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Ixodes ricinus immatures on birds in a focus of Lyme borreliosis

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Abstract. The infestation of birds by immature *Ixodes ricinus* was studied during 6 months in a Swiss woodland, where Lyme borreliosis is endemic. Thirteen passerine species were found to be parasitized by *I. ricinus* subadults and specially *Turdus merula, T. philomelos* and *Erithacus rubecula.* Overall, 300 larvae and 162 nymphs were collected on 95 avian hosts. Prevalence of infestation of nymphs on birds was higher in spring; larvae peaked in summer. The infection of birds by *Borrelia burgdorferi* was also studied using blood cultivation and examinations of ticks. Motionless spirochetes were isolated from two *E. rubecula.* Infected ticks were removed from five species of passerines, and mainly three species of Turdidae (*T. merula, T. philomelos* and *E. rubecula*). Infection rate of larvae and nymphs by spirochetes averaged 16.3% and 21.7%, respectively. These percentages, compared to the infection rate of questing ticks collected through dragging, suggest that some Turdidae may play a role as amplifying hosts for spirochetes in the focus.

The three-host tick, *Ixodes ricinus* L., is the main vector of the Lyme borreliosis spirochete, *Borrelia burgdorferi* Johnson, Schmid, Hyde, Steigenwalt et Brenner, in Europe (Burgdorfer et al. 1983). In Switzerland, *B. burgdorferi* infection is present in all the various populations of *I. ricinus* inspected up to now. Average infection rates of both nymphal and adult *I. ricinus* range from 10 to 50%, depending on the enzootic sites (Aeschlimann et al. 1986, Miserez et al. 1990, Péter 1990). The infection in questing larvae is much lower (3%), indicating that the vertical transmission is rare (Zhioua et al. 1988, Miserez et al. 1990).

A focus of Lyme borreliosis is maintained by a transmission cycle of *B. burgdorferi* involving larvae, nymphs and hosts. The role of imaginal ticks and the transovarial transmission remain unclear in the maintenance cycle of the spirochete. Schematically, infected nymphs transmit spirochetes to hosts, that infect thereafter larval ticks. Such a host is an amplifier, if it transmits the infection to a great number of ticks. It plays a reservoir role, if spirochetes persist in the host for a long period of time. This maintenance cycle of *B. burgdorferi* may occur only if nymphs peaked before larvae on the same host. In Europe, rodents as *Apodemus flavicollis* Melchior, *A. sylvaticus* L. and *Clethrionomys glareolus* Schreber proved to be efficient amplifying hosts (Aeschlimann et al. 1986, Humair et al. 1993) and reservoir hosts for the etiologic agent of Lyme disease (Gern et al., submitted).

Birds, and particularly passerines, are important hosts for larval and nymphal *I. ricinus* (Aeschlimann et al. 1974). In order to determine the eventual role of avian hosts in the maintenance of *B. burgdorferi* in nature, we examined in this report the infestation of birds by subadult *I. ricinus* and the infection of these ticks by borreliae in a well-known focus of Lyme borreliosis. This study was conducted in the same site and during the same period as for the investigation of small mammals (Humair et al. 1993).

MATERIALS AND METHODS

Study area. Birds were captured at the Staatswald (Ins, Canton of Berne, Switzerland, altitude: 433 m), a humid woodland, previously described and where Lyme disease is endemic (Aeschlimann et al. 1986, Humair et al. 1993).

Investigations of birds and ticks. Birds were caught in Japanese mist nets from May through October 1988. The trapping session occurred twice monthly on two to four successive days. Eight to thirteen mist nets were placed in different sites in the forest. Suitable locations for bird captures were selected, depending on bird activities. Birds were identified to species, marked by rectrice-clipping, sexed when possible, examined for ticks, bled and finally released at the capture site. Birds retrapped during later capture sessions were reexamined. The head and neck of each bird were scrupulously inspected for ticks, the body was examined more rapidly. All ticks were removed with tweezers, placed in labelled vials and stored in humid chambers.

Ticks were identified to species and to stage in the laboratory. Whatever its state of repletion, each live tick was dissected and the body contents were smeared on a glass microscope slide. Tick tissues were dried, fixed and prepared for direct immunofluorescence (DI) examination, as described previously (Humair et al. 1993).

