

## Synanthropic Birds Influence the Distribution of *Borrelia* Species: Analysis of *Ixodes ricinus* Ticks Feeding on Passerine Birds<sup>†</sup>

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***Ixodes ricinus* ticks collected from 835 birds and from vegetation in the Czech Republic were analyzed. Host-seeking ticks ( $n = 427$ ) were infected predominantly by *Borrelia afzelii* (25%). Ticks ( $n = 1,012$ ) from songbirds (Passeriformes) were infected commonly by *Borrelia garinii* (12.1%) and *Borrelia valaisiana* (13.4%). Juveniles of synanthropic birds, Eurasian blackbirds (*Turdus merula*) and song thrushes (*Turdus philomelos*), were major reservoir hosts of *B. garinii*.**

In central Europe, including the Czech Republic, the main vector of Lyme disease spirochetes is the *Ixodes ricinus* tick, feeding on a wide range of vertebrate hosts (7). The role of birds as reservoir hosts of *Borrelia* spirochetes, mainly *B. garinii*, has been elucidated in recent years (1, 10). In 2009, based on data acquired during the postbreeding period, we presented a study suggesting a differential role of passerine birds in distribution of *Borrelia* spirochetes (2). Here we show data acquired from *I. ricinus* ticks feeding on birds during the spring migration and/or breeding period at the same location. We compare and synthesize our data from both spring and postbreeding periods, together with 2-year data from host-seeking ticks from the same area and with data from 2 years' collection of ticks from birds in a higher-altitude area of the Czech Republic.

**Bird infestation by ticks was lower in the spring period than in the postbreeding period.** Spring collection at location Certak (370 to 400 m above sea level [ASL], 49°34'N, 17°59'E) (5) was conducted from 31 March to 28 April 2007. Birds were collected with mist nets and then identified, examined, and released after the ticks were removed with tweezers. Host-seeking ticks were collected by blanket dragging. Ticks were placed in 70% ethanol and later in the laboratory were classified according to the species, stage, sex of adults, and blood meal volume as “unfed,” “half fed,” or “fully fed.” All procedures were performed identically to those described in our previous study (2). During spring, the total number of birds captured, including retrapped events, was 835 (Fig. 1; see also Table S1 in the supplemental material). Passerine birds (Passeriformes) represented 99.3% (0.7% belonged to Piciformes). The most common birds were European robins, *Erithacus ru-*

*becula* (39%), and blackcaps, *Sylvia atricapilla* (15%). The overall infestation of birds with ticks was lower in the spring than in the postbreeding period (2), the average number of ticks per bird was 2.1 in the postbreeding period and 1.2 in the spring, and the mean number of ticks per infested bird was 5.1 in the postbreeding period (2) and 3.7 in birds captured in the spring. The difference in infestation by *I. ricinus* ticks was not entirely proportional across the bird species. In certain passerines, we observed little or no increase in infestation in the postbreeding period (*E. rubecula*, 1.3 versus 1.4 [2] ticks/bird; dunnock, *Prunella modularis*, 5.5 versus 7.5 ticks/bird [2]). On the other hand, certain birds were substantially more infested in the postbreeding period (Eurasian blackbird, *Turdus merula*, 8.3 versus 18.7 [2]; winter wren, *Troglodytes troglodytes*, 0.8 versus 4.4 [2]). Generally, ground-foraging species have higher numbers of ticks overall, and the association between foraging behavior and tick infestation was more pronounced in the postbreeding period. There were 1,012 ticks identified as *I. ricinus*, 1 fed larva of *Haemaphysalis concinna*, and 3 nymphs of *Ixodes arboricola*. The proportions of *I. ricinus* larvae and nymphs were 38% and 61%, respectively, with 2 adult females collected as well, whereas in the postbreeding period, 70% of *I. ricinus* ticks were represented by larvae (2). The abundance of larvae on birds in the postbreeding period (August/September) suggests infestation by ticks hatched from eggs laid during earlier months. We observed differences in the proportion of subadult ticks on certain bird species; the vast majority of larvae ( $n = 277$ ) were collected from *E. rubecula*, representing 68% of ticks on these birds (odds ratio [OR], 10.0; 95% confidence interval [CI], 7.5 to 13.4). The proportion of larvae was substantially lower on the following bird species: song thrush *Turdus philomelos*, with 12% (OR, 0.2; 95% CI, 0.1 to 0.5), *Turdus merula* with 19% (OR, 0.3; 95% CI, 0.2 to 0.4), and Dunnock *P. modularis* with 9% (OR, 0.2; 95% CI, 0.1 to 0.3).

