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THE GROUNDHOG TICK *IXODES COOKEI* (ACARI: IXODIDAE): A POOR POTENTIAL VECTOR OF LYME BORRELIOSIS

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ABSTRACT: Evidence for infection with the spirochete, *Borrelia burgdorferi*, was sought in *Ixodes cookei* and in groundhogs (*Marmota monax*) in southern Ontario, Canada, and ticks fed on experimentally inoculated hosts were examined for the spirochete. *Borrelia burgdorferi* was not detected by immunofluorescent examination of 110 larval, nymphal or adult *I. cookei* collected from the environment, or taken from humans and other animals. Three groundhogs inoculated with *B. burgdorferi* developed titers of 1:20 to 1:80 by the indirect immunofluorescent antibody test, but *B. burgdorferi* was not isolated from the spleens, kidneys, or urinary bladders of these animals. One of 30 wild groundhogs had an antibody titer of 1:20 to *B. burgdorferi*. Three (5%) of 59 *I. cookei* larvae fed on *B. burgdorferi*-infected hamsters became infected, in comparison with 23 (28%) of 82 *I. dammini* larvae fed on the same hosts. *Borrelia burgdorferi* was present in 5%, 16% and 4% of molted *I. cookei* nymphs fed on infected hamsters, rats or a groundhog, respectively; prevalences of infection in *I. dammini* fed on the same hosts were significantly ($P < 0.05$) higher (45%, 36% and 23%, respectively), as was the intensity of infection. A naive groundhog on which *I. cookei* nymphs from an infected cohort fed did not become infected with *B. burgdorferi*, but it is uncertain whether an infected tick engorged on the experimental host. *Ixodes cookei* seems to be an inefficient vector of *B. burgdorferi*, and is unlikely to be significant in nature. Groundhogs are potential wildlife reservoirs of *B. burgdorferi*, based on their capacity to transmit infection to *I. dammini*.

Key words: Lyme borreliosis, *Borrelia burgdorferi*, *Ixodes cookei*, groundhog tick, *Ixodes dammini*, *Marmota monax*, groundhog, epidemiology, serology, survey, experimental study.

INTRODUCTION

The Lyme disease spirochete, *Borrelia burgdorferi*, is transmitted mainly by ticks of the *Ixodes ricinus* group; in North America these are represented by *I. dammini*, *I. pacificus*, and *I. scapularis*. The spirochete also has been demonstrated in many other species of hematophagous arthropods, including the ticks *I. dentatus* (Anderson et al., 1989), *Dermacentor variabilis* (Magnarelli and Anderson, 1988), *D. occidentalis* (Lane and Lavoie, 1988), *D. parumpertis* (Rawlings, 1986), *Rhipicephalus sanguineus* (Rawlings, 1986), *Haemaphysalis leporis-palustris* (Lane and Burgdorfer, 1988), *Amblyomma americanum* (Schulze et al., 1984), and *A. maculatum* (Teltow et al., 1991). In addition, *B. burgdorferi* has been found in over a dozen species of biting flies and mosquitoes (Magnarelli et al., 1986; Magnarelli and Anderson, 1988); and the fleas *Ctenocephalides felis* (Rawlings, 1986; Teltow et al., 1991), and *Orchopeas leucopus* (Anderson and Magnarelli, 1984; Lindsay et al., 1991).

Ixodes dammini, *I. pacificus*, *I. scapularis* and *I. dentatus* have been established experimentally to be efficient vectors of Lyme borreliosis, based on transstadial transmission of the agent, or infection of experimental hosts (Burgdorfer et al., 1985; Burgdorfer and Gage, 1986; Telford and Spielman, 1989a; Mather and Mather, 1990). In contrast, vector competence of *A. americanum* and *D. variabilis* seems to be poor (Piesman and Sinsky, 1988; Mather and Mather, 1990; Ryder et al., 1992), and in the case of *D. variabilis*, this is supported by field observations (Lindsay et al., 1991).

In Ontario, Canada, *I. dammini* is

known to be established only at Long Point (43°34′N, 80°00′W), on the north shore of Lake Erie (Barker et al., 1992). Despite this, sporadic cases of Lyme borreliosis are diagnosed in people in Ontario who have not travelled to Long Point, or to known endemic areas elsewhere (LeBer and Njoo, 1990). We hypothesized that an alternative reservoir host and vector cycle might be responsible for human exposures to *B. burgdorferi* in Ontario.

