

Differential Role of Passerine Birds in Distribution of *Borrelia Spirochetes*, Based on Data from Ticks Collected from Birds during the Postbreeding Migration Period in Central Europe[∇]

Lenka Dubska,^{1*} Ivan Literak,¹ Elena Kocianova,² Veronika Taragelova,³ and Oldrich Sychra¹

Department of Biology and Wildlife Diseases, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences, Palackeho 1-3, 612 42 Brno, Czech Republic¹; Institute of Virology, Slovak Academy of Sciences, Dubravská cesta 9, 842 45 Bratislava, Slovakia²; and Institute of Zoology, Slovak Academy of Sciences, Dubravská cesta 9, 845 06 Bratislava, Slovakia³

Received 19 July 2008/Accepted 1 December 2008

***Borrelia* spirochetes in bird-feeding ticks were studied in the Czech Republic. During the postbreeding period (July to September 2005), 1,080 passerine birds infested by 2,240 *Ixodes ricinus* subadult ticks were examined. *Borrelia garinii* was detected in 22.2% of the ticks, *Borrelia valaisiana* was detected in 12.8% of the ticks, *Borrelia afzelii* was detected in 1.6% of the ticks, and *Borrelia burgdorferi sensu stricto* was detected in 0.3% of the ticks. After analysis of infections in which the blood meal volume and the stage of the ticks were considered, we concluded that Eurasian blackbirds (*Turdus merula*), song thrushes (*Turdus philomelos*), and great tits (*Parus major*) are capable of transmitting *B. garinii*; that juvenile blackbirds and song thrushes are prominent reservoirs for *B. garinii* spirochetes; that some other passerine birds investigated play minor roles in transmitting *B. garinii*; and that the presence *B. afzelii* in ticks results from infection in a former stage. Thus, while *B. garinii* transmission is associated with only a few passerine bird species, these birds have the potential to distribute millions of Lyme disease spirochetes between urban areas.**

Lyme borreliosis, the most frequent tick-borne human disease in the northern hemisphere, is a multisystemic disorder caused by spirochetes belonging to the genus *Borrelia* (12). At least seven *Borrelia* species have been obtained from the tick *Ixodes ricinus* (Acari: Ixodidae) in Europe: *B. afzelii*, *B. bissettii*, *B. burgdorferi*, *B. garinii*, *B. lusitaniae*, *B. spielmanii*, and *B. valaisiana* (30, 33). Various reservoir hosts seem to harbor different *Borrelia* species, which is explained by differential properties of the hosts' complement systems (17). Generally, *B. afzelii* is a rodent specialist, *B. garinii* and *B. valaisiana* are associated with birds, and *B. lusitaniae* is associated with lizards (7, 16, 22, 32). The clinical manifestations of Lyme borreliosis in humans differ depending on the *Borrelia* species; *B. garinii* is associated with neurological diseases, while *B. burgdorferi* and *B. afzelii* are more likely to cause arthritis and cutaneous symptoms, respectively (2, 4, 5, 36).

Ixodid ticks may attach to a host for 24 to 48 h, which is sufficient time for some birds to travel hundreds of kilometers along migration routes before ticks complete their feeding and drop off (31). The role of migratory birds in distributing Lyme disease spirochetes to new areas has been established in North America (25, 28, 37).

Recently, it has become clear that the bird host competency for maintaining and transmitting *Borrelia* spirochetes is different in different bird species. Pheasants in the United Kingdom (15, 16) and blackbirds and song thrushes in Central Europe (13, 35) have been shown to be important reservoirs of *B.*

garinii and *B. valaisiana*. However, little is known about the other migrating passerine bird species with respect to their competence as Lyme disease reservoirs and transmission abilities.

Here we characterized tick infestation in migratory passerine bird species captured in the northeastern part of the Czech Republic during the postbreeding migration period. Further, we investigated the incidence of *B. garinii*, *B. valaisiana*, *B. afzelii*, and *B. burgdorferi* (*B. burgdorferi sensu stricto*) in bird-feeding ticks and in questing ticks from the same area in order to evaluate the importance of migrating passerine species as reservoirs and disseminators of Lyme disease spirochetes.

MATERIALS AND METHODS

Study site, birds, and tick collection and determination. The field investigation was conducted at Certak (49°34'N, 17°59'E) in the northeastern part of the Czech Republic. Details concerning the location have been described by Kulich et al. (14). Birds were collected with mist nets during the postbreeding period from 29 July 2005 to 24 September 2005. The birds were identified, examined, and released back into the wild as quickly as possible to minimize disturbance. Ticks were collected from birds using tweezers. On 16 April 2006, host-seeking unfed ticks (nymphs and adults) were collected by dragging a blanket in lower vegetation at the same location. The ticks were placed in 70% ethanol. In the laboratory, the ticks were removed from the ethanol, and the species, stage, and sex of adults were determined using a stereomicroscope. The ticks were classified as "unfed" if there was no marked increase in body volume, as "half-fed" if there was a marked increase, and "fully fed" if the volume of the body was the maximum volume.

DNA isolation and identification and genotyping of *Borrelia* species. Genomic DNA was extracted from ticks by alkaline hydrolysis (8). The mitochondrial cytochrome *b* gene was amplified as a control for DNA extraction for randomly selected *Borrelia*-negative samples (1), which revealed that the efficacy of the DNA isolation procedure was more than 95%. All samples were then subjected to nested PCR targeting a fragment of the *rrf* (5S)-*rrl* (23S) intergenic spacer

* Corresponding author. Mailing address: Department of Biology and Wildlife Diseases, University of Veterinary and Pharmaceutical Sciences, Palackeho 1/3, 612 42 Brno, Czech Republic. Phone: 420 543 136 730. Fax: 420 541 562 631. E-mail: dubska@mou.cz.

[∇] Published ahead of print on 5 December 2008.

(IGS) of *B. burgdorferi* sensu lato (16). Diluted *B. garinii* or *B. valaisiana* DNA was used as a positive control.

The PCR products were analyzed further by performing a reverse line blot assay using DNA probes specific for *B. afzelii*, *B. bissettii*, *B. burgdorferi* sensu lato, *B. burgdorferi* sensu stricto, *B. garinii*, and *B. valaisiana*, as described previously (10, 16). As positive controls, amplicons derived from cultured *B. afzelii*, *B. burgdorferi* sensu stricto, *B. garinii*, and *B. valaisiana* were blotted.