**Birds from higher altitudes are less infested by *Ixodes* ticks.** In 2 to 4 June 2006 and from 27 May to 2 June 2007, field investigation was performed at another location in the northern part of the Czech Republic, Orlicke Zahori (680 m ASL, 50°30'N, 16°44'E). The total number of birds captured was

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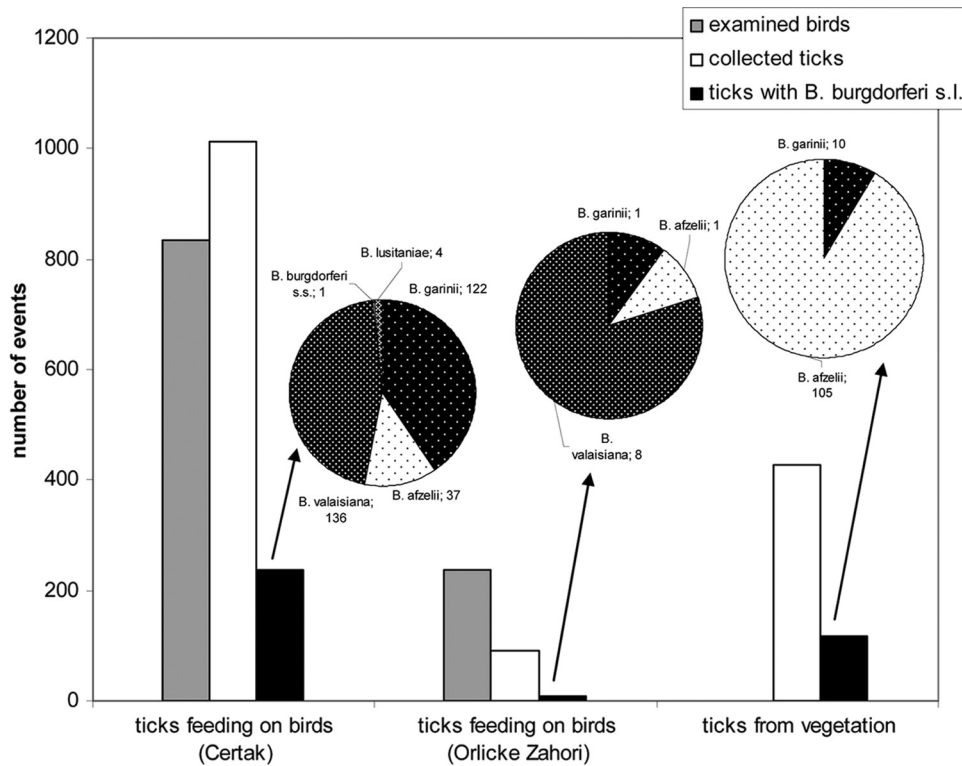


FIG. 1. Overview of the number of examined birds, their infestation with *I. ricinus* ticks, and the presence of *Borrelia* spirochetes in ticks from birds and vegetation, including proportions of *Borrelia* genospecies. s.s., sensu stricto; s.l., sensu lato.