Since *I. cookei* is moderately prevalent on groundhogs (*Marmota monax*) in Ontario (Ko, 1972; Farkas and Surgeoner, 1990), and is one of the ticks most commonly found on dogs and people in the province (Scholten, 1977), we began in 1990 to investigate the possibility that the groundhog and the groundhog tick (*I. cookei*) were a reservoir host and vector, respectively, of Lyme borreliosis. Magnarelli and Swihart (1991) found *B. burgdorferi* in several *I. cookei* nymphs; others have questioned whether *I. cookei* may be a vector of Lyme borreliosis in the eastern USA (Hall et al., 1991; Levine et al., 1991). However, there is recent evidence that it is not a competent vector (Ryder et al., 1992).

We report here a survey for evidence of *B. burgdorferi* infection in groundhogs and their ticks in Ontario, and an assessment of the vector competence of *I. cookei* and of the reservoir competence of the groundhog for *B. burgdorferi*.

MATERIALS AND METHODS

Serum was harvested from clotted blood samples collected by hunters from groundhogs which they shot, or collected from animals live-trapped (Tomahawk Live Trap Co., Tomahawk, Wisconsin, USA) during 1990 and 1991; serum was stored at −70 C until examined. All animals were from within a 100 km radius of Guelph, Ontario (43°06′N, 83°15′W). The spleen, kidney, and urinary bladder were removed aseptically from shot groundhogs or from trapped animals which had been euthanatized with a sodium pentobarbital overdose (100 mg/kg; Euthanyl, MTC Pharmaceuticals, Cambridge, Ontario) and an aliquot of each tissue was triturated in BSK II K-5 medium, modified from Barbour's (1984) medium by the addition of kanamycin and 5-fluorouracil (Johnson et al., 1984). Two hundred μl of the triturate of each organ was inoculated into a 7 ml tube of BSK II K-5 medium, incubated at 33 C and examined by darkfield microscopy for spirochetes weekly for 6 wk.

Ixodes cookei were collected from groundhogs that were live-trapped or shot by hunters; that were removed from dogs or humans; or were collected as free-living stages from groundhog burrows with flannel cloth fixed to the end of a flexible plumber's "snake." Ticks were dissected on multiwell slides, and smears of the mid-gut were examined for the presence of *B. burgdorferi* by indirect immunofluorescent (IFA) staining using a monoclonal antibody (H5332) directed against *B. burgdorferi* OspA (courtesy of Dr. Alan Barbour, University of Texas, San Antonio, Texas, USA), as described by Lindsay et al. (1991).

Female *I. cookei* on naturally infested groundhogs brought into captivity were allowed to feed to repletion, and were collected when they dropped off into a water trap. Individual fed-females oviposited in 15 ml vials with gauze lids, held over damp vermiculite in chambers with a relative humidity >95% in environmental chambers at a constant temperature of 24 C and a light:dark cycle of 12:12 hr. Once oviposition was complete, the female tick was removed and examined for infection with *B. burgdorferi* by IFA. Eggs and newly hatched larvae were held under the same environmental conditions as ovipositing females. Once they were sclerotized, unfed larvae were held in vials in humid chambers at 8 C until used. *Ixodes dammini* larvae were reared in the laboratory under similar conditions. Thirty larvae from each of the pools of unfed *I. cookei* and *I. dammini* larvae used for tick-feeding studies were examined for *B. burgdorferi* by IFA staining; no spirochetes were found.

Antibody to *B. burgdorferi* was sought in groundhog serum by an indirect fluorescent antibody test (IFAT). Immunoglobulin from serum collected from a wild groundhog was precipitated in ammonium sulphate. An initial dose of 2.5 mg and two subsequent doses of 1.0 mg of immunoglobulin, and 100 μg of saponin adjuvant (Quil A, Cedarlane Laboratories, Hornby, Ontario), was inoculated intramuscularly at four week intervals into each of two domestic rabbits. Four weeks after the final inoculation, the rabbits were anesthetized with 30 mg/kg ketamine (Ketaset, Ayerst Laboratories, Saint Laurent, Quebec) and 5 mg/kg xylazine (Haver-Bayvet Division, Chemagro, Concord, Ontario), and exsanguinated. Anti-groundhog serum was separated from the clotted rabbit blood. Indirect FAT were conducted on numbered multiwell slides (Twelve Well Multitest Slide,

Flow Laboratories, ICN Biomedicals Canada Ltd., Mississauga, Ontario) coated with air-dried acetone-fixed *B. burgdorferi* reference strain B31 (American Type Culture Collection #35210) as antigen. Test groundhog sera were applied to slides at doubling dilutions from 1:10 to 1:320. Aliquots of rabbit anti-groundhog serum were applied at optimal dilution (1:50) as the first antibody. The second antibody was fluoresceinated goat anti-rabbit IgG (Kirkegaard & Perry Laboratories Inc., Gaithersburg, Maryland, USA) applied at a dilution of 1:100. Unknown sera with a titer of 1:20 or greater were considered positive, based on serologic test results from known infected groundhogs, reported below.

