SHORT COMMUNICATION

Infection of Field-Collected *Ixodes ricinus* (Acari: Ixodidae) Larvae with *Borrelia burgdorferi* in Switzerland

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ABSTRACT Free-living larvae from natural areas in five regions in Switzerland were collected to determine the infection rate of *Ixodes ricinus* L. larvae by *Borrelia burgdorferi* Johnson. Larvae were examined for the spirochetes using direct fluorescent antibody assay. Of 652 larvae examined, spirochetes were detected in 20 (3.1%). No differences in infection rate among sites were detected. The low prevalence of *B. burgdorferi* in larvae, compared with higher infection rate in nymphs (12.8%) and adults (14.5%), suggests that transovarial transmission is inefficient. These results suggest that *I. ricinus* can serve as a reservoir for *B. burgdorferi* in nature. Further study is needed on the relative importance of ticks, compared with mammals and birds, as reservoirs for *B. burgdorferi*.

KEY WORDS transovarial transmission, *Borrelia burgdorferi*, *Ixodes ricinus*

BECAUSE *Ixodes ricinus* L. is the principal vector of *Borrelia burgdorferi* Johnson, the etiologic agent of Lyme borreliosis in Switzerland (Barbour et al. 1983, Aeschlimann et al. 1986), studies were initiated to obtain data on the relationship of the spirochete with its vector.

In previous studies, the prevalence of spirochete infection in free-living nymphs and adults ranged from 5 to 50% (Aeschlimann et al. 1986, Péter 1989, Miserez et al. 1990). Transovarial transmission rates of 100% and 60% have been reported for the progeny of two field-collected *I. ricinus* females that had fed on a New Zealand white rabbit and deposited eggs (Burgdorfer et al. 1983). In addition, three artificially infected females produced larvae that were 50% positive for the spirochete (Monin et al. 1989). In the United States, studies of transovarial transmission of *B. burgdorferi* in *I. dammini* (= *I. scapularis* Say Spielman, Clifford, Piesman & Corwin [Oliver et al. 1993]) (Bosler et al. 1983, Piesman et al. 1986, Magnarelli et al. 1987), *I. scapularis* (Magnarelli et al. 1986), and *Ixodes pacificus* Cooley & Kohls (Burgdorfer et al. 1985, Lane & Burgdorfer 1987, Schoeler & Lane 1993) have shown very low rates of passage of *B. burgdorferi* from infected females to their progeny (<3%). However, in Europe the transovarial transmission of *B. burgdorferi* in *I. ricinus* is less well known (Burgdorfer et al. 1983, Stanek et al. 1986). In this study, we examined unfed larval *I. ricinus* for the presence of *B. burgdorferi* to determine the infection rate of transovarially infected larvae and the possible role of *I. ricinus* adults as reservoirs for the spirochete.

Materials and Methods

Study Site. Unfed larvae, nymphs, and adults of *I. ricinus* were collected during spring 1987 by flagging in five different regions of Switzerland: Bruzella, Männedorf, Marthalen, Neuchâtel, and Staatswald. All study sites are located on the Swiss Plateau, a region heavily infested with *I. ricinus*, except Bruzella, which is situated on the south side of the Alps (canton of Tessin). In Switzerland, the percentage of infected *I. ricinus* nymphs by *B. burgdorferi* varies from 5 to 50% (Aeschlimann et al. 1986, Péter 1989, Miserez et al. 1990). Woodmice (*Apodemus sylvaticus* L.) and yellow-necked mice (*Apodemus flavicolus* Melchior) are the principal reservoirs of *B. burgdorferi* (Humair et al. 1993). The incidence of Lyme borreliosis is 1.8 cases per 100,000 inhabitants (Chamot 1989, Fahrer et al. 1990, Zhioua 1993).

Spirochete Detection. Field-collected larval ticks were chilled, placed on glass slides, and examined for the presence of spirochetes using a direct fluorescent antibody assay. The body contents were dried for at least 2 h at 34°C and subsequently fixed with acetone for 10 min. Preparations were stained directly with high-titered fluorescent isothiocyanate conjugated rabbit antibody. Serum was obtained from an immunized New Zealand white rabbit challenged with *B. burgdorferi* (Swiss strain). The conjugate was prepared by L. G. according to the method described by Peacock et al. (1971).

