JOURNAL OF CLINICAL MICROBIOLOGY, July 1991, p. 1520–1522
0095-1137/91/071520-03$02.00/0
Copyright © 1991, American Society for Microbiology
Vol. 29, No. 7

Spotted Fever Group Rickettsiae or *Borrelia burgdorferi* in *Ixodes cookei* (Ixodidae) in Connecticut

LOUIS A. MAGNARELLI* AND ROBERT K. SWIHART

Departments of Entomology and Plant Pathology and Ecology, The Connecticut Agricultural Experiment Station, P.O. Box 1106, New Haven, Connecticut 06504*

Received 15 February 1991/Accepted 18 April 1991

Immatures and females of *Ixodes cookei*, a hard-bodied tick, were collected from woodchucks and other mammals in the northeastern United States and examined for spotted fever group rickettsiae and *Borrelia burgdorferi*. Of the 93 nymphs analyzed by a hemolymph test, 4 (4.3%) harbored rickettsiae. Six (15%) of 40 females were also infected. All infected ticks were collected from woodchucks in Connecticut. Indirect fluorescent antibody staining of midgut tissues from 128 nymphs revealed *B. burgdorferi* in two (1.6%) ticks, whereas larval and female ticks were negative. Further consideration should be given to *I. cookei* as a possible vector of spotted fever group rickettsiae or spirochetes that cause Lyme borreliosis.

Ticks transmit viruses, bacteria, and rickettsiae that cause human illnesses. The American dog tick, *Dermacentor variabilis*, and the deer tick, *Ixodes dammini*, are well known for their involvement in Rocky Mountain spotted fever (5, 7, 13, 15) and Lyme borreliosis (6, 22), respectively. However, there are other tick vectors. For instance, *Ixodes cookei* can transmit Powassan virus (17, 18), a cause of human encephalitis (19). Known to parasitize a variety of mammals in eastern North America (1, 8, 11, 15, 17), *I. cookei* is frequently found on woodchucks (*Marmota monax*). Moreover, the occurrence of the nymphal stage of *I. cookei* overlaps with that of *I. dammini* during summers (12), and members of both tick species will bite humans, rodents, mustelids, and canids. There is limited information on the presence of spotted fever group (SFG) rickettsiae or *Borrelia burgdorferi*, the etiologic agent of Lyme borreliosis (6, 22), in *I. cookei*. Accordingly, this study was conducted to determine if these pathogens occur naturally in the motile stages of this tick.

Woodchucks were captured alive in Tomahawk traps in Branford, Guilford, Hamden, and Southington, Conn., from 1987 through 1990. Each individual was anesthetized with xylazine and ketamine hydrochloride (23) and examined for ticks. Following tick removal and an adequate recovery period from anesthetics, the woodchucks were released unharmed into their natural habitat.

Additional *I. cookei* specimens were submitted by citizens in Concord, N.H., upstate New York (Syracuse), and Connecticut (Farmington and New Hartford) during 1989 and 1990. These specimens were removed from eight humans, a domestic cat, a dog, a river otter (*Lutra canadensis*), and a mink (*Mustela vison*). Ticks were kept alive in vials and transported to The Connecticut Agricultural Experiment Station for analyses.

Hemolymph was obtained from ticks to test for SFG rickettsiae. A leg was amputated, and a drop of hemolymph was placed on a glass microscope slide as described previously (4). After being air-dried for 30 to 60 min, the slides were fixed in cold acetone for 10 min. Hemolymph preparations were then stained with fluorescein isothiocyanate-labeled rabbit antibody to *Rickettsia rickettsii*, the causative agent of Rocky Mountain spotted fever. This reagent, prepared at the Centers for Disease Control in Atlanta, Ga., was polyvalent and detected members of the SFG rickettsiae. The following rickettsiae were tested by direct fluorescent antibody (FA) staining to determine specificity: *R. rickettsii* (R strain), *Rickettsia montana* (M/5-6), *Rickettsia prowazekii* (Breinl strain), and *Ehrlichia canis*. Homologous antisera to these antigens were analyzed by indirect FA staining methods with appropriate conjugates to verify antigen reactivity. In direct FA staining, slides were incubated for 30 min at 37°C and washed in phosphate-buffered saline (PBS) solution, whereas two incubation stages and washes were required for indirect FA staining (20). Preparations were mounted with coverslips and buffered glycerol and examined by fluorescence microscopy. Purified suspensions of *R. rickettsii*, *R. montana*, and *R. prowazekii* and homologous antisera were prepared at the Rocky Mountain Laboratories (20) and provided by Robert N. Philip in 1979 and 1982. Antigens of *E. canis* and homologous dog antiserum were supplied by Cynthia J. Holland of the University of Illinois. Positive direct FA reactions were noted for *R. rickettsii* and *R. montana*, members of the SFG. There was no reactivity with *R. prowazekii*, a typhus group rickettsia, or with *E. canis*. All homologous reactions were positive at serum dilutions of 1:80.

