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# Prevalence of *Borrelia burgdorferi* Sensu Lato in Ticks Collected from Migratory Birds in Switzerland

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**The prevalence of ticks infected by *Borrelia burgdorferi* sensu lato on birds during their migrations was studied in Switzerland. A total of 1,270 birds captured at two sites were examined for tick infestation. *Ixodes ricinus* was the dominant tick species. Prevalences of tick infestation were 6% and 18.2% for birds migrating northward and southward, respectively. *Borrelia valaisiana* was the species detected most frequently in ticks, followed by *Borrelia garinii* and *Borrelia lusitaniae*. Among birds infested by infected ticks, 23% (6/26) were infested by *B. lusitaniae*-infected larvae. Migratory birds appear to be reservoir hosts for *B. lusitaniae*.**

It is now clearly established that birds play a role as reservoir hosts in the ecology of Lyme borreliosis (16). In Europe, seven *Borrelia* species belonging to the complex *B. burgdorferi* sensu lato (*Borrelia burgdorferi* sensu stricto, *Borrelia garinii*, *Borrelia afzelii*, *Borrelia valaisiana*, *Borrelia lusitaniae*, *Borrelia bissetti*, and *Borrelia spielmani*) have been detected in the tick vector, *Ixodes ricinus*. The association of at least two of them, *B. garinii* and *B. valaisiana*, with birds is now well documented in Europe (12, 17, 21, 22). Birds play an important role not only in maintaining *B. burgdorferi* sensu lato in areas of endemicity; in fact, some of them, through their migration, also play a role by spreading ticks within and between continents (13, 19, 23–25, 30, 34, 35). In Europe, the role of migratory birds in the dispersal of *B. burgdorferi* sensu lato has been investigated in Scandinavia (26, 27) and on the Baltic Sea (3).

In Switzerland, studies on the infection of birds by *B. burgdorferi* sensu lato are scarce (17, 18). The reservoir competence of the European blackbird (*Turdus merula*) for *B. garinii* and *B. valaisiana* was demonstrated by Humair et al. (17). Recently, Papadopoulos et al. (28) reported the presence of *I. ricinus* on 51 bird species captured in this country. These authors listed 11 tick species infesting birds, with 2 (*Rhipicephalus simus* and *Hyalomma marginatum marginatum*) having been imported by migratory birds (28). Since nothing is known about the role of migratory birds in Switzerland, we investigated the tick infestation of birds during their pre- and postnuptial migrations and the prevalence of *B. burgdorferi* sensu lato in ticks on migratory birds.

The first survey took place at Bolle di Magadino in April and May 2002. This site is a marsh area at 194 m above sea level south of the Alps (Canton Ticino), where local birds and birds migrating further north were caught. The second survey was carried out at the Col de Bretolet (Canton Valais, at the Swiss-French border) from 6 September to 4 November 2002. At this site (1,923 m above sea level), birds were caught during their southwestward migration while crossing this Alpine pass.

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Birds were caught in mist nets during routine bird-catching operations of the Swiss Ornithological Institute (Sempach, Switzerland). All birds belonging to the Turdidae were examined for ticks as well as other species, such as *Anthus trivalis*, *Anthus pratensis*, *Anthus spinoletta*, *Prunella modularis*, *Sylvia borin*, *Sylvia atricapilla*, *Sylvia communis*, and *Sturnus vulgaris*. Ticks were removed from birds with forceps upon visual inspection and placed into plastic vials (one vial/bird). The species and stage of each tick were identified.

Live fully engorged ticks were screened for *B. burgdorferi* sensu lato after their molt by cultivation in BSKII medium (10, 33) either as individuals or in pools of ticks. Pools contained ticks collected from a bird at one time. Culture medium was examined by dark-field microscopy for 45 days. All tubes were investigated for *Borrelia* DNA detection by PCR-restriction fragment length polymorphism according to the method of Postic et al. (29).