Experimental animals (two Wistar rats, *Rattus norvegicus*; four golden hamsters, *Cricetus auratus*; and three groundhogs) each were inoculated subcutaneously with 1.1 to 1.75 $\times$ 10^7 *B. burgdorferi* strain LI-231 at passage level 2. This strain originally was isolated from an engorged *I. dammini* larva from Long Point, Ontario, and was confirmed as *B. burgdorferi* by reaction with monoclone H5332. The rats and hamsters were acquired from Charles River, St. Constant, Quebec, Canada. Groundhogs were juvenile or adult wild animals, tick-free when they were trapped; all were negative for antibody to *B. burgdorferi* by IFAT at a dilution of 1:10 prior to inoculation.

Groundhog 1 was inoculated three times with *B. burgdorferi*, at intervals of 60 days and 110 days after first inoculation, and was used for tick feeding experiments following the first two inoculations; all other animals were inoculated once. Groundhogs 1, 2 and 3 were killed 28 days after primary (groundhogs 2 and 3) or tertiary (groundhog 1) inoculation, and we attempted to isolate *B. burgdorferi* from the spleen, kidney and urinary bladder of each, as described earlier, to confirm the susceptibility of the species to infection with this agent. No attempt was made to isolate *B. burgdorferi* from the rats and hamsters.

Successful transstadial transmission of *B. burgdorferi* in nymphs molting from larvae fed on *B. burgdorferi*-infected hosts was the criterion of vector potential. Trials were conducted by simultaneously placing unfed *I. cookei* and *I. dammini* larvae on hosts previously inoculated with *B. burgdorferi*. The *I. dammini* larvae served as controls for the infection status of the host and for the relative vector potential of *I. cookei*. Hosts were held over water and all engorged larvae were collected, identified, and segregated by species.

In early trials (hamsters 1 and 2), subsets of fully engorged larvae of each species were dissected and examined for infection with *B. burgdorferi* by IFA as described earlier; this was later abandoned, due to the relatively small numbers of ticks available, and the need for molted nymphs to assess transstadial transmission. Engorged larvae were allowed to molt to the nymphal instar and to sclerotize. They then were dissected and examined for infection by IFA, to ascertain whether there had been transstadial transmission of *B. burgdorferi*. The intensity of *B. burgdorferi* infection in mid-gut smears was categorized into four classes: none; few (<10 per entire well); moderate (>10 per well, but <50/field at 200×); many (at least one field containing >50 organisms).

Hamsters 1 and 2 each were infested with ticks twice, 23 and 73 days after inoculation (DAI); hamsters 3 and 4, and rats 1 and 2 were each infested with ticks once, 39 DAI. Groundhog 1 was infested with ticks twice, 17 days after the first, and 14 days after the second, inoculation with *B. burgdorferi* (Table 1). Prevalence and intensity of infection in *I. cookei* and *I. dammini* nymphs were compared statistically, at the 5% level of significance. The generalized linear interactive model (GLIM) (Aitkin et al., 1989) was used; the model was fitted to the data with binomial error distribution and logistic link (Williams, 1982) because there was substantial overdispersion of the data caused by the correlation of the counts within hosts.

A fourth, uninoculated seronegative juvenile groundhog (groundhog 4) was infested with 150 *I. cookei* nymphs which had molted from the second cohort of larvae allowed to engorge on groundhog 1, to ascertain whether they were capable of transmission of *B. burgdorferi* back to an uninfected animal. The *I. cookei* adults which molted from the nymphs fed on groundhog 4 were dissected and examined by IFA for infection with *B. burgdorferi*. Three weeks after the last *I. cookei* nymph dropped off, several hundred *I. dammini* larvae were allowed to feed on groundhog 4. One hundred of these fed larvae were dissected and examined by IFA, to determine whether groundhog 4 had been infected by the *I. cookei* nymphs. Serum was collected from groundhog 4 at the time of *I. cookei* infestation, 27 days later at the time of infestation with *I. dammini* larvae, and at the time that the animal was killed by barbiturate overdose, a further 10 days later. Organs from this animal were cultured as described previously.

