi* ($P < 0.001$, Table I) and partially lytic for both *B. bissettii* CO-501 and DN127 ($P < 0.0414$, Table I). Rabbit serum was partially lytic for all 3 strains of *Borrelia* ($P = 0.021$), whereas mouse serum was nonlytic for all 3 *Borrelia* strains ($P < 0.001$, Table I). Heat inactivation of sera (0.5 hr at 56 C) destroyed all borreliacidal effects (data not shown), suggesting that components of the complement system are involved in bacteriolysis.

Previous studies demonstrated that complement-related killing of *B. burdorferi* sensu lato generally acts through the alternate complement pathway and can be blocked by Factor H (Kraiczy et al., 2001; Stevenson et al., 2002). The spectrum of complement sensitivity of *B. bissettii* appears to be similar to that of European *B. afzelii* in that rodent serum is not lytic to these 2 genospecies (Kurtenbach et al., 1998). This differs from *B. burgdorferi* sensu stricto, which may thrive in both rodents and birds. Interestingly, both *B. bissettii* and *B. afzelii* have been found to

TABLE I. Bacteriolytic activity of selected sera against *Borrelia burgdorferi* sensu stricto and *Borrelia bissettii*.

Animal species	Time (hr)	% Bacteriolysis*		
		B. burgdorferi B31	*B. bissettii* CO-501	*B. bissettii* DN127
Mouse	2	0	0	0
	4	0	0	4.23
	22	2.67	0	7.19
Rabbit	2	52.34†	77.50†	38.33†
	4	40.68†	66.27†	56.00†
	22	40.32†	69.35†	56.25†
Quail	2	1.12‡	20.00‡	47.12‡
	4	10.71‡	63.83‡	81.21‡
	22	0.00‡	72.22‡	77.48‡
Lizard	2	100.00§‖	100.00§‖	97.73§‖
	4	100.00§‖	100.00§‖	100.00§‖
	22	100.00§‖	100.00§‖	100.00§‖
Deer	2	96.43§‖	86.67§#	100.00§‖
	4	96.27§‖	100.00§‖	100.00§‖
	22	100.00§‖	100.00§‖	100.00§‖

* Bacteriolysis calculated using the equation: % mortality = ([no. spirochetes in control − no. spirochetes in serum sample]/[no. spirochetes in control]) × 100, as described in the Material and Methods section.
† Significantly different than mouse serum, $P = 0.021$ (analysis of variance).
‡ Significantly different than mouse serum, $P = 0.0414$.
§ Significantly different than mouse serum, $P < 0.001$.
‖ Significantly different than quail serum, $P < 0.001$.
Not significantly different than quail serum, $P = 0.0605$.

be closely associated with rodents in North America (Postic et al., 1998; Schneider et al., 2000) and Europe (Humair et al., 1995). Previous studies have demonstrated that *B. burgdorferi* anticomplement protein found in tick saliva may determine vector competency for transmission (Valenzuela et al., 2000). However, the studies presented here were accomplished independent of tick transmission and suggest a prominent role for the direct ability of host blood components in determining specific reservoir species in nature.

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