Partially engorged ticks stored in 70% ethanol were examined for *Borrelia* DNA by PCR-reverse line blot (RLB) according to the method of Schouls et al. (32). *Borrelia* DNA was extracted using 0.7 M ammonium hydroxide. Primers used to amplify the variable spacer region between two repeated genes encoding ribosomal 23S and 5S were 5S-Bor and 23S-Bor (4). DNA amplification was performed using a Whatman (Göttingen, Germany) Biometra TGradient Thermocycler 96. Isolates of *B. burgdorferi* sensu stricto (B31), *B. garinii* (NE11), *B. lusitaniae* (PotiB3), *B. afzelii* (NE632), and *B. valaisiana* (VS116) were used as positive controls. Negative controls were included during extraction and PCR. For RLB, the PCR products were hybridized to seven oligonucleotide probes (75 pmol) blotted in lines on an activated Biodyne C membrane (Pall Europe Ltd., Portsmouth, United Kingdom) using a Miniblotter 45 (Immunetic, Cambridge, MA). *B. burgdorferi* sensu lato, *B. burgdorferi* sensu stricto, *B. afzelii*, *and B. garinii* probes were described by Schouls et al. (32) and Alekseev et al. (4). For this study, three new *B. burgdorferi* genospecies-specific oligonucleotide probes were designed: *B. valaisiana* (VSNE) (5′ amino-TATATCTTTTGTTCAATCCATGT), *B. garinii* (GANE) (5′ amino-CAAAAACATAAATATCTAAAAACATAA), and *B. lusitaniae* (LusiNE) (5′ amino-TCAAGATTTGAAGTATAA

TABLE 1. Prevalence and intensity of tick infestation of migratory birds captured at Bolle di Magadino

| Bird species (name) | No. of birds infested/ examined (%) | No. of ticks | Infestation intensity |
|---|---|---|---|
| *Anthus spinoletta* (water pipit) | 0/1 | | |
| *Prunella modularis* (dunnock) | 1/1 (100) | 1 | 1.00 |
| *Erithacus rubecula* (European robin) | 13/219 (5.9) | 29 | 2.23 |
| *Luscinia svecica* (bluethroat) | 0/1 | | |
| *Luscinia megarhynchos* (nightingale) | 1/10 (10.0) | 1 | 1.00 |
| *Phoenicurus phoenicurus* (black redstart) | 2/32 (6.3) | 2 | 1.00 |
| *Saxicola rubetra* (whinchat) | 0/13 | | |
| *Turdus merula* (blackbird) | 0/5 | | |
| *Sylvia communis* (common whitethroat) | 0/1 | | |
| Total | 17/283 (6.0) | 33 | 1.94 |

AATAAAA). Hybridization was visualized after exposing the membrane to X-ray film (Hyperfilm ECL; Amersham Biosciences, Otelfingen, Switzerland).

At Bolle di Magadino, in spring, 283 birds were examined for ticks (Table 1). Seventeen birds of four species were infested by ticks, giving a prevalence of 6%. We observed a mean density (total number of ticks observed divided by total number of examined hosts [20]) of 0.12 tick per bird (33 ticks/283 birds) and an intensity of infestation (total number of ticks observed divided by the number of infested hosts [20]) of 1.94 ticks per bird (33 ticks/17 birds). Intensity of infestation varied from 2.23 to 1 according to bird species (Table 1). All 18 larvae (not identified) were found on *Erithacus rubecula*. Among 15 infesting nymphs, 3 were *H. marginatum* (2 on *Phoenicurus phoenicurus* and 1 on *E. rubecula*), 10 were *Ixodes frontalis* (9 on *E. rubecula* and 1 on *Luscinia megarhynchos*), and 2 were *Ixodes ricinus* (1 on *E. rubecula* and 1 on *P. modularis*). None of the 33 ticks was infected by *Borrelia*.

In autumn, at the Col de Bretolet, 987 birds (21 species) were examined for ticks. One hundred eighty birds of 11 species were infested by 417 ticks (369 larvae and 48 nymphs), giving a prevalence of infestation of 18.2% (Table 2), a mean density of 0.42 tick per bird (417 ticks/987 birds), and an intensity of infestation of 2.32 ticks per infested bird (417 ticks/180 birds). Prevalences and intensity of infestation varied according to bird species (Table 2). The highest intensity of infestation was 7.0 ticks. The maximum number of ticks collected was 30 larvae on one European robin captured in October. The most abundant tick species was *I. ricinus* ($n = 411$). *I. frontalis* ($n = 3$) was collected from one European robin. Three additional ticks could not be identified.

