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## Borreliosis in Free-ranging Black Bears from Wisconsin

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**ABSTRACT:** Blood, kidney and tick samples were obtained from 18 hunter-killed black bears (*Ursus americanus*) from three sites in northern Wisconsin. A *Borrelia* sp., morphologically and antigenically similar to *Borrelia burgdorferi*, was isolated from the blood of two of the animals, and from the kidney of a third. *Ixodes dammini* and *Dermacentor variabilis* were found on the bears. This is the first report of borreliosis in the Ursidae, and of the primary vector of Lyme disease, *I. dammini*, from this host.

**Key words:** *Borrelia* sp., *Ursus americanus*, Lyme disease, *Ixodes dammini*, *Dermacentor variabilis*, survey.

*Borrelia* spp. are members of the family Spirochaetaceae which have the ability to cause disease in man and in a wide range of animal species. They are vectored by both argasid and ixodid ticks, and by lice (Barbour and Hayes, 1986). *Borrelia burgdorferi*, a recently described member of the genus, has been shown to be the causative agent of Lyme disease (LD) in humans (Burgdorfer et al., 1982; Steere et al., 1983). The organism is vectored primarily by ticks of the genus *Ixodes*, with *I. dammini* being responsible for the majority of cases of LD in the United States (Burgdorfer et al., 1985). In Wisconsin, the areas of highest black bear (*Ursus americanus*) density overlap known LD endemic areas (Kohn, 1982; Davis et al., 1984). This paper details the first reported isolation of a *Borrelia* sp. from black bears.

Our investigation took place during the opening weekend of bear season (bait-hunting), on 13 and 14 September 1985. Earlier, hunters had been informed of the upcoming study in a letter accompanying their harvest permits and were requested to leave the kidneys and chest cavity of their bear intact during field-dressing. Three teams of personnel from the Wis-

consin Department of Natural Resources (Madison, Wisconsin 53707, USA) and the Departments of Veterinary Science and Entomology at the University of Wisconsin (Madison, Wisconsin 53706, USA) manned three hunting registration stations in the northern portion of the state (45°41' to 46°20'N, 90°24' to 92°22'W). These were located in the towns of Danbury in Burnett County, Drummond in Bayfield County, and Phillips in Price County.

All bears presented to the registration stations were processed in the following manner: (1) the carcass was searched for ticks by both tactile and visual inspection by combing the fur down to the skin with forceps and fingers, and placing any ticks found into stoppered glass collection vials along with a piece of moist paper towel to prevent desiccation; (2) intact kidneys were removed and placed in individual sealed plastic bags; and (3) the heart was removed and heart blood collected by ventricular needle puncture using a 12-ml syringe with an 18-gauge needle. The blood was immediately transferred into sterile collection tubes. Processing procedures were standardized among the three teams. Knives used to remove specimens were washed and dried after each bear was processed. Blood, kidney, and tick samples were kept on wet ice until they could be processed in the laboratory.

Eighteen bears were processed at the three registration stations; 12 at Phillips, four at Danbury, and two at Drummond. Of these 18 animals, blood samples could be obtained from 14 and kidneys from nine. All were checked for ticks. The period between the time of kill and the time of registration of the bear at the check

station ranged from 2 to 19 hr, with a mean of 12.3 hr.

Samples were processed in a laminar flow hood on 15 September 1986. Attempts to isolate *B. burgdorferi* were done by inoculating 0.1 ml of whole blood into BSK II media (Barbour, 1984). Kidneys were individually stripped of their capsules, and approximately 2 g of renal cortex from each specimen was removed using sterile instruments. The tissue was triturated in 2 ml of sterile phosphate-buffered saline (PBS) using Tenbroeck tissue grinders (Bellco Glass, Vineland, New Jersey 08360, USA). A portion (0.1 ml) of the resultant suspension was inoculated into tubes of BSK II media. All cultures were incubated at 33 C and examined twice weekly for 6 wk by darkfield microscopy. Identification of organisms resembling *Borrelia* spp. was accomplished by direct immunofluorescent staining of air-dried culture smears with a 1:50 dilution of fluorescein isothiocyanate-conjugated rabbit anti-*B. burgdorferi* serum (kindly supplied by W. Burgdorfer, Rocky Mountain Laboratories, Hamilton, Montana 59840, USA). This 1:50 dilution of conjugate had been tested previously against *B. burgdorferi*, *B. turicatae*, *B. hermsii*, and *B. anserina*; and was reactive only with *B. burgdorferi*.