A subset of PCR-positive *B. burgdorferi* sensu lato samples that could not be assigned to species based on the reverse line blot assay results was subjected to nucleotide sequencing of the IGS and/or the flagellin B locus (*flaB*). A partial flagellin gene was amplified by using primers described by Fukunaga et al. (6) and the following PCR conditions: 35 cycles consisting of 94°C for 15 s, 52°C for 30 s, and 70°C for 90 s and then a final extension for 5 min at 70°C. The products of the IGS and *flaB* PCR were purified using a Wizard SV gel and PCR clean-up system (Promega). Both strands of the IGS PCR products were sequenced using the inner primers for the nested PCR. *flaB* PCR products were purified and sequenced in both directions using primers described by Fukunaga et al. (6).

Ability of the host to transmit *Borrelia* to ticks. As *Borrelia* spirochetes are rarely transmitted transovarially (21) or by cofeeding (27), their presence in feeding larvae suggests that they were acquired from the host. Thus, we assumed that a higher proportion of infected fed larvae than of infected unfed larvae supported the conclusion that the host was able to transmit *Borrelia* to ticks.

Data analysis and statistics. We used standard parametric statistics (Student's *t* test and analysis of variance) and determined means and 95% confidence intervals (95% CI). Proportions were evaluated by using odds ratio (OR) procedures with a 95% CI. To estimate the ability to transmit *Borrelia* spirochetes to ticks feeding on birds that might be infected, we calculated a noninfectivity coefficient (n-I) by determining the proportion of noninfected larvae among larvae feeding on a bird carrying ≥ 1 infected tick; an n-I of 1 suggested that a bird species was not able to transmit *Borrelia* spirochetes. Coefficients were calculated for each *Borrelia* species.

Nucleotide sequence accession numbers. Sequences of *Borrelia* DNA have been deposited in the GenBank database under accession numbers EU401776, EU401777, EU401778, EU401779, EU401780, and EU401781 (5S-23S rRNA IGS), as well as EU401782, EU401783, and EU401784 (*flaB* gene).

RESULTS

Tick infestation of birds. The total number of birds captured was 1,084. Of these, 1,080 (99.6%) were passerines (order Passeriformes), 2 (0.2%) were members of the order Piciformes, 1 (0.1%) was common kingfisher (*Alcedo atthis*) (Coraciiformes), and 1 was a corncrake (*Crex crex*) (Gruiformes). Ringing data showed that 857 of the trapped birds were trapped for the first time, while 227 of them had been trapped previously. Both birds that were trapped for the first time and birds that had been trapped previously were examined. The most common birds were blackcaps (*Sylvia atricapilla*) and European robins (*Erithacus rubecula*), which accounted for 29.8 and 18.2% of all examinations, respectively.

Overall, 2,240 ticks were removed from 446 (41.1%) of the 1,084 birds (Table 1). No overall significant decrease in the prevalence of infestation (38.0% in originally trapped birds versus 52.0% in birds that had been trapped previously) or in the infestation level (1.96 ticks/originally trapped bird versus 2.48 ticks/bird that had been trapped previously) was observed for birds that were disinfested and trapped again. Therefore, birds that had been trapped previously were not excluded from the analysis. A total of 273 (25%) of the 1,084 birds were identified as males (172 birds) or females (101 birds). No difference in infestation level between males (1.9 ticks/bird) and females (1.8 ticks/bird) was observed. A total of 1,023 (94%) of the 1,084 birds were identified as young (born in the year when they were examined) (785 birds) or adult (≥ 1 year old) (238 birds). The infestation level was 2.0 ticks/young bird and 2.5 ticks/adult bird, and there was no significant difference

in the level of infestation between birds that were different ages.

The highest prevalences of infestation were found for Eurasian blackbirds (*Turdus merula*) (90.3%; $n = 579$; mean infestation level, 20.7 ticks per infested bird), dunnocks (*Prunella modularis*) (90.9%; $n = 412$; mean infestation level, 8.24 ticks per infested bird), song thrushes (*Turdus philomelos*) (77.4%; $n = 264$; mean infestation level, 6.4 ticks per infested bird), and winter wrens (*Troglodytes troglodytes*) (75.8%; $n = 145$; mean infestation level, 5.8 ticks per infested bird). The mean number of ticks for ground-foraging birds (winter wrens, dunnocks, European robins, song thrushes, bullfinches, gray wagtails, blackbirds, hawfinches, yellowhammers, and marsh warblers) was 4.2 ticks per infested bird, and it was 0.8 tick per infested bird for other bird species.

All ticks found on birds were identified as *I. ricinus* subadults. A total 1,547 (70%) of these ticks were larvae and 676 (30%) were nymphs, and the levels of infestation were 3.5 larvae/infested bird and 1.5 nymphs/infested bird. There were no differences between originally trapped birds and birds that had been trapped previously in the proportions of larvae (68% of all ticks on originally trapped birds and 74% of all ticks on birds that had been trapped previously) and nymphs (32% of the ticks on originally trapped birds and 26% of the ticks on birds that had been trapped previously) or the number of larvae (3.4 larvae/originally trapped infested bird and 4.1 larvae/infested bird that had been trapped previously) or nymphs (1.5 nymphs/originally trapped infested bird and 1.4 nymphs/infested bird that had been trapped previously). However, we observed bird species-related differences in the proportions of larvae and nymphs; most significantly, there were lower proportions of larvae in song thrushes (57%; OR 0.5; 95% CI, 0.4 to 0.7) and dunnocks (60%; OR, 0.6; 95% CI, 0.5 to 0.7) and higher proportions of larvae in winter wrens (95%; OR, 8.9; 95% CI, 4.1 to 19.0). Based on their volumes, 41% of the ticks were identified as unfed, 49% of the ticks were identified as half-fed, and as 9% of the ticks were identified as fully fed. There were no overall differences in proportions among bird species except that dunnocks had significantly higher proportions of unfed ticks (98%; $P < 0.001$).