*I. ricinus* larvae and nymphs collected from 95 birds (76 *E. rubecula*, 2 *P. phoenicurus*, 1 *Oenanthe oenanthe*, 12 *T. philomelos*, 3 *T. merula*, and 1 *P. ochruros* bird) were examined for *Borrelia* infection. Among these birds, 26/95 (27.4%) harbored infected ticks: 14/76 (18.4%) of *E. rubecula*, 7/12 (58.3%) of *T. philomelos*, 2/3 (66.7%) of *T. merula*, 2/2 (100%) of *P. phoenicurus*, and 1/1 (100%) of *P. ochruros*. Most birds (15/26, 57.7%) were infested by larvae only (11 of *E. rubecula*, 2 of *T. merula*, 1 of *P. phoenicurus*, and 1 of *P. ochruros*), 7 (26.9%) were infested by larvae and nymphs (3 of *E. rubecula*, 1 of *P. phoenicurus*, and 3 of *T. philomelos*), and 4 (15.4%) were infested by nymphs only (*T. philomelos*).

*Borrelia* was observed in 21/127 (16.5%) larvae and 9/26 (34.6%) nymphs examined individually (chi-square = 4.475; $P < 0.05$). Five *Borrelia* species were detected in ticks. Mixed infections were observed in six larvae (*B. lusitaniae* and *B. garinii*; *B. lusitaniae* and *B. burgdorferi* sensu stricto; *B. garinii* and *B. valaisiana* [$n = 2$]; *B. lusitaniae*, *B. valaisiana*, and *B.*

TABLE 2. Prevalence and intensity of tick infestation of migratory birds captured at the Col de Bretolet

| Bird species (name) | No. of birds infested/examined (%) | No. of ticks | Infestation intensity |
|---|---|---|---|
| *Alauda arvensis* (skylark) | 0/2 | | |
| *Anthus pratensis* (meadow pipit) | 0/5 | | |
| *Anthus spinoletta* (water pipit) | 0/1 | | |
| *Anthus trivialis* (tree pipit) | 0/18 | | |
| *Erithacus rubecula* (European robin) | 142/636 (22.3) | 327[a] | 2.30 |
| *Luscinia megarhynchos* (nightingale) | 1/1 | 1 | 1.00 |
| *Luscinia svecica* (bluethroat) | 0/1 | | |
| *Oenanthe oenanthe* (northern wheatear) | 1/6 (16.7) | 1 | 1.00 |
| *Phoenicurus ochruros* (black redstart) | 1/20 (5.0) | 7 | 7.00 |
| *Phoenicurus phoenicurus* (common redstart) | 3/24 (12.5) | 9 | 3.00 |
| *Prunella modularis* (dunnock) | 2/17 (11.8) | 1 | 0.50 |
| *Saxicola rubetra* (whinchat) | 0/1 | | |
| *Sturnus vulgaris* (starling) | 2/55 (3.6) | 1 | 0.50 |
| *Sylvia atricapilla* (blackcap) | 1/31 (3.2) | 1 | 1.00 |
| *Sylvia borin* (garden warbler) | 0/1 | | |
| *Turdus iliacus* (redwing) | 1/4 (25.0) | 2 | 2.00 |
| *Turdus merula* (blackbird) | 8/29 (27.6) | 23 | 2.88 |
| *Turdus philomelos* (song thrush) | 18/107 (16.8) | 44 | 2.44 |
| *Turdus pilaris* (fieldfare) | 0/1 | | |
| *Turdus torquatus torquatus* (ring ouzel) | 0/19 | | |
| *Turdus viscivorus* (mistle thrush) | 0/8 | | |
| Total | 180/987 (18.2) | 417 | 2.32 |

[a] Including 3 *I. frontalis* ticks.

