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Ixodes (Pholeoixodes) hexagonus, an efficient vector of *Borrelia burgdorferi* in the laboratory

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Abstract. *Borrelia burgdorferi* Johnson *et al.* was first isolated from the midgut of *Ixodes dammini* Spielman *et al.* in the U.S.A. and from the midgut of *I.ricinus* (L.) in Europe. *I.ricinus* was considered to be the only tick vector of this borrelia, in Europe, until *I.hexagonus* Leach, the hedgehog tick, was found to harbour spirochaetes. This paper reports an evaluation of the vector competence of *I.hexagonus* for the spirochaete *B.burgdorferi*. Transovarial and trans-stadial survival were demonstrated and the spirochaete was transmitted to laboratory mice via the bites of trans-stadially infected *I.hexagonus* females.

Key words. *Ixodes hexagonus*, hedgehog ticks, *Borrelia burgdorferi*, Lyme disease, vector competence.

Introduction

Borrelia burgdorferi Johnson *et al.* was first isolated from the midgut diverticula of *Ixodes dammini* Spielman *et al.* ticks from the United States in 1981 (Burgdorfer *et al.*, 1982), and from the midgut of *I.ricinus* (L.) ticks collected in Switzerland in 1982 (Burgdorfer *et al.*, 1983). Since then, other species of Ixodid ticks have been found infected with spirochaetes: *Amblyomma* (Schulze *et al.*, 1984), *Dermacentor* (Anderson *et al.*, 1985), *Haemaphysalis* (Anderson & Magnarelli, 1984; Lane & Burgdorfer, 1988) and *Rhipicephalus* (Rawlings, 1986). Nevertheless, only members of the *I.ricinus* complex were considered to be competent vectors of *B.burgdorferi*. However, Telford & Spielman (1989) reported that *I.dentatus*, which is not a member of the *I.ricinus* complex, is capable of transmitting *B.burgdorferi*.

I.dammini, *I.scapularis* Say and *I.pacificus* Cooley & Kohls are the vectors of *B.burgdorferi* in North America, and *I.persulcatus* Schulze in Asia. In Europe, *I.ricinus* was considered to be the only tick vector of this spirochaete until *I.(Pholeoixodes)hexagonus* Leach, the hedgehog tick, was found to harbor *B.burgdorferi*-like spirochaetes (Liebisch *et al.*, 1989). Hedgehogs (*Erinaceus europeaus* L.), red foxes (*Vulpes vulpes* L.), martens (*Martes foina* (Erxleben)), stoats (*Mustela erminea* L.), polecats (*M.putoria* L.) and badgers (*Meles meles* L.) are the main hosts for *I.hexagonus* in Switzerland (Toutoungi *et al.*, 1991). If this tick species is shown to serve as a competent vector of *B.burgdorferi*, it may contribute to the establishment of enzootic foci of Lyme borreliosis in regions where *I.ricinus* is absent or infrequent, particularly in urban areas where hedgehogs are present. This paper reports an evaluation of the vector competence of *I.hexagonus* for the spirochaete *B.burgdorferi*.

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Materials and Methods

Tick colonies. Nymphal *I.hexagonus* used in these experiments were derived from our laboratory colony. The breeding conditions will be

the subject of another paper. Briefly, ticks were kept at room temperature, in the dark, in vials with 97% relative humidity. Larvae were fed on Swiss mice, nymphs and adults on rabbits.

Larval *I.ricinus* ticks used for xenodiagnosis, were derived from a laboratory colony that has been maintained for many years (Graf, 1978). Periodic tests using direct immunofluorescence have shown that they are free of *B.burgdorferi* infection.

Infection modes. I.hexagonus nymphs were infected using a modified capillary method described elsewhere (Gern *et al.*, 1990). *B. burgdorferi* strain B31 cultivated in BSK II medium (Barbour, 1984) was employed at a concentration of 10^5-10^6 cells/ml (Helber counting cell chamber) to infect the ticks. Two groups of ticks were used in these experiments: Group I: pre-feeding infected ticks. Unfed nymphs were infected using the capillary method and then placed on rabbit ears to complete engorgement. Group II: post-feeding infected ticks. Engorged nymphs were infected using the capillary method after feeding to repletion on rabbit ears.

Tick feeding. Bloodfeeding of *I.hexagonus* nymphs was carried out using uninfected New Zealand white rabbits which had been tested for *B.burgdorferi* by indirect immunofluorescence. Noninfected Swiss mice were used in the transmission experiments.