***Borrelia* infection in bird-feeding ticks.** *Borrelia* spirochetes were found in 613 (27.4%) of 2,240 *I. ricinus* subadults. For these 613 infected ticks, we detected 856 infection events resulting from infection or coinfection with various *Borrelia* genospecies, and the mean number of infection events was 1.40 events per infected tick. *Borrelia* spirochetes were more common in nymphs than in larvae (31.4% versus 22.4%; OR, 1.6; 95% CI, 1.3 to 1.9), and there was 0.45 infection event per infected nymph, compared with 0.31 infection event per infected larva. The greater infection rate in nymphs was observed for European robins (OR, 12.0; 95% CI, 1.3 to 127.3) and dunnocks (OR, 2.9; 95% CI, 1.3 to 6.5) but not for other bird species.

B. garinii infections were found in 22.2% (497) of the ticks, and the prevalence was higher in nymphs (27.2%) than in larvae (20.2%) (OR, 1.5; 95% CI, 1.2 to 1.8) (Table 2). *B. garinii* DNA was detected far more often in ticks infesting Eurasian blackbirds (55.0%) and song thrushes (50.2%). Intermediate prevalence was observed for great tits (*Parus major*) (15.5% of the ticks were infected) and winter wrens (6.2%

TABLE 1. Birds captured in the Czech Republic during the postbreeding migration period and data on their infestation with *I. ricinus* ticks

Bird species	No. of birds examined	No. of ticks collected	% (no.) of birds infested	Mean no. of ticks per infested bird	Mean no. of ticks per bird
Blackcap (<i>Sylvia atricapilla</i>)	323	275	35.3 (114)	2.41	0.85
European robin (<i>Erithacus rubecula</i>)	197	281	47.2 (93)	3.02	1.43
Great tit (<i>Parus major</i>)	69	174	58.0 (40)	4.35	2.52
Dunnock (<i>Prunella modularis</i>)	55	412	90.9 (50)	8.24	7.49
Song thrush (<i>Turdus philomelos</i>)	53	264	77.4 (41)	6.43	4.98
Chiffchaff (<i>Phylloscopus collybita</i>)	42	5	9.5 (4)	1.25	0.12
Eurasian blackbird (<i>Turdus merula</i>)	31	579	90.3 (28)	20.7	18.67
Blue tit (<i>Cyanistes caeruleus</i>)	29	4	10.3 (3)	0.75	0.14
Winter wren (<i>Troglodytes troglodytes</i>)	33	145	75.8 (25)	5.8	4.4
Pied flycatcher (<i>Ficedula hypoleuca</i>)	23	5	4.34 (1)	5	0.22
Long-tailed tit (<i>Aegithalos caedatus</i>)	23	0	0	NA ^a	0
Marsh tit (<i>Poecile palustris</i>)	21	3	14.3 (3)	1	0.14
Whitethroat (<i>Sylvia communis</i>)	18	29	61.1 (11)	2.64	1.61
Treecreeper (<i>Certhia familiaris</i>)	18	9	33.3 (6)	1.5	0.5
Garden warbler (<i>Sylvia borin</i>)	16	1	6.25 (1)	1	0.06
Red-backed shrike (<i>Lanius collurio</i>)	14	4	21.4 (3)	1.33	0.29
Willow tit (<i>Poecile montanus</i>)	13	9	38.5 (5)	1.8	0.69
Spotted flycatcher (<i>Muscicapa striata</i>)	13	1	7.69 (1)	1	0.08
Yellowhammer (<i>Emberiza citrinella</i>)	13	0	0	NA	0
Gray wagtail (<i>Motacilla cinerea</i>)	12	10	25.0 (3)	3.3	0.83
Nuthatch (<i>Sitta europaea</i>)	10	5	30 (3)	1.7	0.5
Bullfinch (<i>Pyrrhula pyrrhula</i>)	9	6	44.4 (4)	1.5	0.67
Grasshopper warbler (<i>Locustella naevia</i>)	8	10	25 (2)	5	1.25
Chaffinch (<i>Fringilla coelebs</i>)	8	4	12.5 (1)	4	0.5
Willow warbler (<i>Phylloscopus trochilus</i>)	5	0	0	NA	0
Collard flycatcher (<i>Ficedula albicollis</i>)	5	0	0	NA	0
Redstart warbler (<i>Phoenicurus phoenicurus</i>)	4	3	25 (1)	3	0.75
Wood warbler (<i>Phylloscopus sibilatrix</i>)	4	0	0	0	0
Goldcrest (<i>Regulus regulus</i>)	3	0	0	NA	0
Icterine warbler (<i>Hippolais icterina</i>)	2	0	0	NA	0
Hawfinch (<i>Coccothraustes coccothraustes</i>)	1	2	100 (1)	2	2
Corncrake (<i>Crex crex</i>)	1	0	0	NA	0
Common kingfisher (<i>Alcedo atthis</i>)	1	0	0	NA	0
Lesser whitethroat (<i>Sylvia curruca</i>)	1	0	0	NA	0
Spotted woodpecker (<i>Dendrocopos major</i>)	1	0	0	NA	0
Gray-headed woodpecker (<i>Picus canus</i>)	1	0	0	NA	0
Total	1,084	2,240	51.8 (562)	5.06	2.06

^a NA, not applicable.

of the ticks were infected). The lowest n-I for *B. garinii* were obtained for song thrushes (0.34), great tits (0.42), and blackbirds (0.45). Although we did not find *B. garinii* in cofeeding nymphs, we detected *B. garinii* in 25 larvae from eight Eurasian

blackbirds, in 9 larvae from four song thrushes, in 2 larvae from two European robins, in 1 larva from a blackcap, in 6 larvae from four dunnocks, in 3 larvae from 3 winter wrens, in 5 larvae from 2 great tits, and in 1 larva from a common treecreeper