TABLE 3. *B. burgdorferi* sensu lato infection in *I. ricinus* feeding on the different bird species (including mixed infections and infections of pooled ticks)

| Bird species | Frequency of *Borrelia* species in larvae and nymphs (no. of birds with infected ticks)[a] | | | | | |
|---|---|---|---|---|---|---|
| | Ba | Bg | Bv | Bb | Bl | Bsp |
| *Erithacus rubecula* | 4*/0 (4) | 1/0 (1) | 7*/0 (6) | 3/0 (3) | 2/1 (3) | 2/1 (3) |
| *Turdus philomelos* | 0/1 (1) | 1/3 (4) | 1/1 (2) | 1/0 (1) | 2/0 (2) | 1/1 (2) |
| *Turdus merula* | 0/0 (0) | 0/1 (1) | 2**/0 (2) | 0/0 (0) | 1**/0 (1) | 0/0 (0) |
| *Phoenicurus phoenicurus* | 0/0 (0) | 2/0 (2) | 1/0 (1) | 0/0 (0) | 1/0 (1) | 0/0 (0) |
| *Phoenicurus ochruros* | 0/0 (0) | 0/0 (0) | 2/0 (1) | 1/0 (1) | 0/0 (0) | 0/0 (0) |
| Total | 4/1 (5) | 4/4 (8) | 13/1 (12) | 5/0 (5) | 6/1 (7) | 3/2 (5) |

[a] *, included a pool of two larvae infected by *B. afzelii* and *B. valaisiana* and considered as one larva with a mixed infection; **, included one pool of three larvae infected by *B. valaisiana* (considered as one larva) and one pool of two larvae infected by *B. valaisiana* and *B. lusitaniae* and considered as one larva with a mixed infection. Ba, *B. afzelii*; Bg, *B. garinii*; Bv, *B. valaisiana*; Bb, *B. burgdorferi* sensu stricto; Bl, *B. lusitaniae*; Bsp, unidentified *Borrelia* species.

*garinii*; and *B. lusitaniae*, *B. valaisiana*, and *B. burgdorferi*) and one nymph (*B. garinii* and *B. valaisiana*). In addition to these individually examined ticks, 68 larvae and 2 nymphs collected on 9 *E. rubecula*, 2 *T. merula*, and 1 *T. philomelos* bird were examined as 12 pools (1 for each bird). *B. valaisiana* and *B. afzelii* were detected in one pool of two larvae collected from one *E. rubecula* bird, *B. valaisiana* and *B. lusitaniae* in one pool of two larvae collected from one *T. merula* bird, and *B. valaisiana* in 1 pool of 3 larvae collected from one *T. merula* bird.

*B. valaisiana* was the most frequent species in ticks ($n = 14$), followed by *B. garinii* ($n = 8$), *B. lusitaniae* ($n = 7$), *B. afzelii* ($n = 5$), *B. burgdorferi* sensu stricto ($n = 5$), and unidentified species ($n = 5$) (Table 3). Five *Borrelia* species were detected in ticks feeding on *E. rubecula* and *T. philomelos*, whereas *T. merula* and *P. phoenicurus* were infested by ticks infected by *B. garinii*, *B. valaisiana*, and *B. lusitaniae*. Ticks collected from *P. ochruros* were infected by *B. valaisiana* and *B. burgdorferi* sensu stricto only. *E. rubecula* birds harboring infected ticks were infested in 11 cases by larvae only and by larvae and nymphs in 3 cases. Five isolates were obtained from ticks: NE3928 (*B. afzelii*), NE3929 (*B. garinii*), NE3930 (*B. valaisiana* and *B. lusitaniae*), and NE3931 and NE3032 (*B. valaisiana*).

*I. ricinus*, *I. frontalis*, and *H. marginatum* were infesting migratory birds during the northward and southward migrations. *I. ricinus* clearly dominated on birds captured during the southward autumn migration, whereas *I. frontalis* dominated among ticks identified from local or northward spring-migrating birds. *I. ricinus* is very frequently found on birds in Switzerland (2, 17, 18, 28). In this country, *I. ricinus* is known as the vector of *B. burgdorferi* sensu lato, the tick-borne encephalitis virus, *Rickettsia helvetica*, *Babesia divergens*, *Babesia microti*, *Dipetalonema rugosicauda*, and a trypanosome related to *Trypanosoma theileri* (1, 5, 8, 28).

*I. frontalis* is widely distributed in Europe and is rather rare in Switzerland compared to regions with a milder climate, such as Spain (28). Robins appear to be its most common hosts (28), as confirmed here. Papadopoulos et al. (28) also described its presence on a song thrush, and we observed *I. frontalis* for the first time in Switzerland on *L. megarhynchos*.