236, of which 43 birds were infested by a total of 92 ticks (Fig. 1; see also Table S2 in the supplemental material); 91 *I. ricinus* ticks (62% larvae and 38% nymphs) and 1 *I. arboricola* subadult tick. The most common birds were blackcaps, *S. atricapilla*, and common whitethroats, *Sylvia communis*, which accounted for 18% and 17%, respectively. The mean number of ticks per bird was 0.4, which is significantly less than findings from a lower-altitude (Certak) location and corresponds to the altitude-dependent tick density (9).

**The presence of *Borrelia* spirochetes is lower in bird-feeding ticks from higher altitudes.** DNA was extracted from ticks by alkaline hydrolysis, and borrelial DNA was detected by the nested PCR targeted *ffr* (5S)-*rrl* (23S) intergenic spacer, followed by a reverse line blotting assay for genospecies identification. In analyzing the spring tick collection from Certak, *Borrelia* spirochetes were found in 238 (23.4%) *I. ricinus* ticks, specifically 122 cases of *B. garinii*, 37 of *B. afzelii*, 136 of *Borrelia valaisiana*, 1 of *B. burgdorferi* sensu stricto, and 4 *Borrelia lusitaniae* infections (Fig. 1), thus resulting in 1.26 infection events per infected tick caused by frequent coinfections; e.g., 5.8% of examined ticks were infected by both *B. garinii* and *B. valaisiana*. Adult females of *I. ricinus* and other tick species found on birds were not infected. *Borrelia* spirochetes were more frequent in nymphs (32.6%) than in larvae (9.2%). In 92 ticks collected at Orlicke Zahori, *B. garinii* was found in 1 nymph from the Eurasian blackbird, *T. merula*, *B. afzelii* was found in 1 nymph from the gray wagtail, *Motacilla cinerea*, and *B. valaisiana* was found in 8 ticks feeding on 2 Eurasian blackbirds (*T. merula*; 4 ticks), 1 fieldfare, *Turdus pilaris* (2), and 2 *S.*

*atricapilla* robins (2) (see Table S2 in the supplemental material).

**The overall level of *B. garinii* infections in bird-feeding ticks is lower in the spring than in the postbreeding period.** To estimate the ability to transmit *Borrelia* spirochetes to ticks feeding on a bird host that might be infected, we did the following: (i) compared the proportions of infected fed and unfed larvae, assuming that a higher proportion of fed infected larvae reflects the host's ability to transmit *Borrelia* to ticks, (ii) compared the proportion of infected nymphs and infected larvae, assuming that a low or no difference in the infection rate between subadult stages reflects the ability of the current host to transmit *Borrelia* to ticks rather than infection from a former stage, and (iii) calculated the noninfectivity coefficient (n-I) by determining the proportion of noninfected larvae among larvae feeding on a bird carrying  $\geq 1$  infected tick. Proportions were evaluated using odds ratio (OR) procedures with 95% confidence intervals (95% CI) (2). *B. garinii* (see Table S3 in the supplemental material) was detected in 12.1% of *I. ricinus* ticks, more frequently in nymphs than in larvae (OR, 5.7; 95% CI, 3.2 to 10.0), suggesting that a substantial number of infected nymphs were the result of infection during a former stage. *B. garinii* infection was associated with ticks feeding on *T. merula* (29.3%), *T. philomelos* (26.0%), and to a lesser extent the great tit, *Parus major* (12.5%). This was accompanied by decreased noninfectivity indices (n-I) in these bird species. Comparing the proportions of *B. garinii* infection in fed and unfed larvae (OR, 5.1; 95% CI, 0.7 to 40), a significant association was not found between blood volume and the pres-

ence of *B. garinii* during the spring period. The nymph-to-larva infection rate was substantially higher in the spring (OR, 5.7; see Table S3) than in the postbreeding period (OR, 1.5) (2), suggesting also an increased risk of *B. garinii* acquisition by ticks from spirochetemic birds in the postbreeding period. We observed a decreased infection rate in ticks feeding on adult birds in late summer compared to that in the spring. This suggests that the statistically increased proportion of *B. garinii* infections during the postbreeding period (22.2%) (2) reflects mainly the activity of juvenile *Turdus merula* and *T. philomelos* after breeding season.