TABLE 2. Birds with *B. garinii* spirochete-infected subadult ticks

Bird species	No. of larvae	No. of nymphs	No. (%) of infected larvae	No. (%) of infected nymphs	OR ^a	95% CI	No. (%) of infected ticks	n-I	No. (%) of birds with infected ticks
Blackcap (<i>Sylvia atricapilla</i>)	215	60	1 (0.5)	1 (1.7)	3.6	0.2–58.9	2 (0.7)	0.75	2 (0.6)
European robin (<i>Erithacus rubecula</i>)	226	55	2 (0.9)	2 (3.6)	4.2	0.6–30.7	4 (1.4)	0.67	4 (2.0)
Great tit (<i>Parus major</i>)	135	33	20 (14.8)	6 (18.2)	1.3	0.5–5	26 (15.5)	0.42	7 (10.0)
Dunnock (<i>Prunella modularis</i>)	252	167	5 (2.0)	4 (2.4)	1.2	0.3–4.6	9 (2.1)	0.92	7 (12.7)
Song thrush (<i>Turdus philomelos</i>)	147	112	70 (47.6)	60 (53.6)	1.3	0.8–2.1	130 (50.2)	0.34	25 (47.2)
Eurasian blackbird (<i>Turdus merula</i>)	384	189	206 (53.6)	109 (57.6)	1.2	0.8–1.7	315 (55.0)	0.45	24 (77.4)
Winter wren (<i>Troglodytes troglodytes</i>)	138	7	8 (5.8)	1 (14.3)	2.7	0.3–25.3	9 (6.2)	0.83	5 (15.2)
Whitethroat (<i>Sylvia communis</i>)	18	11	0 (0)	0 (0)			0 (0)		0 (0)
Treecreeper (<i>Certhia familiaris</i>)	4	6	1 (25)	0 (0)			1 (10)		1 (5.6)
Grasshopper warbler (<i>Locustella naevia</i>)	5	5	0 (0)	0 (0)			0 (0)		0 (0)
Gray wagtail (<i>Motacilla cinerea</i>)	1	9	0 (0)	1 (11.1)			1 (10)		1 (8.3)
Total	1,547	676	312 (20.2)	185 (27.2)	1.5	1.2–1.8	497 (22.2)		76 (7.0)

^a OR for infection in nymphs compared to infection in larvae.

TABLE 3. Birds with *B. valaisiana* spirochete-infected subadult ticks

Bird species	No. of larvae	No. of nymphs	No. (%) of infected larvae	No. (%) of infected nymphs	OR ^a	95% CI	No. (%) of infected ticks	n-I	No. (%) of birds with infected ticks
Blackcap (<i>Sylvia atricapilla</i>)	215	60	2 (0.9)	1 (1.7)	1.8	0.2–20.3	3 (1.1)	0.83	3 (0.9)
European robin (<i>Erithacus rubecula</i>)	226	55	0 (0)	1 (1.8)			1 (0.4)	1.0	1 (0.5)
Great tit (<i>Parus major</i>)	135	33	5 (3.7)	0 (0)			5 (3.0)	0.62	1 (1.4)
Dunnock (<i>Prunella modularis</i>)	252	167	5 (2.0)	3 (1.8)	0.9	0.2–3.8	8 (1.9)	0.94	6 (10.9)
Song thrush (<i>Turdus philomelos</i>)	147	112	33 (22.4)	17 (15.2)	0.6	0.3–1.2	50 (19.3)	0.60	17 (32.1)
Eurasian blackbird (<i>Turdus merula</i>)	384	189	139 (36.2)	73 (38.6)	1.1	0.8–1.6	212 (37.0)	0.64	24 (77.4)
Winter wren (<i>Troglodytes troglodytes</i>)	138	7	4 (2.9)	0 (0)			4 (2.8)	0.85	4 (12.1)
Whitethroat (<i>Sylvia communis</i>)	18	11	0 (0)	1 (9.1)			1 (3.3)		1 (5.6)
Treecreeper (<i>Certhia familiaris</i>)	4	6	0 (0)	0 (0)			0 (0)		0 (0)
Grasshopper warbler (<i>Locustella naevia</i>)	5	5	0 (0)	0 (0)			0 (0)		0 (0)
Gray wagtail (<i>Motacilla cinerea</i>)	1	9	0 (0)	1 (11.1)			1 (10)		1 (8.3)
Total	1,547	676	188 (12.2)	97 (14.3)	1.1	0.9–1.5	285 (12.8)		58 (5.4)

^a OR for infection in nymphs compared to infection in larvae.

(*Certhia familiaris*). The prevalence of *B. garinii* was significantly higher in larvae feeding on young birds than in larvae feeding on birds more than 1 year old for Eurasian blackbirds (72% versus 6%; OR, 42.2; 95% CI, 17.7 to 100.5) and for song thrushes (63% versus 20%; OR, 6.0; 95% CI, 2.5 to 14.3).

B. valaisiana was found in 12.7% (285) of the ticks, and there was no statistically significant difference between the prevalence in nymphs (14.3%) and the prevalence in larvae (12.2%) (OR, 1.1; 95% CI, 0.9 to 1.5) (Table 3). For song thrushes and dunnocks, we observed a lower level of infection in nymphs than in larvae. *B. valaisiana* was detected mostly in ticks infesting Eurasian blackbirds (37.0%), and the prevalence in ticks infesting song thrushes was intermediate (18.6%). In ticks feeding on other bird species, including great tits and winter wrens, the level of *B. valaisiana* infection was low. For all bird species, the n-I of *B. valaisiana* was greater than the n-I of *B. garinii*, and the lowest values were obtained for song thrushes (0.60), great tits (0.62), and blackbirds (0.64). Although we did not find *B. valaisiana* in cofeeding nymphs, we detected *B. valaisiana* in 23 larvae from six Eurasian blackbirds, in 11 larvae from seven song thrushes, in 2 larvae from two blackcaps, in 4 larvae from two dunnocks, in 3 larvae from three winter wrens, and in 5 larvae from a great tit. A higher prevalence of *B. valaisiana* in larvae feeding on young birds was observed for Eurasian blackbirds (71% versus 17%; OR, 12.2; 95% CI, 6.9 to 22.0) but not for song thrushes (20% versus 27%; OR, 0.7; 95% CI, 0.3 to 1.6).

B. afzelii infection was found in 1.6% (36) of the ticks, and the prevalence was substantially higher in nymphs (5.0%) than in larvae (0.1%) (OR, 40.0; 95% CI, 9.8 to 170.8) (Table 4). The levels of infection with *B. afzelii* were approximately equal in ticks infesting great tits (2.3%), dunnocks (2.4%), song thrushes (1.1%), and Eurasian blackbirds (1.9%). *B. afzelii* was not present in ticks feeding on blackcaps and winter wrens. *B. afzelii* was detected in 2.3% of the unfed nymphs and in only 1.0% of the nymphs whose body volume was markedly increased (OR, 2.35; *P* < 0.02). Estimated absolute noninfectivity was observed for all bird species except great tits (n-I, 0.92). Although we did not find *B. afzelii* in cofeeding nymphs, we detected *B. afzelii* in one larva from a great tit and in one larva from a gray wagtail (*Motacilla cinerea*).