Spirochete detection. Ticks were examined for spirochaetes using the direct immunofluorescent antibody test (DFA). Tick organ smears were treated with a fluorescein isothiocyanate-labelled conjugate prepared from the serum of a New Zealand white rabbit that had been immunized against *B.burgdorferi* (Peacock *et al.*, 1971). The adults were dissected and the various organs were screened separately for spirochaetal infection; larval and nymphal ticks were squashed on slides and then examined.

Results

Trans-stadial transmission

In order to determine whether a spirochaetal infection of *I.hexagonus* could be maintained trans-stadially, we examined adults derived from nymphs that had been infected pre- and post-engorgement.

Group I: pre-feeding infected nymphs. Twenty unfed nymphs infected using the capillary method were placed on rabbit ears to complete feeding. Eighteen engorged nymphs were collected from the rabbit. To determine whether the spirochaetes survived in *I.hexagonus* midgut, six of the replete nymphs were examined 1 week after detachment from the rabbit: four of the ticks were found to harbour *B.burgdorferi*. The trans-stadial survival of spirochetes in the twelve resulting adults was determined 10 days after moulting: seven of eight female ticks were found to be infected and only one of four males. The prevalence of infection for adults derived from pre-feeding infected nymphs was 66.6%.

Group II: post-feeding infected nymphs. In this experiment fifty-three nymphs were infected by the capillary method after feeding on a rabbit; forty-four nymphs moulted to the adult stage. Of these, twenty-one males and fourteen females were tested by DFA, 1 week after moulting. Most of these ticks (71.4%) were found to be infected (15/21 males and 10/14 females).

The overall infection prevalence for *I.hexagonus* adults was 70.2%. The slight discrepancy observed in the evidence of infection of adults derived from pre- or post-feeding infected nymphs has also been reported in similar experiments conducted with *I.ricinus* ticks (Monin *et al.*, 1989). The results demonstrate that trans-stadial persistence of *B.burgdorferi* occurs in artificially infected *I.hexagonus*.

Transmission of B.burgdorferi to the host

To determine whether *I.hexagonus* can transmit spirochaetes to a vertebrate host, five females derived from nymphs of group II were placed on four uninfected mice (Table 1). Following engorgement, three females were dissected and examined for spirochaetal infection by DFA; *B.burgdorferi* was detected in two of them. The negative tick had fed on mouse 3 (Table 1). The two remaining females were examined after egg laying (see transovarial transmission). The success of the transmission of *B.burgdorferi* by *I.hexagonus* females to the mice was determined by xenodiagnosis. For this purpose, larval *I.ricinus* were allowed to feed on mice 1 week and 2 weeks after repletion of the *I.hexagonus* adults. A total of seventy-two engorged *I.ricinus* larvae were recovered and examined 6 days later for *B.burgdorferi* infection by DFA; most

Table 1. Xenodiagnosis (using *I.ricinus*) of mice exposed to infected *I.hexagonus* females.

	I.ricinus	
Mouse	No. tested	No. infected (%)
1	6	5 (83%)
2	24	16 (67%)
3*	21	19 (90%)
4	21	13 (62%)

* Two *I.hexagonus* females were placed on this mouse.

of them (53/72; 73.6%) were shown to be infected (Table 1). The results demonstrate that *I.hexagonus* females can transmit the spirochaete to laboratory-bred mice.

Transovarial transmission

Two females from group II were examined after egg laying (see transmission of *B.burgdorferi* to the host). Both contained spirochaetes in their tissues: one in the midgut only, the other in the midgut and in the ovary. Larval *I.hexagonus* derived from these females were examined 5 weeks after hatching (10 weeks after egg laying). Spirochaetes were found in nine of twenty-five larvae from one female and none of thirty-two larvae from the second. Thus *I.hexagonus* females are able to transmit transovarially the *B.burgdorferi* infection to their progeny.

Discussion

In Europe, Ixodidae in genera other than *Ixodes* have never been shown to contain *B.burgdorferi*. Furthermore, Lyme borreliosis spirochaetes were described in species other than *I.ricinus* only recently. Liebisch *et al.* (1989) found spirochaetes in *I.hexagonus* in Germany. Using dark field microscopy, these authors examined fourteen larvae, 178 nymphs and 111 adults collected from hedgehogs (*E.europaeus*), and found that 4.3% of them were infected. This percentage appeared to be greater if they considered the females only: 11.7% of 111 females contained the spirochaete. However, when tested using immunofluorescence, only 2.7% of the females and none of the nymphs and larvae were positive for the spirochaete. More recently, Doby *et al.* (1990) observed spirocheates, using the immunofluorescence test, in two other *Ixodes* sp. collected in France, *I.trianguliceps* (10/106) and *I.acuminatus* (1/32).

