APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 1995, p. 1641–1642 Vol. 61, No. 4
0099-2240/95/$04.00+0

Detection of Lyme Disease Spirochetes in the Skin of Naturally Infected Wild Sika Deer (*Cervus nippon yesoensis*) by PCR

KOICHI KIMURA,[1] EMIKO ISOGAI,[2]* HIROSHI ISOGAI,[3] YURI KAMEWAKA,[4] TAKESHI NISHIKAWA,[5] NORIHISA ISHII,[6] AND NOBUHIRO FUJII[1]

Department of Microbiology[1] and Animal Experimental Center,[3] School of Medicine, and Division of Molecular Biology, Cancer Research Institute,[5] Sapporo Medical University, Sapporo 060, Department of Preventive Dentistry, School of Dentistry, Health Sciences University of Hokkaido, Ishikari-Tobetsu 1757, Hokkaido 061-02,[2] Health Center of Rumoi, Rumoi, Hokkaido 077,[4] and Department of Dermatology, Yokohama City University of Medicine, Yokohama 236,[6] Japan

Received 14 October 1994/Accepted 27 January 1995

We demonstrated the presence of *Borrelia burgdorferi* sensu lato DNA in the skin tissues of naturally infected wild sika deer, using PCR. The risk of transmission of *B. burgdorferi* sensu lato is recognized in sika deer.

The etiological agent of Lyme disease is the spirochete *Borrelia burgdorferi* and associated species (2, 9), which are primarily transmitted by ixodid ticks (1, 5, 10). Although it is well known that *Ixodes* ticks feed on deer species (10, 19), the role played by deer species in the epidemiology of Lyme disease spirochetes is not completely understood. Several reports have suggested deer as reservoirs of these spirochetes (4, 6, 14, 18), while other investigators have concluded that deer are incompetent reservoirs for *B. burgdorferi* (8, 17). In this experiment, we searched for spirochetes in the skin of wild sika deer (*Cervus nippon yesoensis*) by the PCR method. The detection of Lyme disease spirochetes in skin samples obtained from wild animals by the culture method is difficult, since the growth of the spirochetes is very slow and skin samples from animals in the wild are usually contaminated by many other bacterial species. However, it is very important to detect the etiological agent in skin to confirm the risk of transmission of *B. burgdorferi*, since ticks pick up the spirochetes while feeding on skin.

We obtained skin samples from eight wild sika deer (1 to 2 years old) in East Hokkaido, Japan (43°12′ to 43°30′N, 143°24′ to 144°12′E), in August 1991, with the kind support of M. Suzuki, N. Ohtaishi, and members of the Wild Life Research Workshop. First, the sika deer were examined for feeding ticks, and adults and nymphs of *Ixodes persulcatus* and other species were found on five of the eight deer. In tick-positive deer, one skin sample without ticks adjacent to the tick-infected area was taken from each deer. Nymphal and adult ticks feeding on the skin area were also collected. All five deer had feeding ticks clustered on their ears (30 to 40 ticks on a square 5 by 5 cm), and we therefore obtained all tick samples and all skin biopsy specimens, including those from the three tick-negative deer, from the ears. To examine the penetration of Lyme disease spirochetes into the body of the deer, healthy skin specimens were also obtained from the backs of tick-positive deer. The specimens (ear, blocks 3 by 3 by 3 mm; back, squares 3 by 3 mm and 10 mm in depth) were cut into small pieces and homogenized in TE buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA). DNA was extracted with SepaGene (Sanko-Junyaku, Tokyo, Japan). Modified PCR, based on the method described by Picken (15), was used to detect the *B. burgdorferi* sensu lato gene for the flagellum-associated 41-kDa antigen (flagellin), as previously described (7). The amplified 167-bp DNA fragment was analyzed by 4% agarose gel electrophoresis. In this protocol, lysis of at least 10 to 100 Lyme borrelias was sufficient to obtain a positive result, and no cross-reactivity with other bacteria, such as *Escherichia coli* or *Leptospira interrogans*, was observed (7).

Isolation of *B. burgdorferi* sensu lato from the tick midgut was carried out according to the method described by Miyamoto et al. (12) with Barbour-Stoenner-Kelly medium (3). *B. burgdorferi* sensu lato was isolated from the adult and nymphal ticks obtained from three of the five tick-positive sika deer. The borrelia-positive rate in ticks obtained from the three deer was 81% (17 of 21 ticks from the three deer were positive), which was much higher than the rate in field-swept ticks (less than 16%) (11), suggesting the high risk of infection on the skin of the deer. No spirochetes were detected in any ticks taken from the other two tick-positive sika deer, as determined by culture.