B. burgdorferi was found in only 0.3% (6) of the ticks feeding on birds, and there was not a striking association with a particular bird species (Table 5). Although we did not find *B. burgdorferi* in cofeeding nymphs, we detected *B. burgdorferi* in two larvae from a winter wren, for which the n-I was 0.91.

TABLE 4. Birds with *B. afzelii* spirochete-infected subadult ticks

Bird species	No. of larvae	No. of nymphs	No. (%) of infected larvae	No. (%) of infected nymphs	OR ^a	95% CI	No. (%) of infected ticks	n-I	No. (%) of birds with infected ticks
Blackcap (<i>Sylvia atricapilla</i>)	215	60	0 (0)	0 (0)			0 (0)		0 (0)
European robin (<i>Erithacus rubecula</i>)	226	55	0 (0)	1 (1.8)			1 (0.4)	1.0	1 (0.5)
Great tit (<i>Parus major</i>)	135	33	1 (0.7)	4 (12.1)	13.4	1.3–133.3	5 (3.0)	0.92	4 (5.8)
Dunnock (<i>Prunella modularis</i>)	252	167	0 (0)	10 (6.0)			10 (2.4)	1.0	6 (10.9)
Song thrush (<i>Turdus philomelos</i>)	147	112	0 (0)	3 (2.7)			3 (0.5)	1.0	2 (3.8)
Eurasian blackbird (<i>Turdus merula</i>)	384	189	0 (0)	11 (5.8)			11 (1.9)	1.0	8 (25.8)
Winter wren (<i>Troglodytes troglodytes</i>)	138	7	0 (0)	0 (0)			0 (0)		0 (0)
Whitethroat (<i>Sylvia communis</i>)	18	11	0 (0)	3 (27.3)			3 (10.3)		3 (16.7)
Treecreeper (<i>Certhia familiaris</i>)	4	6	0 (0)	0 (0)			0 (0)		0 (0)
Grasshopper warbler (<i>Locustella naevia</i>)	5	5	0 (0)	1 (20)			1 (10)		1 (12.5)
Gray wagtail (<i>Motacilla cinerea</i>)	1	9	1 (100)	1 (11.1)			2 (20)		2 (16.7)
Total	1,547	676	2 (0.1)	34 (5.0)	40.0	9.8–170.8	36 (1.6)		27 (2.5)

^a OR for infection in nymphs compared to infection in larvae.

TABLE 5. Birds with *B. burgdorferi* sensu stricto spirochete-infected subadult ticks

Bird species	No. of larvae	No. of nymphs	No. (%) of infected larvae	No. (%) of infected nymphs	OR ^a	95% CI	No. (%) of infected ticks	n-I	No. (%) of birds with infected ticks
Blackcap (<i>Sylvia atricapilla</i>)	215	60	0 (0)	0 (0)			0 (0)		0 (0)
European robin (<i>Erithacus rubecula</i>)	226	55	0 (0)	1 (1.8)			1 (0.4)	1.0	1 (0.5)
Great tit (<i>Parus major</i>)	135	33	0 (0)	0 (0)			0 (0)		0 (0)
Dunnock (<i>Prunella modularis</i>)	252	167	0 (0)	1 (0.6)			1 (0.2)	1.0	1 (1.8)
Song thrush (<i>Turdus philomelos</i>)	147	112	0 (0)	0 (0)			0 (0)		0 (0)
Eurasian blackbird (<i>Turdus merula</i>)	384	189	0 (0)	2 (1.1)			2 (0.3)		2 (6.5)
Winter wren (<i>Troglodytes troglodytes</i>)	138	7	2 (1.4)	0 (0)			2 (1.4)	0.91	1 (3.0)
Whitethroat (<i>Sylvia communis</i>)	18	11	0 (0)	0 (0)			0 (0)		0 (0)
Treecreeper (<i>Certhia familiaris</i>)	4	6	0 (0)	0 (0)			0 (0)		0 (0)
Grasshopper warbler (<i>Locustella naevia</i>)	5	5	0 (0)	0 (0)			0 (0)		0 (0)
Gray wagtail (<i>Motacilla cinerea</i>)	1	9	0 (0)	0 (0)			0 (0)		0 (0)
Total	1,547	676	2 (0.1)	4 (0.6)	4.6	0.8–25.2	6 (0.3)		5 (0.5)

^a OR for infection in nymphs compared to infection in larvae.

The reverse line blotting technique allowed identification of spirochete species in all cases except organisms that infected 10 ticks that were identified by sequencing of the flagellin or 16S rRNA gene as *B. garinii* for nine bird-infesting ticks and one case of *B. afzelii* in an adult questing tick. Two variants of *B. garinii* (accession numbers EU401782 and EU401783) isolated from two ticks infesting a song thrush were identical to isolates obtained by Taragel'ova et al. (35) and to a spirochete found in *I. ricinus* in Moscow province (23). Using BLAST searches, we found a *B. afzelii* partial *flaB* sequence (accession number EU401784) from a questing male tick that differed from the sequences of the most similar isolates by two nucleotides.

Greater infection in fed (half-fed and fully fed) larvae than in unfed larvae was observed for Eurasian blackbirds (OR, 4.6; 95% CI, 2.9 to 7.3) and for song thrushes (OR, 2.5; 95% CI, 1.2 to 5.3), as well as (without statistical significance) for great tits (OR, 2.4; 95% CI, 0.5 to 11.5) and for winter wrens (OR, 1.5; 95% CI, 0.5 to 4.0). Specifically, for Eurasian blackbirds and song thrushes, the correlation between the volume of larvae and *Borrelia* infection was stronger for *B. valaisiana*, for which the OR was 5.7 and the 95% CI was 3.1 to 10.3 for blackbirds and the OR was 9.0 and the 95% CI was 2.1 to 39.7 for song thrushes, than for *B. garinii*, for which the OR was 3.1 and the 95% CI was 2.0 to 4.9 for blackbirds and the OR was 2.1 and the 95% CI was 1.0 to 4.4 for song thrushes.