Isolation of spirochetes. Blood was drawn from the brachial vein of birds by means of a needle and a miniature pipette. Whole blood (0.01 to 0.2 ml) was inoculated into 4 ml BSK II medium (Barbour 1984), incubated at 33 °C and examined by dark-field microscopy for the presence of spirochetes at fortnightly intervals for the first 2 months and then monthly to 10 months post-inoculation.

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Statistical analysis. Fisher's exact test (significant difference: $p \leq 0.05$) was used to examine the proportions of tick-infested hosts, hosts parasitized by infected ticks and infected ticks. Statistical analyses of means of larvae and nymphs per host were performed by Student *t*-test (significant difference: $p \leq 0.05$). Median numbers of ticks were compared by the Mann-Whitney U test (significant difference: $p \leq 0.05$).

RESULTS

One hundred and seventy-eight birds, representing 20 species, were captured in mist nets at the Staatswald from May through October 1988. In addition to the 13 species of Passeriformes listed in Table 1, five other passerine species were collected (*Phylloscopus trochilus* L. (n=2), *Regulus regulus* L. (n=4), *R. ignicapilla* Temminck (n=1), *Ficedula hypoleuca* Pallas (n=1) *Parus montanus* Conrad (n=3)), as well as one Coraciiforme (*Alcedo atthis* L. (n=1)) and one Piciforme (*Dendrocopos major* L. (n=2)). Only 12 passerines were recaptured during subsequent trapping days: most were retrapped once (n=11) and one twice. All of the 178 birds captured were examined for ticks. *I. ricinus* was the predominant bird-infesting tick. Only one larva of *Ixodes frontalis* Panzer was collected on a great tit (*Parus major* L.). Because of its infrequent occurrence, *I. frontalis* specimen was not examined further.

Of the 20 bird species inspected, 13 passerine species were shown to be parasitized by *I. ricinus* immatures (Table 1). The seven bird species listed above were not found parasitized by ticks. Among species at least once infested, 95 of 164 passerines (57.9%) proved to be hosts of subadult *I. ricinus*. The Turdidae (*Erithacus rubecula* L., *Turdus philomelos* Brehm and *T. merula* L.) were significantly more frequently (83.1%) infested with *I. ricinus* than the other avian species (41.4%) (Fisher's exact test: $p < 0.001$). *I. ricinus* larvae were almost twice as abundant as nymphs. 75% of larvae and 83% of nymphs were found on Turdidae, and even half of larvae were collected on robins (153/300, 51.0%) and half of nymphs on blackbirds (87/162, 53.7%). Except *Troglodytes troglodytes* L., the mean numbers of larvae were the highest on *T. philomelos, E. rubecula* and *T. merula,* showing no statistical difference between one another. The mean numbers of nymphs collected on *T. merula* and *T. philomelos* were significantly higher than those on *E. rubecula* (Student *t*-test: respectively $p<0.001$ and $p=0.001$). However, the infestation of robin by nymphs is frequent, as 60% of the tick-infested robins (21/35) were parasitized by the nymphal stage.

The number of Turdidae (*T. merula, T. philomelos* and *E. rubecula*) captured was unevenly distributed throughout the season. Numbers of juveniles peaked mainly in June and July, as determined by analyses of weight and observation of feathers. Most of the Turdidae were parasitized

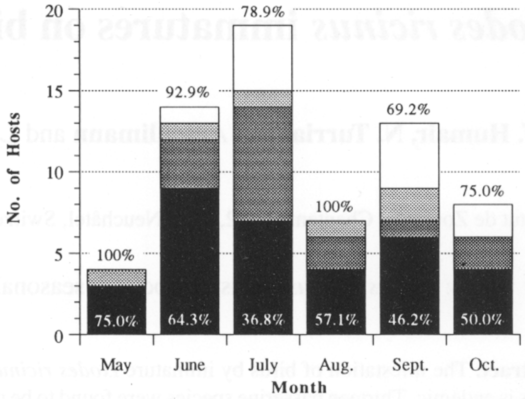

Fig. 1. Seasonal distribution of Turdidae infested with *Ixodes ricinus* immatures, Staatswald, Switzerland, May – October 1988.

☐ Number of hosts without ticks
▩ Number of hosts with larvae only
▨ Number of hosts with nymphs only
■ Number of hosts with larvae and nymphs

Percentages above the columns represent the prevalence of hosts parasitized by ticks and those inside the columns the prevalence of hosts parasitized simultaneously by larvae and nymphs.

