THE ROLE OF BIRDS IN THE TRANSMISSION OF LYME DISEASE A LITERATURE REVIEW

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ABSTRACT

During the vast distances in migration, sometimes through thousands of kilometers, the birds carry ticks very far away from their nesting sites. Up to this moment 136 species of hard ticks Ixodidae have been frequently found on birds. Larvae, nymphs and adults of 25 species of the genus *Ixodes*, parasitize on birds. By transporting ticks infected with *B. burgdorferi* s.l., the birds introduce the infection indirectly to new geographical regions. Once imported, the spirochetes may adapt to the local ecological areas and reservoir hosts. Migratory birds which carry *Borrelia* spp. in their tissues or blood, are able to infect the ticks in the new area. This determines the reservoir competence of the birds and their participation in the ecology and the epidemiology of Lyme disease. The selective transmission of *Borrelia garinii* from birds to ticks has been detected; as well as the fact that migratory birds are able to carry Lyme disease as a latent infection for several months.

Keywords: *birds, ticks, Borrelia spp., reservoir competence*

BIRDS AS DISSEMINATORS OF THE VECTORS OF LYME DISEASE

The risk for humans in certain regions to be infected with *Borrelia* spp. and to develop Lyme borreliosis (LB) largely depends on the tick abundance. In Europe, the primary vector tick is the widely spread *Ixodes ricinus* (1), in Asia - *Ixodes persulcatus* (2), and in the USA - *Ixodes scapularis* and *Ixodes pacificus* (3).

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Mammals are the most common hosts for Ixodid ticks (4). A total of 136 hard ticks have been found on birds (Aves) so far. Larvae, nymphs and adults of 25 species of the genus *Ixodes* parasitize on birds, including the vectors of Lyme disease (5). During their long-distance migration, sometimes through thousands of kilometers, birds carry ticks far away from the nesting habitats. The slow feeding process of ticks for several days, makes their passive transportation possible.

I. ricinus is a polyphagous that feeds on a broad range of vertebrates (6). There is a large number of studies on its distribution and host-spectrum in Russia. Among birds, some species of Galliformes and Passeriformes, such as the song thrush (*Turdus philomelos*), the tree pipit (*Anthus trivialis*) and the Eurasian jay (*Garrulus glandarius*), serve as hosts for nymphs (7). Larvae prefer to suck the blood of the common chaffinch (*Fringílla coélebs*), the European crested tit (*Parus cristatus*) and the European robin (*Erithacus rubecula*).

A survey in Cyprus showed that the larvae and nymphs of *I. ricinus* parasitize on migratory passerines (8). *Haemaphysalis punctata* and *Haemaphysalis concina* larvae were also found. These two species may play the role of vectors of Lyme disease (9, 10).

Almost 100 million birds migrate every spring to or through Sweden to their breeding sites (11). At the same time, they carry a great number of ticks. Out of 22 998 studied Aves, 2% were infested with 967 ticks (12). The most-abundant tick species was *I. ricinus* (98.3%). The authors estimated that more than 6.8 million new ticks were carried to Sweden by the migratory birds every spring. Every year, during the autumn flights 4.7 million ticks passively leave the country and move to the south. The taiga tick, *I. persulcatus*, was found for the first time in the Scandinavian region during the study period, one nymph from willow warbler (*Phylloscopus trochilus)*. *I. persulcatus* is one of the most opportunistic hard ticks. It takes blood meal on more than 200 species of mammals and over 120 species of birds (13). Birds are apparently preferred by nymphs (14). An interesting fact is that the ones that mainly parasitize on humans are the *Ixodes persulcatus* adults (15), unlike *I. ricinus* and its nymphal stage which is the one that most frequently bites humans (16).

Ixodes scapularis, widespread in the USA and Canada, feeds on more than 125 vertebrates, of which 57 are birds (17). Immature stages were found on birds (18, 19, 20). A little less diversity of hosts has *Ixodes pacificus*. A review article showed a total number of 108 vertebrates, 48 of which are Aves (21).

BIRDS AS RESERVOIRS FOR *BORRELIA BURGDORFERI* SENSU LATO (S.L.)