The spirochaetes present in the midgut of *I.hexagonus*, *I.trianguliceps* and *I.acuminatus* were neither characterized using monoclonal antibodies nor isolated from these tick species. Hence their identification as *B.burgdorferi* was not confirmed. Moreover, ticks were collected on hosts and examined in their engorged state. It is possible that the spirochaete was present in the bloodmeal and only survived in the midgut for a short period. In fact, no tests were carried out to demonstrate the ability of these tick species to transmit the spirochaete or to maintain it trans-stadially.

This paper is the first to describe the ability of a European tick species other than *I.ricinus* to transmit *B.burgdorferi* to a host. The successful infection of four mice, using five trans-stadially infected *I.hexagonus* females, demonstrated that this tick species is able to transmit *B.burgdorferi* to laboratory animals. In addition, the laboratory tests conducted in this study showed that *I.hexagonus* maintained infection trans-stadially from nymphal to adult stages (70.2%) and transmitted transovarially the spirochaetes to the larval ticks (15.8%). The numbers of infected ticks in our experiments are generally high compared with those observed in natural populations of this tick species. Liebisch *et al.* (1989) reported that 4.3% of *I.hexagonus* collected from hedgehogs were positive and 11.7% if females only were considered. In our experiment, 70.2% of the unfed adults harboured *B.burgdorferi*. This could be explained by the experimental conditions and especially by the high dose of *B.burgdorferi* that the ticks imbibed. Alternatively, the observed discrepancy may be due to the tick host. Liebisch *et al.* (1989) collected ticks from an unique host species captured in one region only. The hedgehog may be a poor reservoir host of *B.burgdorferi*.

As shown in our experiments, *I.hexagonus* is a very efficient potential vector of *B.burgdorferi*. Thus, the fact that this tick species has an host spectrum and a geographical distribution that differ from those of *I.ricinus* (Toutoungi *et al.*, 1991) suggests that *I.hexagonus* may maintain an alternative transmission cycle of this bor-

reliosis in nature. This implies that the geographical distribution of *B.burgdorferi* may be greater than that predicted on the basis of the known distribution of *I.ricinus*. Furthermore, if *I.trianguliceps* and *I.acuminatus* are also confirmed as vectors of *B.burgdorferi*, this will mean that the borrelia may be transmitted in other silent foci (i.e. no disease in humans) devoid of bridge vectors such as *I.ricinus*.

If *I.hexagonus* (and possibly other *Ixodes* tick species such as *I.trianguliceps* and *I.acuminatus*) is a competent vector under natural conditions, it may be an additional reason why the distribution of *B.burgdorferi* is greater than that of TBE virus in Switzerland (Gern *et al.*, 1991). Small mammals have been found to be a source of *B.burgdorferi* infection for *I.ricinus* larvae and nymphs for long periods: more than 2 years in the laboratory (Vittoz *et al.*, 1990). These rodents are very efficient reservoirs of *B.burgdorferi* and they could also occasionally be a source of infection for *I.hexagonus* since they can be infested by this tick species (Manhert, 1971).

The presence of *I.hexagonus* on hedgehogs and martens indicates that this tick species is found in locations where *I.ricinus* is rare or absent, e.g. in suburban and urban areas.

Furthermore, *I.hexagonus* parasitizes domestic animals such as dogs and cats (Toutoungi *et al.*, 1991). Hence *I.hexagonus* can be suspected as a vector of *B.burgdorferi* to these animals. Clinical cases of Lyme borreliosis in dogs have been reported in Switzerland by Pfister *et al.* (1989). As described by Arthur (1953), *I.hexagonus* seems to feed rarely on humans. Failure to parasitize human hosts suggests that this tick poses little danger to public health (Matuschka *et al.*, 1990) but the presence of infected *I.hexagnous* on dogs may represent a source of *B.burgdorferi* infection for the persons who remove the ticks from these animals.

Under laboratory conditions, the vector competence of *I.hexagonus* proved to be very high. Additional experiments are required to assess the vector competence of this tick under natural conditions. This assessment is important as it may identify other independent cycles of transmission of *B.burgdorferi*, including other reservoir hosts such as insectivores and carnivores, which were not described in the current zoonotic model of Lyme borreliosis in Europe.

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