**The presence of other *Borrelia* species in ticks from birds does not differ between the spring and the postbreeding period.** *B. valaisiana* (see Table S4 in the supplemental material) infection was detected in 13.4% of *I. ricinus* ticks and was more frequent in nymphs than in larvae (OR, 2.6; 95% CI, 1.7 to 4.0). The presence of *B. valaisiana* in ticks was associated with *T. merula* (37.2%) and *T. philomelos* (30.0%), with low n-I coefficients. We did not observe overall or bird species-related association between blood volume and the presence of *B. valaisiana* in the spring collection. *B. afzelii* (see Table S5) was found only in nymphs with a n-I equal to 1.0, and the infection in nymphs (5.0%) did not differ from that in the postbreeding period (6.0% [2]). A negative association between blood volume and the presence of *B. afzelii* (OR, 10.9; 95% CI, 1.3 to 9397) supports the theory of an inhibiting effect of bird blood, presumably complement based, on the viability of *B. afzelii* (6). *B. burgdorferi* sensu stricto was found in one half-fed nymph feeding on *P. major*. *B. lusitanae* was detected in 4 nymphs: 1 unfed on *E. rubecula*, 1 full on *T. merula*, and 1 half-fed and 1 full on the same *T. merula* individual retrapped 5 days later; thus, we cannot rule out the capability of *T. merula* to transmit *B. lusitanae*.

**Host-seeking ticks are infected mainly by *B. afzelii*.** Host-seeking ticks were collected from vegetation by blanket dragging at Certak at the time of bird netting. All 427 collected ticks were identified as *I. ricinus*: 3 larvae, 366 nymphs, 22 females, and 36 males. We detected presence of *B. garinii* in 2.3% of host-seeking ticks and *B. afzelii* in 24.6%, respectively (Fig. 1; see also Table S6 in the supplemental material).

Our summary data show that *B. garinii* and *B. valaisiana* are associated with *I. ricinus* ticks feeding on synanthropic passerines, such as Eurasian blackbirds (*T. merula*), song thrushes (*T. philomelos*), and great tits (*P. major*) and that the *B. garinii* occurrence is attenuated at higher altitudes. Although the tick infestation rate reflects foraging behavior, it is not the key to the *Borrelia* infection rate. One determinant may be the tick's ability to feed on certain bird species. For example, despite a high infestation of *P. modularis*, the low infection rate is in accordance with low blood meal volume in ticks feeding on *P. modularis*. This can be related to the differences in the host T-cell-mediated immune response, presumably to tick salivary

proteins, as is described for tick-sensitive and tick-resistant animal hosts (3, 8). Bird immune response to *Borrelia* pathogens may be another biological determinant of a competent reservoir host. Birds are likely naturally resistant to *B. afzelii*, and rodents are the main reservoir hosts of *B. afzelii* in Europe (4). Some individuals of Turdidae and *P. major* (Paridae) are competent reservoir hosts for *B. garinii* and *B. valaisiana* depending on acquired resistance. This is implicated by a decreasing *B. garinii* infection rate in ticks feeding on *T. merula* according to age consecution: juvenile bird, 72%; breeding adult bird, 29%; adult bird during postbreeding, 6% (see Table S3 in the supplemental material) (2). Comparing data from host-seeking and bird-feeding ticks, we conclude that the occurrence of *B. garinii* and *B. valaisiana* in ticks is associated with current-host (bird) feeding. This suggests that transstadial transmission of *B. garinii* and *B. valaisiana* may be less efficient, although possible, based on an increased infection in nymphs compared to larvae and the presence of *B. garinii* in adult ticks. Thus, we conclude that major competent reservoir hosts of the bird-associated Lyme disease spirochete *B. garinii* are juvenile Eurasian blackbirds and song thrushes, with epidemiological relevance due to their synanthropic behavior and distribution potential. The biological determinants of competent bird reservoirs of *B. garinii* need to be elucidated.

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