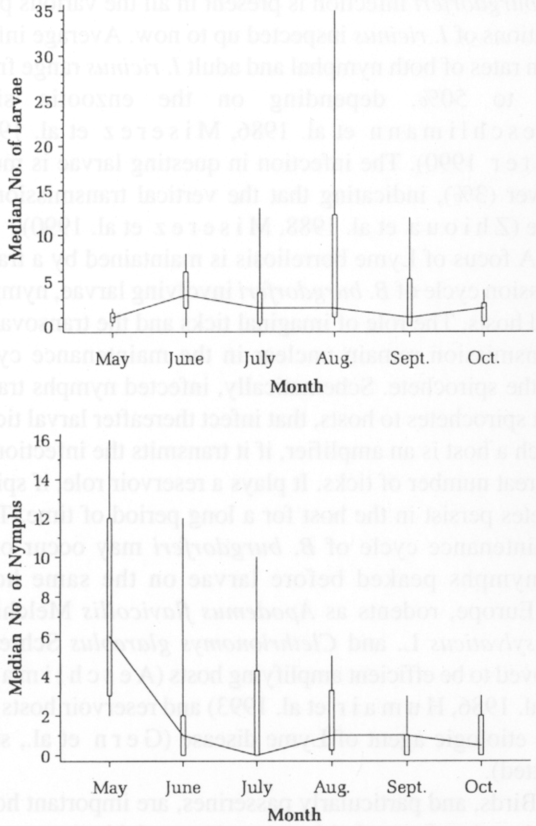

Fig 2. Seasonal distribution of the median number of *Ixodes ricinus* larvae (2a, above) and nymphs (2b, below) per Turdidae.
— median number of tick per host
▯ delimits the percentiles 25 and 75
| delimits minimal and maximal values

Table 1. Passerine birds infested with larval and nymphal *Ixodes ricinus*. Staatswald, Switzerland, May - October 1988.

Host species	No. hosts examined	No. hosts infested (%)		Larvae		Nymphs	
				No.	Mean No. per host ± SD	No.	Mean No. per host ± SD
Troglodytes troglodytes L. Wren	2	2	**(100)**	12	**6.0 ± 7.1**	1	**0.5 ± 0.7**
Erithacus rubecula L. Robin	42	35	**(83.3)**	153	**3.6 ± 6.1**	33	**0.8 ± 1.1**
Turdus merula L. Blackbird	17	14	**(82.4)**	39	**2.3 ± 2.1**	87	**5.1 ± 5.0**
Turdus philomelos Brehm Song Thrush	6	5	**(83.3)**	24	**4.0 ± 3.6**	15	**2.5 ± 1.5**
Sylvia borin Bodd Garden Warbler	7	1	**(14.3)**	0		1	**0.1 ± 0.4**
Sylvia atricapilla L. Blackcap	33	13	**(39.4)**	13	**0.4 ± 0.7**	4	**0.1 ± 0.3**
Parus palustris L. Marsh Tit	7	2	**(28.6)**	3	**0.4 ± 0.8**	2	**0.3 ± 0.8**
Parus ater L. Coal Tit	5	1	**(20.0)**	6	**1.2 ± 2.7**	0	
Parus caeruleus L. Blue Tit	8	1	**(12.5)**	2	**0.3 ± 0.7**	0	
Parus major L. Great Tit	24	14	**(58.3)**	39	**1.6 ± 2.4**	8	**0.3 ± 0.8**
Sitta europaea L. Nuthatch	9	3	**(33.3)**	2	**0.2 ± 0.4**	5	**0.6 ± 1.0**
Garrulus glandarius L. Jay	1	1	**(100)**	1		1	
Fringilla coelebs L. Chaffinch	3	3	**(100)**	6	**2.0 ± 1.0**	5	**1.7 ± 2.9**
	164	95	**(57.9)**	300	**1.8 ± 3.7**	162	**1.0 ± 2.3**

simultaneously by larval and nymphal *I. ricinus* (Fig. 1). *E. rubecula* was more inclined than *Turdus* sp. to be "mono-infested"with either larvae or nymphs. This phenomenon was particularly pronounced in July. The seasonal variations in the number of larvae and nymphs on Turdidae are illustrated in Figs. 2a and 2b, respectively. Medians were calculated in order to reduce the importance of extreme values, which tend to influence means. No statistical difference was observed between the median numbers of larvae, although this stage was particularly abundant in August (Fig. 2a). The Mann-Whitney U test showed that the median number of nymphs in May was significantly higher than the values from June, September and October. Thus, the seasonal distribution of nymphs presented two peaks: one in May and another in July and August (Fig. 2b).