We also frequently observed mixed infections with *Borrelia* species. If infection with *Borrelia* spirochetes in ticks were independent, we would expect that 2.8% of the ticks would be infected with both *B. garinii* and *B. valaisiana*; in fact, the value was substantially higher, 10.4%. Infections with three species, mostly infections with *B. garinii*, *B. valaisiana*, and *B. afzelii*, were also detected for nine ticks.

***Borrelia* spirochetes in host-seeking unfed ticks.** A total of 109 questing ticks (24 unfed nymphs, 39 adult males, and 46 adult females) were collected from vegetation by dragging a blanket. *B. afzelii* was detected in seven ticks (6.4%), including three nymphs, three adult males, and one adult female. Moreover, we detected a tick containing both *B. garinii* and *B. valaisiana* (one female tick [0.9%]).

DISCUSSION

In this paper, we report data obtained in a large-scale study of *Borrelia* spirochete distribution in passerine bird-feeding ticks collected from birds during the postbreeding migration period, and we assess the roles of certain bird species as reservoirs and distributors of *Borrelia* species. We found significantly higher infestation rates in ticks on ground-feeding birds connected with high levels of infection with *Borrelia* spirochetes in Eurasian blackbirds and song thrushes, but not dunnocks or European robins. Interestingly, dunnocks were heavily infested with ticks, but their infection rates were very low, which may have been associated with the fact that 98% of the ticks on dunnocks were starving. On the other hand, over 60% of the ticks feeding on European robins had a blood meal. Thus, ground-foraging behavior in birds results in a significantly greater risk of tick infestation. It is not, however, the crucial biological determinant for competence for transmission of *Borrelia* spirochetes to *I. ricinus*.

Overall, the *Borrelia* infection data for ticks obtained from passerine birds in our study were equivalent to findings for birds captured during 2001 and 2002 in Slovakian woodlands (35), but the values were substantially higher than the values for ticks obtained from birds examined in the spring and autumn of 2001 in southeastern Sweden (3). This suggests that migratory passerine birds from Central Europe are prominent reservoir hosts of *Borrelia* species. Almost 90% of *B. garinii* infections were detected in song thrushes and Eurasian blackbirds. The significantly higher proportion of *B. garinii* in larvae feeding on young song thrushes and Eurasian blackbirds than in larvae feeding on adult birds suggests that juvenile birds are prone to spirochetemia. It has been shown that migratory restlessness in young redwing thrushes (*Turdus iliacus*) can reactivate a latent *B. garinii* infection (9). This may be due to an increase in endogenous glucocorticoids during migratory restlessness (19) that adversely affects the immune system and leads to a decrease in the bactericidal response (24). Juvenile birds might have substantial migration-associated hormonal changes or less developed immune responses (20, 29) that increase their susceptibility to infectious diseases.

Based on noninfectivity ability and the cluster of *B. garinii* infections in great tits, we concluded that these passerine birds can also transmit *B. garinii* to feeding ticks under certain conditions. Interestingly, all five great tits yielding three to nine *B. garinii*-infected larvae had previously been captured. And for one great tit examined for the first time there was 1 nymph among the 16 ticks infected with *B. garinii*; then after capture on days 9, 10, and 12, 16 of 17 new larvae were infected. This suggests that trap-induced stress may have been a triggering mechanism for spirochetemia (9, 24). The n-I less than 1 for other passerine birds and the presence of infected larvae but not *B. garinii*-infected nymphs suggest that blackcaps, European robins, dunnocks, and treecreepers may play minor roles in transmitting *B. garinii*. Compared to the noninfectivity of *B. garinii*, however, the noninfectivity of *B. valaisiana* was higher, and Eurasian blackbirds and song thrushes were key reservoirs of this spirochete species. Moreover, our data suggest that juvenile Eurasian blackbirds, but not juvenile song thrushes, comprise a subgroup that is very efficient for *B. valaisiana* transmission. The role of other passerine birds as reservoirs of *B. valaisiana* is minor, and transmission competence is likely restricted to a few individuals.

The prevalence of *B. afzelii* in bird-feeding ticks was low, and there was no robust evidence that birds had transmission competence for this spirochete species. Together with the fact that the vast majority of infections were detected in nymphs, we suggest that these ticks were infected with *B. afzelii* as larvae feeding on nonbird hosts (likely rodents). Moreover, the significantly higher prevalence of *B. afzelii* in unfed nymphs supports the conclusion of Kurtenbach et al. (18) that avian blood induces selective complement killing of *B. afzelii* in ticks. Nevertheless, our finding that 13 bird-feeding nymphs were infected with *B. afzelii* suggests that the immune elimination of this *Borrelia* species by a bird host is not absolute. However, we observed two larvae positive for *B. afzelii*, one from a great tit and one from a gray wagtail, which may be attributed to a low transmission rate of *B. afzelii* in birds, to transovarial transmission, or to cofeeding transmission (26).

B. burgdorferi sensu stricto was rare in bird-feeding ticks. However, since we found two larvae containing *B. burgdorferi* on a treecreeper, we cannot exclude the possibility that birds have the potential to transmit this *Borrelia* genospecies.

Based on data for bird-feeding subadult ticks and questing adult ticks and nymphs, the estimated transstadial transmission is more significant for *B. afzelii* than for other *Borrelia* genospecies. Compared to the strikingly higher prevalence of *B. afzelii* in nymphs as a result of accumulated infection in the former stage during consecutive feeding events, we found a less markedly higher proportion of *B. garinii* in nymphs, and there was no difference between nymph infection with *B. valaisiana* and larva infection with *B. valaisiana*. This suggests that *B. garinii* and *B. valaisiana* spirochetes are transmitted in ticks transstadially with lower efficiency and possibly that their levels decrease during blood meal digestion, which is supported by the fact that there was a strong correlation between the tick volume and the presence of *B. valaisiana*.