Three skin biopsy specimens obtained from ears of the three deer with spirochete-positive ticks were positive by PCR. In contrast, the two samples obtained from the two deer with spirochete-negative ticks were negative by PCR. The three samples obtained from the healthy skin of the three deer without ticks were also negative by PCR. (Fig. 1). These findings indicate that Lyme disease spirochetes are present in the skin of wild sika deer infested with spirochete-positive ticks. However, PCR was negative in the healthy skin samples apart from the tick-infested area obtained from the backs of the deer with spirochete-positive ticks (data not shown), suggesting local infection by the spirochetes. The ticks emit pheromones and therefore tend to gather on the skin of infested animals (16). Indeed, we observed clusters of ticks on the deer. Many spirochete-negative ticks infesting the deer would be infected with spirochetes through the deer skin if there was one spirochete-positive tick in the cluster. Therefore, it could be said that general infection of the deer with Lyme borrelias is not necessarily needed for the efficient transmission of Lyme borrelias from one tick to many ticks in the cluster. The wood mouse (*Apodemus speciosus ainu*) is a competent reservoir for Lyme disease spirochetes transmitted by *I. persulcatus* in Hokkaido, Japan. Both the larvae and the nymphs of *I. persulcatus* obtained from *A. speciosus ainu* showed a high incidence of infection with the spirochetes (13). Wild sika deer are commonly exposed to two stages of vector ticks, adult and nymphal *I.*

* Corresponding author. Mailing address: Department of Preventive Dentistry, School of Dentistry, Health Sciences University of Hokkaido, Ishikari-Tobetsu 1757, Hokkaido 061-02, Japan.

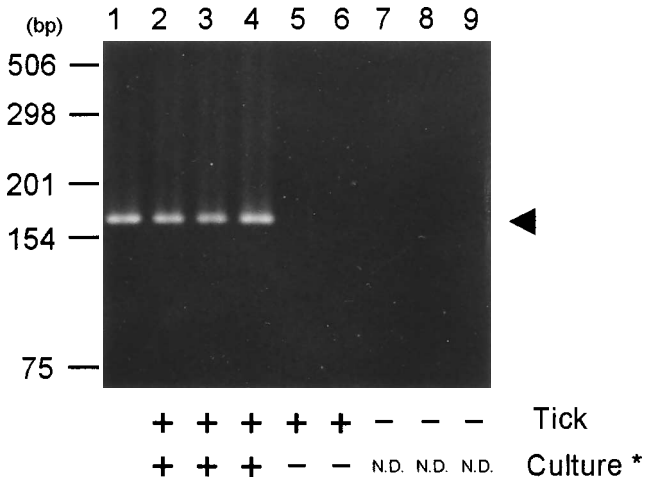

FIG. 1. Agarose gel electrophoresis of amplification products of the partial-length (167-bp) flagellin gene of *B. burgdorferi* sensu lato. Lane 1, *Borrelia garinii* (*B. burgdorferi* HP3) DNA; lanes 2 to 9, skin specimens obtained from the ears of eight sika deer. Ticks were found on five deer (lanes 2 to 6). *B. burgdorferi* sensu lato was isolated from ticks infesting three of these five sika deer (lanes 2 to 4). *, detection of Lyme disease spirochetes from the midgut of *I. persulcatus* feeding on sika deer by the culture method. N.D., not done. The arrowhead indicates amplified products (167 bp).

persulcatus, similarly to *A. speciosus ainu*. In this study, we detected spirochetes in the skin lesions of sika deer by a PCR method and spirochetes in the midgut of two stages of *I. persulcatus* feeding on sika deer by a culture method. We therefore conclude that, in the wild, deer could be a source of Lyme disease spirochetes in ticks and could play an important role in spreading the spirochetes in ticks of different generations.

We thank Masatsugu Suzuki and Noriyuki Ohtaishi, Department of Anatomy, Faculty of Dental Medicine, Hokkaido University; Natsuki Hama, Ohji Zoo; Naomi Shimada, Department of Biochemistry, Faculty of Veterinary Medicine, Hokkaido University; and members of the Wild Life Research Workshop (a wild animal lovers' society) for their kind support.