Midgut and associated tissues were dissected from ticks to test for *B. burgdorferi*. These tissues were smeared on a glass microscope slide, air-dried overnight at 37°C, and fixed in cold acetone for 10 min. Undiluted murine monoclonal antibodies (H5332), specific for outer surface protein A of *B. burgdorferi* (2, 3), were used to form antigen-antibody complexes during incubation for 30 min at 37°C (14). After being washed in PBS solution, the slides were air-dried and stained with a 1:60 dilution of fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulins (H and L chain specific) purchased from Organon Teknika Corporation, Durham, N.C. When present, spirochetes were visible by fluorescence microscopy. For a positive control, a suspension of washed whole cells of cultured *B. burgdorferi* (16) was coated to glass microscope slides and tested by indirect FA staining with monoclonal antibodies and conjugated anti-mouse immunoglobulins. Slides coated with suspensions of yolk sac (20) lacking *B. burgdorferi* were included as negative controls.

* Corresponding author.

TABLE 1. Prevalence of larval and nymphal *I. cookei* infected with SFG rickettsiae or *B. burgdorferi* in Connecticut from 1987 to 1990

Dates of collection	No. tested[a]/no. positive (%) for:		
	SFG rickettsiae[b] in nymphs	*B. burgdorferi*[c] in:	
		Larvae	Nymphs
June–July 1987	21/2 (9.5)	NT	22/1 (4.6)
August–September 1987	5/0	NT	5/0
April–May 1988	11/1 (9.1)	4/0	19/0
June 1988	22/1 (4.6)	9/0	28/0
August–September 1988	21/0	10/0	22/0
April–May 1989	NT	NT	NT
June–July 1989	13/0	1/0	11/0
April 1990	NT	NT	NT
June–July 1990	NT	NT	18/1 (5.6)
August–September 1990	NT	5/0	3/0
Total	93/4 (4.3)	29/0	128/2 (1.6)

[a] NT, none tested.
[b] No dual infections of rickettsiae and *B. burgdorferi* were determined.
[c] Tick midguts were tested by an indirect FA staining method.

Immatures or adults of *I. cookei* parasitized 102 woodchucks, eight humans, a mink, one river otter, a domestic cat, and a domestic dog. All motile stages were observed on woodchucks, whereas only nymphs were removed from the mink ($n = 2$) and the humans. Female ticks also parasitized the river otter, cat, and dog. Of the 93 nymphs tested for SFG rickettsiae, 4 (4.3%) were positive (Table 1). Infected ticks were removed from woodchucks captured in Branford, Guilford, and Southington from April through June. In analyses of 40 females collected from woodchucks (Table 2), 6 (15%) contained SFG rickettsiae in hemocytes. Two positive ticks were removed from the same woodchuck.

Examination of midgut tissues revealed a low number of *I. cookei* carrying *B. burgdorferi*. Of the 128 nymphs screened, 2 (1.6%) were infected (Table 1). Larval and female ticks were negative. Positive ticks were removed from woodchucks captured in Guilford and Southington during June and July.

The presence of *I. cookei* on a variety of mammalian hosts in the eastern United States and Canada is well established (1, 8, 11, 17). However, nymphs feeding on humans in areas that are highly endemic for Lyme borreliosis require special attention. Nymphs of *I. cookei* and *I. dammini* are abundant during late spring and summer. Presence of *I. cookei* on humans can confuse physicians and patients because these small ticks are often assumed to be *I. dammini*. If these ticks are misidentified, unnecessary prophylactic antibiotic treatment for Lyme borreliosis can result (9, 12). Compared with *I. dammini*, *I. cookei* has a low vector potential, but ticks removed from humans should be accurately identified by trained personnel.