Thus, we concluded that migratory passerine birds, specifically Eurasian blackbirds, song thrushes, and great tits, are competent for transmitting the Lyme disease spirochete *B. garinii*, and that migratory restlessness may influence the in-

fectivity (9, 35). The Eurasian blackbird, the song thrush, and the great tit commonly inhabit Central Europe, from lowlands up to the upper tree line at 1,400 m above sea level (34). The Eurasian blackbird population density is highest in urban areas, especially in parks. The total number of pairs in the Czech Republic is estimated to 2 to 4 million, and this population shows long-term stability. From the data collected in this study, we estimated that the Czech population of blackbirds may contain as many as 50×10^6 *B. garinii*-infected *I. ricinus* subadult ticks. The total population of the song thrush in the Czech Republic is estimated to be 400,000 to 800,000, also shows long-term stability, and carries approximately 2×10^6 *B. garinii*-infected *I. ricinus* subadult ticks. The total population of the great tit in the Czech Republic is estimated to be 3 to 6 million and carries approximately 1×10^6 to 2×10^6 *B. garinii*-infected *I. ricinus* subadult ticks. Synanthropization processes are obvious in the cases of the Eurasian blackbird, song thrush, and great tit, and thus their roles in the natural cycle of *B. garinii* may substantially impact the epidemiology of Lyme disease. However, the transstadial transmission of *B. garinii* spirochetes is a crucial step that influences the risk of infection for humans, and the efficiency of this process in *I. ricinus* ticks remains to be fully elucidated. The passerine bird migration habits need to be considered in the ecology of *Borrelia* spirochetes. Eurasian blackbirds from the Czech Republic winter mainly in France and northern Italy. Although only one-third to two-thirds of Czech Eurasian blackbirds migrate seasonally, they potentially carry millions of Lyme disease spirochete-infected ticks. In addition, the entire Czech population of song thrushes winters in France, northern Italy, and the Mediterranean, including Spain and North Africa. The vast majority of great tits reside in the Czech Republic, and a minor part of the population migrates southwest to Germany, Austria, northern Italy, and eastern France (11). Thus, we concluded that passerine birds play different roles in transmitting Lyme neuroborreliosis agents. Eurasian blackbirds, song thrushes, and great tits are transmission-competent hosts of *B. garinii* and prominent reservoirs of this spirochete due to their high densities in urban habitats and high rates of infestation by *I. ricinus*. During migration periods, moreover, they may transfer millions of *Borrelia*-infected ticks and thus establish new endemic foci of Lyme disease in southwest Europe and North Africa.

ACKNOWLEDGMENTS

This study was funded by the Ministry of Education, Youth and Sports of the Czech Republic (grant MSM6215712402) and by the Czech Science Foundation (grant 524-08-P139). V.T. received funds from the Slovak Research and Development Agency (grant APVV-51-009205), and E.K. was funded by the Scientific Grant Agency of the Ministry of Education and the Slovak Academy of Sciences (grant 2/7020).

We thank Eva Suchanova for her excellent technical cooperation.

REFERENCES

- Black, W. C., IV, and R. L. Roehrdanz. 1998. Mitochondrial gene order is not conserved in arthropods: prostriate and metastriate tick mitochondrial genomes. *Mol. Biol. Evol.* 15:1772-1785.
- Busch, U., C. Hizo-Teufel, R. Bohmer, V. Fingerle, D. Rossler, B. Wilske, and V. Preac-Mursic. 1996. *Borrelia burgdorferi* sensu lato strains isolated from cutaneous Lyme borreliosis biopsies differentiated by pulsed-field gel electrophoresis. *Scand. J. Infect. Dis.* 28:583-589.
- Comstedt, P., S. Bergstrom, B. Olsen, U. Garpmo, L. Marjavaara, H. Meljon, A. G. Barbour, and J. Bunikis. 2006. Migratory passerine birds as reservoirs of Lyme borreliosis in Europe. *Emerg. Infect. Dis.* 12:1087-1095.