All *H. marginatum* ticks were collected from northward spring-migrating birds, as reported by Papadopoulos et al. (28). All individuals of this Mediterranean *Hyalomma* species were fully engorged, showing that they had been attached for a few days and were ready to drop off. This suggests that exotic ticks are imported into Switzerland by birds coming from the south. This tick species is considered the main vector of Crimean-Congo hemorrhagic fever virus in Eastern Europe, the Balkan countries, and Transcaucasia (14) and has been found infected by the West Nile virus (6).

During the southward migration in autumn, the prevalence of tick infestation was higher in birds (18.2%) than during the northward migration in spring (6.0%). In autumn, *E. rubecula* and *T. merula* had a tick infestation prevalence of 22% and 28%, respectively. For *E. rubecula*, this prevalence is much higher than that reported by Olsen et al. (26) in Scandinavia (2.2%). The mean density of ticks on birds was also higher in autumn (0.45 tick per bird) than in spring (0.12 tick per bird). A previous study in Egypt (15) also showed a higher infestation of birds in autumn than in spring. In contrast, other studies have shown a higher prevalence of tick infestation and mean density in spring than in autumn (3, 26). In our study, considering bird species, the rate of infestation of *E. rubecula* by *I. ricinus* was almost five times higher in autumn than in spring, while the sample size was too small with the other species for comparisons between seasons. The higher proportion of birds carrying ticks and the higher mean density in autumn than in spring or vice versa reflect the activity of ticks at these times of the year at stopover sites used by birds. It is clear that the lower the latitude (as with Switzerland compared to Sweden, Denmark, and Baltic Sea), the higher the infestation in autumn, since birds captured during the southward autumn migration over the Alps traveled through areas where *I. ricinus* biotopes are widespread and where *I. ricinus* may show a second peak of questing activity at this period of the year. At the opposite, in spring, birds traveled through the southern part of Europe before arriving in Switzerland and therefore had few contacts with *I. ricinus*. This explains why birds migrating to the south were three times more infested by ticks than those migrating northwards.

Most of the migrants caught in autumn originate from areas of the northeast of Switzerland, up to Fennoscandia and Russia. Among the species with a sample size >20 and which carried infected ticks, *E. rubecula*, *T. philomelos*, *T. merula*, and *P. ochruros* are wintering in southern France, Italy, the Iberian peninsula, Morocco, and Algeria, whereas *P. phoenicurus* is wintering in sub-Saharan Africa.

Among birds migrating southward, five species (*E. rubecula*, *T. philomelos*, *T. merula*, *P. ochruros*, and *P. phoenicurus*) carried *B. burgdorferi* sensu lato-infected ticks. Infection rates of examined *I. ricinus* ticks were 16.5% for larvae and 34.6% for nymphs. These results are similar to those observed in Scandinavia (26).

In Europe, *B. garinii*, *B. burgdorferi* sensu stricto, and *B. afzelii* were reported in ticks from migrating birds (3, 26). In our study, *B. valaisiana*, *B. garinii*, and *B. lusitaniae* were the most frequently found species, followed by *B. afzelii* and *B. burgdorferi* sensu stricto. Interestingly, robins and song thrushes were harboring ticks infected by five *Borrelia* species. Most robins (78.6%) were infested by larvae alone, suggesting that birds transmitted these *Borrelia* species to feeding larvae. The origin of the *Borrelia* species found in ticks feeding on song thrushes is less clear; larvae may have been infected through cofeeding transmission (9). The role played in our results by the reactivation of *Borrelia* infection in migrating birds (11) is unknown.

The most surprising part of our results is the abundance of *B. lusitaniae* in ticks feeding on birds. Not only was this *Borrelia* species identified in larvae in most cases, but it was above all present in larvae feeding on birds (6/7) infested by larvae only. This strongly suggests that birds are reservoir hosts for *B. lusitaniae*. This *Borrelia* species appears to be infrequent in *I. ricinus* in most areas of Europe (10), but *B. lusitaniae* has been described as the dominant species in southwest Europe (7) and in North Africa (31, 36, 37). Since some bird species, such as *E. rubecula* and *T. philomelos*, are migrating between southwestern Europe/North Africa and northern Europe, their role in the dispersal of *B. lusitaniae*, according to our results, is highly suspected.

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