Ticks removed from bird were examined for spirochetes before molting. About 16% of larval and 22% of nymphal *I. ricinus* were infected with borreliae (Table 2). Six species of passerines harboured spirochete-infected *I. ricinus* subadults: robin, blackbird, song thrush, blackcap, great tit and chaffinch. The proportion of Turdidae parasitized by infected ticks was significantly higher than that of the other species ($p<0.001$). No statistical difference was observed between the three species of Turdidae.

The seasonal distribution of Turdidae parasitized by spirochete-infected *I. ricinus* immatures was uneven (Fig. 3). A greater number of Turdidae captured in spring and in summer was shown to harbour infected ticks than did those captured in autumn. Spirochetes were isolated in haemocultures of two *E. rubecula*. Both isolates, detected about 5 months after inoculation of BSK II medium, were motionless and appeared to grow very slowly. Inoculations of culture tubes occurred in the field causing problematic bacterial contaminations.

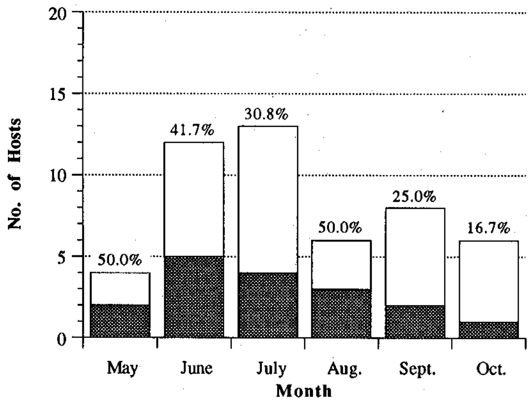

Fig. 3. Seasonal distribution of Turdidae parasitized by infected *Ixodes ricinus* ticks.

☐ Number of hosts without infected ticks

■ Number of hosts with infected ticks

Percentages above the columns represent the prevalence of hosts with infected ticks.

DISCUSSION

Birds, and particularly species classified in the order Passeriformes, are notable hosts for *I. ricinus* subadults. Three species of Turdidae (*T. merula, T. philomelos* and *E. rubecula*) are more frequently exposed to tick bites than the other avian species. Generally, passerines are parasitized by both larval and nymphal *I. ricinus*. Larvae are more abundant on *E. rubecula* while nymphs are more numerous on *T. merula*. Both prevalence and intensity of tick infestation of Turdidae show that these species are most often affected by *I. ricinus,* as previously observed (Aeschlimann et al. 1974). Considering the tick vertical distribution (Gigon 1985), ground-frequenting bird species are most likely to come into contact with ticks. This observation is in accordance with the report of Colquhoun and Morley (1943), who showed a vertical zonation of bird species in a woodland: blackbirds (*T. merula*), robins (*E. rubecula*) and wrens (*T. troglodytes*) are more active near the ground level than great tits (*P. major*) and marsh tits (*Parus palustris* L.). Seemingly, the infestation of an avian species with *I. ricinus* ticks must be correlated with its frequentation rate in the ground and the low strata zones (Rosický and Balát 1954).

If we compare the infestation of micromammals by *I. ricinus* subadults (Mermod et al. 1973, Matuschka et al. 1990) with that of birds, small mammals are generally preferential hosts for larvae while birds for nymphs.

I. ricinus larvae and nymphs are present on Turdidae throughout the time of maximal tick activity in the biotope (April – October) (Mermod et al. 1973). In 1988 in the study

Table 2. Prevalence of spirochete-infected immature *Ixodes ricinus* parasitizing passerine birds.

Host species	No. hosts examined	No. hosts with infected ticks (%)		Larvae			Nymphs		
				No. examined	No. infected (%)		No. examined	No. infected (%)	
Troglodytes troglodytes	2	0	(0.0)	10	0	(0.0)	1	0	(0.0)
Erithacus rubecula	30	7	**(23.3)**	129	23	**(17.8)**	28	1	**(3.6)**
Turdus merula	14	7	**(50.0)**	30	6	**(20.0)**	75	23	**(30.7)**
Turdus philomelos	5	3	**(60.0)**	18	7	**(38.9)**	11	5	**(45.5)**
Sylvia borin	1	0	(0.0)	0			1	0	(0.0)
Sylvia atricapilla	9	1	**(11.1)**	8	1	**(12.5)**	3	0	(0.0)
Parus palustris	2	0	(0.0)	2	0	(0.0)	2	0	(0.0)
Parus ater	1	0	(0.0)	5	0	(0.0)	0		
Parus caeruleus	1	0	(0.0)	1	0	(0.0)	0		
Parus major	12	2	**(16.7)**	35	3	**(8.6)**	8	0	(0.0)
Sitta europaea	3	0	(0.0)	2	0	(0.0)	4	0	(0.0)
Garrulus glandarius	1	0	(0.0)	1	0	(0.0)	1	0	(0.0)
Fringilla coelebs	3	1	**(33.3)**	5	0	(0.0)	4	1	**(25.0)**
	84	21	**(25.0)**	246	40	**(16.3)**	138	30	**(21.7)**