RESULTS

Ixodes cookei was found on 11 (21%) of 58 wild groundhogs. *Borrelia burgdorferi* was not detected by IFA in any of the partially engorged *I. cookei* larvae (n = 46), nymphs (n = 34), or partially or fully

TABLE 1. Prevalence of fed larval, and molted nymphal *Ixodes cookei* and *Ixodes dammini* positive by IFA for *Borrelia burgdorferi* following engorgement on inoculated hamsters, rats and groundhogs.

Species	Animal no.	DAI[a]	Engorged larvae				Molted nymphs			
			I. cookei		*I. dammini*		*I. cookei*		*I. dammini*	
Hamster	1-1[b]	23	0/10[c]		0/10		1/12 (1,0,0)[d]		17/24 (5,11,1)	
	1-2[e]	73	0/13		6/30 (1,5,0)		—[f]		—	
	2-1[b]	23	1/15 (0,1,0)		5/12 (2,3,0)		2/12 (1,1,0)		17/21 (4,13,0)	
	2-2[e]	73	2/21 (0,2,0)		12/30 (1,11,0)		—		—	
	3	39	—		—		2/39 (2,0,0)		29/93 (2,20,7)	
	4	39	—		—		0/29		59/133 (8,47,4)	
	Total		3/59 (0,3,0)	5%[g]	23/82 (4,19,0)	28%	5/92 (4,1,0)	5%	122/271 (19,91,12)	45%
Rats	1	39	—		—		4/34 (4,0,0)		2/12 (1,1,0)	
	2	39	—		—		4/16 (3,1,0)		23/58 (7,11,5)	
	Total						8/50 (7,1,0)	16%	25/70 (8,12,5)	36%
Groundhog	1-1[b]	17	0/24		0/12		0/98		0/13	
	1-2[e]	14	—		—		4/100 (4,0,0)	4%	13/57 (3,9,1)	23%

[a] DAI, number of days after inoculation with *B. burgdorferi* that tick feeding was initiated.
[b] First infestation by ticks.
[c] Number of ticks positive for *B. burgdorferi*/number tested.
[d] Number of infected ticks in each *B. burgdorferi* infection class (few, moderate, many).
[e] Second infestation by ticks.
[f] None tested.
[g] Percent positive.

engorged females (n = 26) collected from groundhogs, dogs or humans in southern Ontario. Nor was it detected in the four unengorged nymphs of this species collected from groundhog burrows.

Antibody to *B. burgdorferi* was not present at a titer ≥1:10 prior to inoculation of the three experimental groundhogs with *B. burgdorferi*; all three animals developed to a titer of 1:20 to 1:80 by 28 days following inoculation. One of 30 wild groundhogs examined serologically had a titer of 1:20 by IFAT to *B. burgdorferi*; five others had titers at the screening dilution of 1:10. *Borrelia burgdorferi* was not cultured from the organs of 27 wild groundhogs, nor was it recovered from the organs of any of the three experimentally inoculated animals (groundhogs 1, 2, 3).

Larvae of *I. cookei* and *I. dammini* fed to repletion on all hosts, and with the exception of the first cohort of ticks fed on groundhog 1, *I. dammini* which fed on each individual host became infected at a prevalence of 16 to 81% (2/12 to 17/21 ticks examined) (Table 1). A small proportion (0 to 25%) of *I. cookei* nymphs were infected with generally low numbers of *B. burgdorferi* after feeding as larvae on inoculated hosts, but overall, the groundhog tick appeared to have poor potential as a vector in comparison with *I.*

dammini (Table 1). There was variation in the results among species, among individual hosts, and between trials on the same hosts. However, over all trials, the prevalence and intensity of infection with *B. burgdorferi* was significantly lower ($P \leq 0.05$) in *I. cookei* nymphs compared with *I. dammini* nymphs.

None of the 54 *I. cookei* from the infected cohort which had fed successfully as nymphs on groundhog 4 and ecdysed to the adult instar was infected when examined by IFA. Groundhog 4 remained seronegative; *B. burgdorferi* was not isolated from its organs, nor was *B. burgdorferi* present in 100 *I. dammini* larvae which fed on this animal 3 weeks after it had been exposed to the *I. cookei* nymphs.

DISCUSSION

There is no substantive evidence that *B. burgdorferi* is cycling in *I. cookei* and groundhogs in southern Ontario. A single wild groundhog had antibodies at the threshold titer established on the basis of experimental inoculation of three groundhogs, but in a non-enzootic area there is a high probability that the few seroreactors encountered will be false positive (Martin et al., 1987).

The low probability of such a cycle is supported by the experimental studies. At best, *I. cookei* may be an inefficient vector of *B. burgdorferi* infection.