Together with the knowledge on the feeding behavior of the vectors and the competent reservoirs among mammals (22, 23), assumptions appear that some Passeriformes may be also a source of *Borrelia* spp., causing Lyme disease in humans. In the early 1980s entomologists at the Connecticut Agricultural Experiment station isolated spirochetes from the blood of six species of birds (18). Spirochetes were also detected in the larvae and the nymphs of *I. dammini* (*scapularis*), removed from some of bird species in the investigated area. The identification of the spirochetes was still unknown. Two years later, again in Connecticut, spirochetes were isolated and identified as *Borrelia burgdorferi*. The isolates were from a liver of a veery (*Catharus fuscescens*) and *I. dammini* larvae derived from other passerines (24). The recovery of *B. burgdorferi* from the liver of a veery was the first isolation of this pathogen from a bird. To confirm the infectious nature of *B. burgdorferi* to birds, the veery liver isolate was inoculated in laboratory bred chicks. Borreliae were successfully isolated from the kidney and the spleen of an inoculated chick. During field investigations in Minnesota, spirochetes were observed in the blood culture from a song sparrow *Melospiza melodia* (25). The bird was naturally infected and the isolate was confirmed as *B. burgdorferi* by immunofluorescence assay. Ticks feeding on song sparrow were not tested, even though it was already noticed that *Melospiza melodia* is a species that is frequently infested (26). Another study on the reservoir role of birds was conducted on an island near Massachusetts (27). It was proven that one of the most common birds on the island (*Dumetella carolinensis*) was not a source of infection for ticks but only a blood meal source. None of the individual birds in the study were able to infect the larvae, although *Dumetella carolinensis* is frequently attacked by ticks and the rate of tick infection with *B.burgdorferi* on the island is significant (28). These results proved that the high density of a certain bird species in a certain area and the frequent tick infestation of birds do not mean that the bird should be considered as a competent reservoir. Most probably that bird species is not susceptible to Lyme disease infection and it is not capable of transmitting the pathogens to vectors. The authors concluded that in the endemic LB areas a regular research on the infection of the common bird species with spirochetes was necessary.

An increase in LB cases in Scandinavia during the 90s lead to determination of the role of migratory birds in the maintenance of the natural infection cycle . Around 1.3 million potentially infected with *B. burgdorferi* s.l. ticksleave Sweden during the bird migration every autumn. Larvae of *I. ricinus* were examined for *Borrelia* spp. by molecular genetic methods of polymerase chain reaction (PCR) (12). *B. garinii* was the most frequently found among ticks, in 23 of 33 infected ticks (70%), followed by *B. burgdorferi* s.s. (20%) and *B. afzelii* (9%). Results showed that all three *Borrelia* species associated with LB in Europe could be detected in bird transported ticks. Moreover, seven out of eight analyzed Turdidae birds carried larvae infected with *B. burgdorferi* s.l. The authors assumed that some ground foraging species are hosts of both ticks and LB spirochetes. Another study in Sweden (2006) also identified *B. garinii* as the most dominant genospecies in ticks feeding on Passeriformes, especially among the larvae (29). Considering the rare transovarial transmission of Lyme disease agents (30), the researchers supposed that the infected larval stage most probably got spirochetes by birds and the putative reservoir role of birds depends on their feeding habits.

Spirochetes of the genus *Borrelia* were found in *Ixodes uriae* ticks collected from Alcidae seabirds, on an island in the Baltic Sea (31). DNA of the spirochete-infected *I. uriae* ticks was isolated, purified and amplified by PCR. A presence of *B. burgdorferi* was detected. *B. burgdorferi* DNA was also observed in a biopsy from the foot web of one razorbill. One of the two ticks found on the same bird was also positive for *Borrelia* spp. It contained *B. burgdorferi* DNA with the same size of the flagellin gene fragment as the *Borrellia* DNA of the biopsy. According to the authors the results supposed bird involvement in the transmission cycle of *B. burgdorferi* s.l. This is also supported by the fact that the island is free from mammals. The evidence that *I. uriae* parasitizes on razorbills and is capable of harboring *B. garinii* (32), prompted the hypothesis that the presence of seropositive Faeroe Islanders who were regularly bitten by *I. uriae* might be due to a transfer of *B. garinii* to humans by this tick species (33). The principal vector of Lyme disease, *I. ricinus*, is not established on the Faroe Islands. The major participants in the enzootic cycle are *I. uriae*, *B. garinii* and seabirds such as puffins.