site, the nymphal activity on Turdidae was bimodal: one peak occurred in May and a small one in July and August. Although no statistical evidence allowed to show a peak larval abundance on Turdidae, larvae were, however, more numerous in August on these hosts, suggesting that a maximal activity occurred at that time, as observed in the case of rodent hosts in the same site during the same year (Humair et al. 1993). Thus, the inverted pattern of seasonal abundance of immature *I. ricinus* (nymphs peak before larvae) occurred on avian hosts and small mammals as well (Humair et al. 1993).

Our study was conducted in a woodland area, previously described as natural focus of Lyme disease (Aeschlimann et al. 1986). In this site, the *B. burgdorferi* infection rate of questing *I. ricinus* larvae reached 3% and that of questing nymphs and adults averaged 25% (Aeschlimann et al. 1986, Zhioua et al. 1988). Rodents as *A. flavicollis, A. sylvaticus* and *C. glareolus* play a role as amplifying hosts, as they may infect a great number of larvae (Humair et al. 1993) and play a reservoir role, as these hosts maintain spirochetes for a long period of time and remain infectious for ticks (Gern et al., submitted). In this report, borreliae were found in both larval and nymphal *I. ricinus* collected from passerines. The infection rate of larvae engorged on *T. merula, T. philomelos* and *E. rubecula* were all significantly higher than that of starved larvae (3%) (Fisher's exact test: $p<0.001$), suggesting that larvae acquire the borrelial infection while feeding on Turdidae. The infection rate of nymphs engorged on *T. merula* and *T. philomelos* were slightly higher than that of questing nymphs collected through dragging (about 25%) (Aeschlimann et al. 1986), but the difference observed was not statistically significant. Finally, since the Turdidae are highly exposed to nymphal tick bites, they are highly subjected to spirochetal infection. *T. merula, T. philomelos* and *E. rubecula* may be spirochetemic, as supported by the isolation of spirochetes from the blood of 2 robins. And once infected, these passerines may be infective to *I. ricinus* larvae and nymphs and play a role as amplifying hosts.

The role of avian hosts in a focus of Lyme borreliosis is quite controversial on both sides of the Atlantic (Anderson and Magnarelli 1984, Anderson et al. 1986, Schultze et al. 1986, Anderson 1988, 1989, Mather et al. 1989, Weisbrod and Johnson 1989, Anderson et al. 1990, Manweiler et al. 1990, McLean et al. 1992, Matuschka and Spielman 1992, Olsen et al. 1992). Our observations show that avian hosts may transmit *B. burgdorferi* to *I. ricinus* ticks. This capacity of transmission is limited to some ground-frequenting passerines, highly exposed to subadult tick bites. Other bird species are not involved in the transmission cycle of *B. burgdorferi* in the focus. Some infective birds, like *T. merula, T. philomelos* and *E. rubecula,* may be considered as amplifier hosts, since they may diffuse the spirochetal infection to ticks initially uninfected. Quantitatively, the amplifying role of *E. rubecula* should be more important than that of *Turdus* sp., since the former species harbours mainly larvae and therefore may infect a greater number of larvae.

Further studies are needed to clarify the role of birds as amplifier and reservoir hosts in foci of Lyme borreliosis in both the Old and the New Worlds. In Central Europe, the Turdidae such as robins, blackbirds and thrushes should be particularly investigated. Besides their role of local amplifiers, birds may be short- and long-distance dispersal agents for *B. burgdorferi*-infected *I. ricinus* to other areas in Europe, and even in North Africa. In any case, we think that the contribution of rodents as reservoirs for *B. burgdorferi* is certainly much greater than any other hosts for *I. ricinus* subadults, because of their high population density, their high exposure to larval ticks and their high infectivity to the vectors.

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