REFERENCES

1. **Anderson, J. F., and L. A. Magnarelli.** 1984. Avian and mammalian hosts for spirochetes-infected ticks and insects in a Lyme disease focus in Connecticut. Yale J. Biol. Med. **57:**627–641.
2. **Baranton, G., D. Postic, I. Saint Girons, P. Boerlin, J.-C. Piffaretti, M. Assous, and P. A. D. Grimont.** 1992. Delineation of *Borrelia burgdorferi* sensu stricto, *Borrelia garinii* sp. nov., and group VS461 associated with Lyme borreliosis. Int. J. Syst. Bacteriol. **42:**378–383.
3. **Barbour, A. G.** 1984. Isolation and cultivation of Lyme disease spirochetes. Yale J. Biol. Med. **57:**521–525.
4. **Bosler, E. M., B. G. Orimston, L. J. Coleman, J. P. Hanranhan, and J. L. Benach.** 1984. Prevalence of the Lyme disease spirochetes in populations of white-tailed deer and white-footed mice. Yale J. Biol. Med. **57:**651–659.
5. **Burgdorfer, W., A. G. Barbour, S. F. Hayes, J. L. Benach, E. Grundwald, and J. P. Davis.** 1982. Lyme disease: a tick-borne spirochetosis? Science **216:** 1317–1319.
6. **Isogai, E., H. Isogai, T. Masuzawa, Y. Yanagihara, N. Sato, S. Hayashi, T. Maki, and M. Mori.** 1991. Serological survey for Lyme disease in sika deer (*Cervus nippon yesoensis*) by enzyme-linked immunosorbent assay (ELISA). Microbiol. Immunol. **35:**695–703.
7. **Isogai, E., S. Tanaka, I. S. Braga III, C. Itakura, H. Isogai, K. Kimura, and N. Fujii.** 1994. Experimental *Borrelia garinii* infection of Japanese quail. Infect. Immun. **62:**3580–3582.
8. **Jaenson, T. G. T., and L. Talleklint.** 1992. Incompetence of roe deer as reservoir of the Lyme borreliosis spirochetes. J. Med. Entomol. **29:**813–817.
9. **Johnson, R. C., G. P. Schmid, F. W. Hyde, A. G. Steigerwalt, and D. J. Brenner.** 1984. *Borrelia burgdorferi* sp. nov.: etiological agent of Lyme disease. Int. J. Syst. Bacteriol. **34:**496–497.
10. **Lane, R. S., J. Piesman, and W. Burgdorfer.** 1991. Lyme borreliosis: relation of its causative agent to its vectors and hosts in North America and Europe. Annu. Rev. Entomol. **36:**587–609.
11. **Miyamoto, K., M. Nakano, K. Uchikawa, and H. Fujita.** 1992. Prevalence of Lyme borreliosis spirochetes in ixodid ticks of Japan, with special reference to a new potential vector, *Ixodes ovatus* (Acari: Ixodidae). J. Med. Entomol. **29:**216–220.
12. **Miyamoto, K., M. Nakao, N. Sato, and M. Mori.** 1991. Isolation of Lyme disease spirochetes from an ixodid tick in Hokkaido, Japan. Acta Trop. **49:**65–68.
13. **Nakao, M., and K. Miyamoto.** 1993. Reservoir competence of wood mouse, *Apodemus speciosus ainu*, for the Lyme disease spirochetes, *Borrelia burgdorferi*, in Hokkaido, Japan. Jpn. J. Sanit. Zool. **44:**69–84.
14. **Oliver, J. H., Jr., D. Stallknecht, F. W. Chandler, A. M. James, B. S. Mcguire, and E. Howerth.** 1992. Detection of *Borrelia burgdorferi* in laboratory-reared *Ixodes dammini* (Acari: Ixodidae) fed on experimentally inoculated white-tailed deer. J. Med. Entomol. **29:**980–984.
15. **Picken, R. N.** 1992. Polymerase chain reaction primers and probes derived from flagellin gene sequences for specific detection of the agents of Lyme disease and North American relapsing fever. J. Clin. Microbiol. **30:**99–114.
16. **Sonenshine, D. E.** 1991. Tick pheromones, p. 331–369. *In* D. E. Sonenshine (ed.), Biology of ticks, vol. 1. Oxford University Press, Oxford.
17. **Telford, S. R., III, T. N. Mather, S. I. Moore, M. L. Wilson, and A. Pielman.** 1988. Incompetence of deer as reservoirs of the Lyme disease spirochete. Am. J. Trop. Med. Hyg. **39:**105–109.
18. **Wilson, M. L., G. H. Adler, and A. Spielman.** 1985. Correlation between deer abundance and that of the tick, *Ixodes dammini* (Acari: Ixodidae). Ann. Entomol. Soc. Am. **78:**172–176.
19. **Wilson, M. L., S. R. Telford III, J. Piesman, and A. Spielman.** 1988. Reduced abundance of immature *Ixodes dammini* (Acari: Ixodidae) following elimination of deer. J. Med. Entomol. **25:**224–228.