All existing knowledge on the reservoirs of *B. burgdorferi* s.l in Europe was collated in a review article from 1998 (34). A total of 16 species of Passeriformes, Charadriiformes (Alcidae) and Galliformes (Phasianidae) were listed in the paper, which appear capable of participating in the natural transmission of *B. burgdorferi* s.l. The criteria for reservoir status were also described. To identify the species which maintain the circulation in the specific area, the researchers recommended the method of xenodiagnosis or a combination of other methods : collection of engorged larvae from wild animals and their examination after moulting under laboratory conditions; analysis of blood meal remnants in the gut of unfed nymphs for identification the host that the nymphs fed on as larvae; isolation of *B. burgdorferi* or detection of *B. burgdorferi* DNA in vertebral tissue; detection of antibodies to *B. burgdorferi* in vertebrate blood.

Richter et al. (2000), who explored the reservoir competence of the American robin (*Turdus migratorius*) in North America, stated that the competent reservoir host tolerates feeding by both subadult stages of vector ticks (35). These authors considered that the competent animal reservoir for LB agents had three major characteristics: 1) readily acquires infection from vector ticks; 2) permits spirochetes to proliferate; 3) readily infects vector ticks.

SELECTIVE TRANSMISSION OF *BORRELIA BURGDORFERI* S.L. BY BIRDS

Together with the studies on the reservoir role of birds, some researchers formulated the hypothesis that the transmission cycle of various *Borrelia* spp. in the nature is associated with specific vertebrate animal groups (36). This hypothesis was supported by the results for Japan, showing that more than 60% of *I. persulcatus* larvae from birds were infected with *B. garinii*. No isolates of *B. garinii* were found among the isolates from rodent-feeding *I. persulcatus* larvae. Only *B. afzelii* were detected in them. The observations suggested that two enzootic cycles in nature maintain borreliae specifically: birds-ticks and rodents-ticks. Another Japanese study in 2000 year showed that *B. garinii* is most probably imported in the country by migratory birds from North-East China via Korea (37).

Researches on the transmission of borreliae in England showed the very high ability of *Phasianus colchicus* to infect ticks with *B. garinii* and *B. valaisiana* (38). Over 50% of *I. ricinus* nymphs detached from pheasants carried both *Borrelia* spp. A xenodiagnostic experiment was applied in order to assay the spirochetal transmission from rodents to ticks. Non-infected *I. ricinus* larvae were placed to feed on wild rodents captured from the pheasant breeding territory. The diagnostic method indicated that rodents were not capable of transmitting *B. garinii* to the larvae, although a later performed biopsy of the internal organs showed that 19% (9/47) of them harbored *B. garinii*. The rodents only transmitted *B. burgdorferi* s.s. Data for pathogen presence in questing adult ticks from the same site showed a prevalence of *B. garinii*, followed by *B. valaisiana*. That data was similar to the one obtained for the ticks from pheasants. This gave basis to researchers to think that pheasants, which are the major host for nymphs in the area, selectively infect with *B. garinii* and *B. valaisiana*. The researchers considered that different genospecies of *B. burgdorferi* s.l. could be maintained in some regions of Europe by distinct transmission cycles involving the same vector tick species but different vertebrate host species. The assumptions for an existing association between the birds and *B. garinii* were based on the optimum temperature growth of the borreliae causing LB. The optimum temperature for *in vitro* cultivation of Lyme disease spirochetes is between 34 and 37^{0}C (39). This might mean that *in vivo* growth is not possible in some animals with relatively high body temperature. The average body temperature of some Passeriformes may reach 40,6^{0}C (40). Most probably *B. garinii* tolerates a higher one (41).

The experimental results obtained for the common pheasants are somewhat contradictory to the results for the reservoir competence of the great tit *Parus major* (42). In south Belgium, the ticks from *Parus major* carried five genospecies of *B. burgdorferi* s.l. and some of them were associated with mammals *B. afzelii* and *B. spielmanii*. The authors explained that it could be due to the fact that the European *B. afzelii* strains have a longer co-evolutionary history with the native birds than with the introduced pheasants. Findings of Polish, German and Spanish researchers also proved the presence of *B. afzelii* in bird-derived ticks (43, 44, 45). Swiss researchers found that migratory birds infect *I. ricinus* with one more *Borrelia* species (46). It was *B. lusitaniae* which is distributed mainly in Southwestern Europe (47) and North Africa (48). *B. lusitaniae* was the third most prevalent genospecies in Switzerland following *B. valaisiana* and *B. garinii*. Only *B. lusitaniae*-infected larvae were detected in that study. The authors concluded that migratory Aves appear to be reservoir hosts for *B. lusitaniae. B. lusitaniae* was also found in non-engorged, field-collected Bulgarian *I. ricinus* ticks (49).