4. Demaerschalck, I., A. Ben Messaoud, M. De Kesel, B. Hoyois, Y. Lobet, P. Hoet, G. Bigaignon, A. Bollen, and E. Godfroid. 1995. Simultaneous presence of different *Borrelia burgdorferi* genospecies in biological fluids of Lyme disease patients. *J. Clin. Microbiol.* **33**:602–608.
5. Floris, R., G. Menardi, R. Bressan, G. Trevisan, S. Ortenzio, E. Rorai, and M. Cinco. 2007. Evaluation of a genotyping method based on the ospA gene to detect *Borrelia burgdorferi* sensu lato in multiple samples of Lyme borreliosis patients. *New Microbiol.* **30**:399–410.
6. Fukunaga, M., K. Okada, M. Nakao, T. Konishi, and Y. Sato. 1996. Phylogenetic analysis of *Borrelia* species based on flagellin gene sequences and its application for molecular typing of Lyme disease borreliae. *Int. J. Syst. Bacteriol.* **46**:898–905.
7. Gern, L., and P. F. Humair. 1998. Natural history of *Borrelia burgdorferi* sensu lato. *Wien. Klin. Wochenschr.* **110**:856–858.
8. Guy, E. C., and G. Stanek. 1991. Detection of *Borrelia burgdorferi* in patients with Lyme disease by the polymerase chain reaction. *J. Clin. Pathol.* **44**:610–611.
9. Gylfe, A., S. Bergstrom, J. Lundstrom, and B. Olsen. 2000. Reactivation of *Borrelia* infection in birds. *Nature* **403**:724–725.
10. Hanincova, K., V. Taragelova, J. Koci, S. M. Schafer, R. Hails, A. J. Ullmann, J. Piesman, M. Labuda, and K. Kurtenbach. 2003. Association of *Borrelia garinii* and *B. valaisiana* with songbirds in Slovakia. *Appl. Environ. Microbiol.* **69**:2825–2830.
11. Hudec, K. 2003. Fauna ČSSR, Ptáci. Aves III/2. Academia, Prague, Czech Republic.
12. Jaenson, T. G. 1991. The epidemiology of Lyme borreliosis. *Parasitol. Today* **7**:39–45.
13. Kipp, S., A. Goedecke, W. Dorn, B. Wilske, and V. Fingerle. 2006. Role of birds in Thuringia, Germany, in the natural cycle of *Borrelia burgdorferi* sensu lato, the Lyme disease spirochaete. *Int. J. Med. Microbiol.* **296**(Suppl. 40): 125–128.
14. Kulich, P., E. Roubalova, L. Dubska, O. Sychra, B. Smid, and I. Literak. 2008. Avipoxvirus in blackcaps (*Sylvia atricapilla*). *Avian Pathol.* **37**:101–107.
15. Kurtenbach, K., D. Carey, A. N. Hoodless, P. A. Nuttall, and S. E. Randolph. 1998. Competence of pheasants as reservoirs for Lyme disease spirochetes. *J. Med. Entomol.* **35**:77–81.
16. Kurtenbach, K., M. Peacey, S. G. Rijpkema, A. N. Hoodless, P. A. Nuttall, and S. E. Randolph. 1998. Differential transmission of the genospecies of *Borrelia burgdorferi* sensu lato by game birds and small rodents in England. *Appl. Environ. Microbiol.* **64**:1169–1174.
17. Kurtenbach, K., H. S. Sewell, N. H. Ogden, S. E. Randolph, and P. A. Nuttall. 1998. Serum complement sensitivity as a key factor in Lyme disease ecology. *Infect. Immun.* **66**:1248–1251.
18. Kurtenbach, K., S. M. Schafer, H. S. Sewell, M. Peacey, A. Hoodless, P. A. Nuttall, and S. E. Randolph. 2002. Differential survival of Lyme borreliosis spirochetes in ticks that feed on birds. *Infect. Immun.* **70**:5893–5895.
19. Landys, M. M., J. C. Wingfield, and M. Ramenofsky. 2004. Plasma corticosterone increases during migratory restlessness in the captive white-crowned sparrow *Zonotrichia leucophrys gambelli*. *Horm. Behav.* **46**:574–581.
20. Lee, K. A., M. Wikelski, W. D. Robinson, T. R. Robinson, and K. C. Klasing. 2008. Constitutive immune defences correlate with life-history variables in tropical birds. *J. Anim. Ecol.* **77**:356–363.
21. Magnarelli, L. A., J. F. Anderson, and D. Fish. 1987. Transovarial transmission of *Borrelia burgdorferi* in *Ixodes dammini* (Acari:Ixodidae). *J. Infect. Dis.* **156**:234–236.
22. Majlathova, V., I. Majlath, M. Derdakova, B. Vichova, and B. Pet'ko. 2006. *Borrelia lusitaniae* and green lizards (*Lacerta viridis*), Karst region, Slovakia. *Emerg. Infect. Dis.* **12**:1895–1901.
23. Masuzawa, T., I. G. Kharitonov, T. Kadosaka, N. Hashimoto, M. Kudeken, N. Takada, K. Kaneda, and Y. Imai. 2005. Characterization of *Borrelia burgdorferi* sensu lato isolated in Moscow province—a sympatric region for *Ixodes ricinus* and *Ixodes persulcatus*. *Int. J. Med. Microbiol.* **294**:455–464.
24. Matson, K. D., B. I. Tieleman, and K. C. Klasing. 2006. Capture stress and the bactericidal competence of blood and plasma in five species of tropical birds. *Physiol. Biochem. Zool.* **79**:556–564.
25. Morshed, M. G., J. D. Scott, K. Fernando, L. Beati, D. F. Mazerolle, G. Geddes, and L. A. Durden. 2005. Migratory songbirds disperse ticks across Canada, and first isolation of the Lyme disease spirochete, *Borrelia burgdorferi*, from the avian tick, *Ixodes auritulus*. *J. Parasitol.* **91**:780–790.
26. Ogden, N. H., P. A. Nuttall, and S. E. Randolph. 1997. Natural Lyme disease cycles maintained via sheep by co-feeding ticks. *Parasitology* **115**:591–599.
27. Piesman, J., and C. M. Happ. 2001. The efficacy of co-feeding as a means of maintaining *Borrelia burgdorferi*: a North American model system. *J. Vector Ecol.* **26**:216–220.
28. Rand, P. W., E. H. Lacombe, R. P. Smith, Jr., and J. Ficker. 1998. Participation of birds (Aves) in the emergence of Lyme disease in southern Maine. *J. Med. Entomol.* **35**:270–276.
29. Rappole, J. H., S. R. Derrickson, and Z. Hubalek. 2000. Migratory birds and spread of West Nile virus in the Western Hemisphere. *Emerg. Infect. Dis.* **6**:319–328.
30. Rauter, C., and T. Hartung. 2005. Prevalence of *Borrelia burgdorferi* sensu lato genospecies in *Ixodes ricinus* ticks in Europe: a metaanalysis. *Appl. Environ. Microbiol.* **71**:7203–7216.
31. Reed, K. D., J. K. Meece, J. S. Henkel, and S. K. Shukla. 2003. Birds, migration and emerging zoonoses: West Nile virus, Lyme disease, influenza A and enteropathogens. *Clin. Med. Res.* **1**:5–12.
32. Richter, D., and F. R. Matuschka. 2006. Perpetuation of the Lyme disease spirochete *Borrelia lusitaniae* by lizards. *Appl. Environ. Microbiol.* **72**:4627–4632.
33. Richter, D., D. Postic, N. Sertour, I. Livey, F. R. Matuschka, and G. Baranton. 2006. Delineation of *Borrelia burgdorferi* sensu lato species by multilocus sequence analysis and confirmation of the delineation of *Borrelia spielmanii* sp. nov. *Int. J. Syst. Evol. Microbiol.* **56**:873–881.
34. Stastny, K., V. Bejcek, and K. Hudec. 2006. Atlas hnízdniho rozšíření ptaku v České republice 2001–2003. Aventinum, Prague, Czech Republic. (In Czech with English summary.)
35. Taragel'ova, V., J. Koci, K. Hanincova, K. Kurtenbach, M. Derdakova, N. H. Ogden, I. Literak, E. Kocianova, and M. Labuda. 2008. Blackbirds and song thrushes constitute a key reservoir of *Borrelia garinii*, the causative agent of borreliosis in Central Europe. *Appl. Environ. Microbiol.* **74**:1289–1293.
36. van der Heijden, I. M., B. Wilbrink, S. G. Rijpkema, L. M. Schouls, P. H. Heymans, J. D. van Embden, F. C. Breedveld, and P. P. Tak. 1999. Detection of *Borrelia burgdorferi* sensu stricto by reverse line blot in the joints of Dutch patients with Lyme arthritis. *Arthritis Rheum.* **42**:1473–1480.
37. Wright, S. A., D. A. Lemenager, J. R. Tucker, M. V. Armijos, and S. A. Yamamoto. 2006. An avian contribution to the presence of *Ixodes pacificus* (Acari: Ixodidae) and *Borrelia burgdorferi* on the Sutter Buttes of California. *J. Med. Entomol.* **43**:368–374.