TABLE 2. Examination of female *I. cookei* for SFG organisms or *B. burgdorferi* in Connecticut from 1987 to 1990

Dates of collection	No. tested/no. positive (%) for:	
	SFG rickettsiae[a]	*B. burgdorferi*[b]
June–July 1987	11/0	12/0
August–September 1987	1/0	1/0
April–May 1988	5/2 (40)	9/0
June 1988	4/0	4/0
August–September 1988	4/2 (50)	4/0
April–May 1989	3/1 (33.3)	3/0
June–July 1989	5/0	7/0
April 1990	2/0	2/0
June–July 1990	5/1 (20)	4/0
August–September 1990	NT	3/0
Total	40/6 (15)	49/0

[a] Tick hemocytes.
[b] Tick midgut tissues.

Prevalence of *I. cookei* infected with SFG rickettsiae varied. Infectivity for nymphs (4.3% positive) was relatively low and consistent with figures published for *D. variabilis* (3.6 to 7.8%), a known vector of *R. rickettsii*, in Connecticut (13, 15). However, infectivity of female *I. cookei* (15%) was relatively high. Since the fluorescein isothiocyanate-conjugated antibodies used in direct FA staining are not species specific, it is unknown what percentage of infected ticks were carrying *R. rickettsii*. Other SFG rickettsiae, such as *R. montana*, are not known to cause human illness and can occur in tick populations. Therefore, rickettsiae should be isolated from *I. cookei* and characterized by biochemical and serological procedures.

The presence of SFG rickettsiae in nymphs and females may not necessarily mean they were acquired during feedings on woodchucks. Some rickettsiae, such as *R. rickettsii*, are transovarially transmitted (7). Also, uninfected larval ticks that feed on other mammals may ingest rickettsiae and transstadially pass these microorganisms to the next life stage.

Detection of *B. burgdorferi* in midgut tissues of *I. cookei* adds yet another tick species to the growing list of potential vectors. However, the infectivity rate of immatures is low, and it does not appear that woodchucks are important vertebrate reservoirs for *B. burgdorferi*. Some authors suggest that this tick could be an important vector to humans (10). Granted, *I. cookei* can harbor *B. burgdorferi*, and the hosts chosen by this tick are likewise parasitized by *I. dammini* (1, 8, 11), but transstadial passage and transmission efficiency for *I. cookei* have not been determined. Uninfected larval and nymphal ticks should be allowed to feed on spirochetemic hosts, and after molting, the ticks should refeed on uninfected hosts to determine if transmission occurs. Until these laboratory experiments have been conducted, *I. cookei* should be cautiously viewed as a possible secondary vector. With much higher infection rates (6), clearer association with multiple human cases in several states (22), and evidence of infected salivary glands (21), *I. dammini* remains the most important vector of *B. burgdorferi* in the United States.

We thank Alan G. Barbour of the University of Texas (San Antonio) for providing monoclonal antibody H5332 and Peter Picone for technical assistance.

REFERENCES

1. **Anderson, J. F., and L. A. Magnarelli.** 1980. Vertebrate host relationships and distribution of ixodid ticks (Acari: Ixodidae) in Connecticut, USA. J. Med. Entomol. **17**:314–323.
2. **Barbour, A. G., R. A. Heiland, and T. R. Howe.** 1985. Heterogeneity of major proteins in Lyme disease borreliae: a molecular analysis of North American and European isolates. J. Infect. Dis. **152**:478–484.
3. **Barbour, A. G., S. L. Tessier, and W. J. Todd.** 1983. Lyme disease spirochetes and ixodid tick spirochetes share a common surface antigenic determinant defined by a monoclonal anti-