Statistical Analysis. To test whether the percentage of infected larvae is homogeneous across

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Table 1. Prevalence of *B. burgdorferi* infection in field-collected *I. ricinus* larvae in Switzerland

Locations	No. examined larvae	No. infected larvae (%)
Bruzella	211	7 (3.31)
Männedorf	118	5 (4.20)
Marthalen	122	0
Neuchâtel	30	0
Staatswald	171	8 (4.67)
Total	652	20 (3.06)

the study sites, a chi-square test statistic was calculated. Because the expected count for at least one study site is smaller than 1, the distribution of the test statistic may not be chi-square. Furthermore, the number of test larvae and study sites made an exact test unwieldy. A Monte Carlo simulation of the distribution of the test statistic was, thus, generated in the following way. As discussed in Agresti et al. (1979), 17,000 simulations were determined to be sufficient to estimate the *P*-value. In each simulation, 20 infected and 632 noninfected larvae were allotted randomly to study sites. Hence, the marginal distribution of infected larvae across sites was the same as in the original data. The chi-square test statistic was then calculated for each sample.

Results

In total, 652 *I. ricinus* larvae were examined for *B. burgdorferi*. The infection prevalence was 3.1% (20/652). Eight infected larvae were collected from Staatswald, five from Männedorf, and seven from Bruzella. No infected larvae were collected at the Neuchâtel and Marthalen study sites (Table 1). Because sample sizes were small, we used a Monte Carlo simulation to estimate the chi-square statistic. Of the 17,000 simulations, 2,267 yielded a higher test statistic than the original data. The estimated *P*-value is 0.133 (2,267/17,000). Hence, the percentage of infected larvae did not differ significantly among study sites.

Concurrently, we investigated the prevalence of infected *I. ricinus* nymphs and adults in the same regions. Of 258 field-collected nymphs, 33 (12.8%) harbored the etiologic agent of Lyme borreliosis. Of 55 field-collected *I. ricinus* females, 8 (14.5%) were infected by *B. burgdorferi*. The prevalence of spirochete infection in larvae was lower ($\chi^2 = 36.17$, df = 1, $P = 0.0001$) than in nymphal and adult *I. ricinus*.

Discussion

We found the prevalence of infected field-collected *I. ricinus* larvae in nature to be 3.1%. By contrast, laboratory studies reported transovarial transmission rates of up to 100% from field-collected *I. ricinus* females and from capillary-infected ticks (Burgdorfer et al. 1983, Stanek et al. 1986, Monin et al. 1989). Those studies included only a small number of ticks and probably do not reflect the transovarial transmission rate in nature.

Other studies on the prevalence of *B. burgdorferi* in unfed field-collected *I. ricinus* larvae showed comparable results. In France, percentages of infected larvae of 0% (of 351 larvae) and 4.8% (355 larvae) have been reported (Doby et al. 1989, 1990). The percentage of unfed field-collected larvae was 1.1% (367 larvae) in Germany (Wilske et al. 1987). The absence or low prevalence of infection in unfed field-collected larvae suggests a low transovarial transmission rate of *B. burgdorferi* in *I. ricinus*.

Transovarial transmission is possible only when spirochetes disseminate and invade ovarian tissues. However, the prevalence of unfed *I. ricinus* ticks with systemic infection seems to be rather low. Burgdorfer et al. (1983) described the presence of spirochetes in the ovary and synganglion in six of 112 *I. ricinus* from Staatswald on the Swiss Plateau. A deficient immunological system in some tick individuals might allow generalized infection in unfed *I. ricinus* females. It is also possible that some *B. burgdorferi* strains are more capable than others of producing generalized infections in ticks.

During the blood meal, in some gut-infected ticks, spirochetes may cross the midgut wall and induce a systemic infection in various organs, including ovaries (Benach et al. 1987; Ribeiro et al. 1987; Burgdorfer et al. 1988, 1989; Monin et al. 1989; Zung et al. 1989; Gern et al. 1990). Thus, an increased percentage of ticks with systemic infection is observed in engorged ticks. Nevertheless, ovarian infection does not necessarily mean that larvae will become infected. Oocytes heavily infected with spirochetes do not seem to be viable, and spirochetes present in too low numbers seem to gradually die off during oogenesis (Burgdorfer et al. 1988, 1989).

These factors may account for the rarity of infected field-collected larvae and should be studied more intensively. Low spirochete prevalence in larval ticks could result from low frequency or rate of transovarial transmission, low passage from eggs to the resulting filial ticks, or inefficient transovarial transmission in most (but not all) females.

The low prevalence of infected field-collected *I. ricinus* larvae observed in this study generally prevails among tick species of the *I. ricinus* complex (data concerning the transovarial transmission of *B. burgdorferi* in *Ixodes persulcatus* Schulze are not available). We conclude that there is a low prevalence of transovarially infected *I. ricinus* and that larval ticks acquire *B. burgdorferi* mainly by feeding on infected hosts, such as small mammals (Aeschlimann et al. 1986, Humair et al. 1993). The epidemiological role of

I. ricinus adults as possible reservoirs of *B. burgdorferi* is minor.

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