Only 5% of engorged *I. cookei* larvae which fed on hamsters 1 and 2 were infected (Table 1). Hence, the low prevalence of infection seen in molted *I. cookei* nymphs was likely a function of failure of larvae to acquire infection, rather than due to poor transstadial transmission. Based on the similar infection rates of *I. cookei* larvae and nymphs, we suggest that *B. burgdorferi* does persist in ticks which are successfully infected as larvae. Spirochetes replicate relatively rapidly in *I. dammini* larvae after feeding and remain numerous, though diminished in number, after the molt (Piesman et al., 1990). It was not possible to compare meaningfully the intensity of infection with *B. burgdorferi* in engorged larvae of the two tick species. But based on the low intensity of infection in infected *I. cookei* nymphs, in comparison with *I. dammini*, we suggest that at least one of the innate characteristics that make *I. dammini* a good vector of *B. burgdorferi* is lacking in *I. cookei*. It may be that low numbers of organisms were acquired at feeding by the few larvae which did become infected; that significant replication may not occur in *I. cookei* which do become infected; or that few spirochetes persist through the molt in this tick.

Our attempt to transmit *B. burgdorferi* to an uninfected groundhog with *I. cookei* nymphs was inconclusive, but it is consistent with a low potential for a groundhog-*I. cookei* cycle of *B. burgdorferi* infection in nature, given the relatively poor propensity for this tick to become infected with the Lyme disease agent. We were unable to ascertain the infection status of individual live ticks, and we had only a limited number of nymphs from the infected cohort for feeding trials. Though we placed all 150 nymphs available on the test groundhog, only 54 fed to repletion. With an estimated 4% prevalence of infection in this cohort of ticks, there is an 89% probability that one of the fed ticks was infected (Snedecor and Cochran, 1980). However, none was found to be infected following the molt to the adult. Hence, there is no proof that groundhog 4 actually was exposed to an infected tick.

In contrast with the low prevalence of *B. burgdorferi* infection we found in *I. cookei*, Ryder et al. (1992) were unable to detect *B. burgdorferi* in *I. cookei* fed on infected hamsters, despite a very high prevalence of infection in *I. dammini* fed concurrently on the same animals. However, they examined only 66 *I. cookei*, which would give a 95% probability of detecting an infected tick at a prevalence of infection $\geq 4.5\%$. This is approximately the prevalence we encountered in *I. cookei* fed on hamsters; if the prevalence of infection in their study was similar to or

lower than ours, small sample size may have precluded detecting infection. Similarly, their failure to transmit *B. burgdorferi* with *I. cookei* does not preclude the possibility that a low proportion of ticks might have been infected, since only 16 nymphs fed to repletion.

The minimum prevalence of infection with *B. burgdorferi* in tick populations, and the intensity of infection in individual ticks, necessary to maintain transmission among a population of hosts, have not been determined for any tick-reservoir system. Prevalence of infection of vectors will depend on host and vector density, among other variables. However, individual *I. cookei* larvae are about two to nine times less likely to become infected with *B. burgdorferi* than *I. dammini* larvae feeding on the same host (Table 1); and if an *I. cookei* larva does become infected, there is a significant probability that the intensity of infection with *B. burgdorferi* will be lower than in *I. dammini*. Despite the evidence for transstadial infection, the capacity of *I. cookei* to act as a vector of *B. burgdorferi* is as yet unproven by successful experimental feeding trials. An independent tick-reservoir host cycle of *B. burgdorferi* infection involving *I. cookei* and groundhogs, analogous to that involving *I. dentatus* and rabbits (Telford and Spielman, 1989b), was not detected in southern Ontario and has yet to be described elsewhere.

The detection of *I. cookei* infected with *B. burgdorferi* at several localities in the eastern USA (Magnarelli and Swihart, 1991; Hall et al., 1991; Levine et al., 1991) implies that these ticks may have been feeding on hosts infected at a high prevalence, probably through the mediation of another, more efficient, vector. As suggested by Magnarelli and Swihart (1991), the risk of *I. cookei* transmitting *B. burgdorferi* to humans or domestic animals seems relatively low, even in areas where infection of reservoir hosts is maintained by other vectors.

Groundhogs are capable of acting as a wildlife reservoir for Lyme borreliosis, based on the successful infection of *I. dammini* fed on an experimentally inoculated animal. All three experimentally inoculated groundhogs developed antibodies; failure of groundhog 1 to be infectious for *I. dammini* following the first inoculation, and failure to isolate *B. burgdorferi* in culture from these animals are unexplained. The same method and medium have been used for successful isolations from other small mammals in this laboratory, and were capable of detecting <10 *B. burgdorferi* in an inoculum of active culture.

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