body. Infect. Immun. **41**:795–804.
4. **Burgdorfer, W.** 1970. Hemolymph test. A technique for detection of rickettsiae in ticks. Am. J. Trop. Med. Hyg. **19**:1010–1014.
5. **Burgdorfer, W.** 1977. Tick-borne diseases in the United States: Rocky Mountain spotted fever and Colorado tick fever. Acta Trop. **34**:103–126.
6. **Burgdorfer, W., A. G. Barbour, S. F. Hayes, J. L. Benach, E. Grunwaldt, and J. P. Davis.** 1982. Lyme disease—a tick-borne spirochetosis? Science **216**:1317–1319.
7. **Burgdorfer, W., and L. P. Brinton.** 1975. Mechanisms of transovarial infection of spotted fever rickettsiae in ticks. Ann. N.Y. Acad. Sci. **266**:61–72.
8. **Cooley, R. A., and G. M. Kohls.** 1945. The genus *Ixodes* in North America. Natl. Inst. Health Bull. **184**:1–246.
9. **Falco, R. C., and D. Fish.** 1988. Ticks parasitizing humans in a Lyme disease endemic area of southern New York State. Am. J. Epidemiol. **128**:1146–1152.
10. **Hall, J. E., J. W. Armine, Jr., R. D. Gais, V. P. Kolanko, B. E. Hagenbuch, V. F. Gerencser, and S. M. Clark.** 1991. Parasitization of humans in West Virginia by *Ixodes cookei* (Acari: Ixodidae), a potential vector of Lyme borreliosis. J. Med. Entomol. **28**:186–189.
11. **Ko, R. C.** 1972. Biology of *Ixodes cookei* Packard (Ixodidae) of groundhogs (*Marmota monax* Erxleben). Can. J. Zool. **50**:433–436.
12. **Magnarelli, L. A., and J. F. Anderson.** 1989. Infected ticks feeding on persons in areas endemic for Lyme disease and Rocky Mountain spotted fever. J. Infect. Dis. **160**:729–730.
13. **Magnarelli, L. A., J. F. Anderson, W. Burgdorfer, R. N. Philip, and W. A. Chappell.** 1985. Spotted fever group rickettsiae in immature and adult ticks (Acari: Ixodidae) from a focus of Rocky Mountain spotted fever in Connecticut. Can. J. Microbiol. **31**:1131–1135.
14. **Magnarelli, L. A., J. F. Anderson, and D. Fish.** 1987. Transovarial transmission of *Borrelia burgdorferi* in *Ixodes dammini*. J. Infect. Dis. **156**:234–236.
15. **Magnarelli, L. A., J. F. Anderson, R. N. Philip, W. Burgdorfer, and W. A. Chappell.** 1983. Rickettsiae-infected ticks (Acari: Ixodidae) and seropositive mammals at a focus for Rocky Mountain spotted fever in Connecticut, USA. J. Med. Entomol. **20**:151–156.
16. **Magnarelli, L. A., J. M. Meegan, J. F. Anderson, and W. A. Chappell.** 1984. Comparison of an indirect fluorescent-antibody test with an enzyme-linked immunosorbent assay for serological studies of Lyme disease. J. Clin. Microbiol. **20**:181–184.
17. **Main, A. J., A. B. Carey, and W. G. Downs.** 1979. Powassan virus in *Ixodes cookei* and Mustelidae in New England. J. Wildl. Dis. **15**:585–591.
18. **McLean, D. M., A. de Vos, and E. J. Quantz.** 1964. Powassan virus: field investigations during the summer of 1963. Am. J. Trop. Med. Hyg. **13**:747–753.
19. **McLean, D. M., and W. L. Donohue.** 1959. Powassan virus: isolation of virus from a fatal case of encephalitis. Can. Med. Assoc. J. **80**:708–711.
20. **Philip, R. N., E. A. Casper, R. A. Ormsbee, M. G. Peacock, and W. Burgdorfer.** 1976. Microimmunofluorescence test for the serological study of Rocky Mountain spotted fever and typhus. J. Clin. Microbiol. **3**:51–61.
21. **Ribeiro, J. M. C., T. N. Mather, J. Piesman, and A. Spielman.** 1987. Dissemination and salivary delivery of Lyme disease spirochetes in vector ticks (Acari: Ixodidae). J. Med. Entomol. **24**:201–205.
22. **Steere, A. C.** 1989. Lyme disease. N. Engl. J. Med. **321**:586–596.
23. **Wright, J. M.** 1983. Ketamine hydrochloride as chemical restraint for selected small mammals. Wildl. Soc. Bull. **11**:76–79.