Collected ticks were identified to species level. Eight of the ticks were then surface disinfected using successive rinses of 70% ethyl alcohol, 2% hydrogen peroxide, and two rinses of sterile PBS. The midgut of each tick was dissected onto a drop of sterile PBS and inoculated into BSK II media.

*Borrelia* sp. was isolated from three of the 18 bears (17%). Isolations were from the kidney of one bear and from the blood of two others. All three of these animals were from the Phillips registration station. These isolates reacted positively when stained with the 1:50 dilution of anti-*B. burgdorferi* conjugate. The isolated borreliae had an average of 17 tight, very regular coils, and ranged from approximately 15 to 30  $\mu\text{m}$  in length. Growth of all the cultures was sparse, and the organ-

isms appeared nonmotile. None of the isolates survived beyond the second culture passage.

Ticks were found on eight of the bears. These consisted of 28 adult *I. dammini* and three adult *D. variabilis*. Ticks were found on four of four bears from Danbury, four of 12 from Phillips, and none of two from Drummond. The greatest number of ticks found on one bear was nine. Only one of the three animals which were culture positive for *Borrelia* sp. had any ticks on it, that animal having one partially engorged *I. dammini* female. All tick midgut cultures were negative for *Borrelia* sp., including the tick from the spirochete positive bear.

Published reports on ectoparasites of free-ranging bears are scarce. A study in northeastern Minnesota and northern Michigan reported *D. variabilis* and *D. albipictus* on live-trapped bears (Rogers, 1975). A survey on ectoparasites of bears from northern Wisconsin in 1974 and 1975 revealed the presence of *D. variabilis* and *I. scapularis* (Manville, 1978). However, it is likely that the latter species was actually *I. dammini*, because the northern populations of *I. scapularis* more recently have been reclassified as *I. dammini* (Spielman et al., 1979).

The *Borrelia* sp. isolated from three of the bears unfortunately was lost before more definitive speciation using monoclonal antibodies could be performed. It is our opinion that the organism was *B. burgdorferi*. The isolate fits the morphologic characteristics of the LD spirochete (Barbour and Hayes, 1986). Although the polyvalent conjugate used in this study could cross react with other *Borrelia* spp., we observed no reactivity at the working dilution of 1:50 with three other members of the genus. The lack of reactivity with *B. hermsii* has particular significance, since that species is the most closely related to *B. burgdorferi* of all borreliae tested, based on DNA homology studies (Johnson et al., 1984).

Although *B. burgdorferi* has a wide host

range, its ability to produce disease in wild animal species is undetermined. In humans, infection can result in neurologic, cardiac, ocular and rheumatologic abnormalities, and may produce birth defects (Steere et al., 1983, 1985; Markowitz et al., 1986). Clinical disease attributable to Lyme borreliosis has also been reported in dogs and horses (Kornblatt et al., 1985; Burgess et al., 1986). The significance of the infection for the black bear population is unknown. Because of their relatively low numbers, estimated at 4,200 in Wisconsin (Kohn, 1982), black bears are probably not significant in the epidemiology of human or domestic animal borreliosis in the state. However, if the organism isolated in this study was *B. burgdorferi*, as we believe the evidence suggests, it is likely that Wisconsin bear hunters are at greater risk of exposure to the LD spirochete since the procedures of handling and field-dressing their bear may result in crushing infected *I. dammini* ticks and contact with spirochetemic blood.

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