Association of *B. garinii* and *B. valaisiana* with Aves was observed several times in Central and Western European countries (50, 51, 52). Czech researchers revealed that although only three bird species (common blackbird, song thrush and great tit) mainly infect the ticks, they have the potential to spread millions of spirochetes in urban areas (53). In a three-year period, blood samples of 1254 Passeriformes belonging to 42 species were examined in a neighboring Polish region, an endemic area of *B. burgdorferi* s.l. infection in ticks (54). *B. burgdorferi* s.l. was detected in 4.2% of all blood samples. Nine birds were positive. Although in this study ticks were not tested, the results indicated that the incidence of infected birds was higher in April-June, when *I. ricinus* abundance is increased. Later, in July-August, the incidence of infected birds was lower and it corresponded to the decreased abundance of parasites. In conclusion, the authors assumed a presence of infected ticks in the observed region of northeastern Poland, as well as bird hosts susceptible to the pathogens. *Borrelia* infection in bird-derived ticks was examined a decade later (2016) in the same region (43). *B. garinii* was the most prevalent genospecies, in 16 of all 35 ticks, or 45.7 %.

The important role of birds in the epidemiology of Lyme disease is also supported by findings for Germany (55). The prevalence of B. burgdorferi s.l in Thuringia is 25% as showed the testing of 141 bird-derived *Ixodes ricinus*. The majority of the ticks carried *B. garinii* (53%), followed by *B. valaisiana* (28%). Unusually high *Borrelia* prevalence (29%) was found in larvae. Among the unfed ticks in the same area, a total of 256 *Ixodes ricinus* were tested, and *Borrelia* DNA was found in 39 of them (15.2%). The bird-associated *B. valaisiana* was the predominant species.

In Western Europe, data about Spain indicated that the common blackbird and the song thrush were the most frequently infested. (45). Ticks detached form birds were not examined, but only questing nymphs collected in the same site by dragging a white flannelette over the vegetation. Vertebrate DNA in the blood remnants in the nymph gut was identified by PCR amplification. Sixty-one nymphs were processed. Unambiguous pathogen and/or host detection was only achieved for 25 specimens. Most blood remnants (17 out 25) were from Passeriformes and Galliformes. A few blood meal samples were from rodents, wild boars and *Cervus* sp. *B. afzelii*, *B. garinii* and *B. valaisiana* were detected as well.

A recent article summarized the data for tick infestation of Passeriformes in 11 European countries - Czech Republic, Estonia, Finland, Germany, Greece, Hungary, Netherlands, Portugal, Slovenia, Spain and Sweden (56). A total of 2,308 ticks were collected from 843 birds. A total of 656 *Ixodes* ticks were analyzed for *B. burgdorferi* s.l. and 244 were positive (37.2%). *B. burgdorferi* s.l prevalence in larvae was 20% (22/110), and in nymphs 41% (214/521), almost twice as higher. *Ixodes ricinus* was the most infected tick species, 40.2% (210/522). The fieldfare *Turdus pilaris* turned to be carrying ticks with the highest *Borrelia* prevalence (92%), followed by the common blackbird (58%). Sequencing the *flaB* gene showed that the most prevalent genospecies was the *B. garinii* (60.7%, 116/191), followed by *B. valaisiana* (23.6%, 45/191), *B. afzelii* (9.4%, 18/191), *B. turdi* (5.2%, 10/191), *B. lusitaniae* (0.5%, 1/191) and one novel genospecies (0.5%, 1/191). The article also focused on the genetic characterization of *B. garinii* at the European and transcontinental scale. The diversity of the common avian-associated *B. garinii* genospecies and potential phylogeographical patterns were determined using a multilocus sequence typing scheme (a technique in molecular biology). Little overlap of the sequence types among the continents Europe, Asia and North America was found but no structuring geographical population in Europe was noticed. Furthermore, according to the authors, no pattern of geographical population structuring was observed according to the isolation source (bird-derived ticks or questing ticks/ human isolates), probably due to *B. burgdorferi* s.l./

or ticks' dispersal promoted by birds. These results taken together provided evidence that birds act as important reservoirs for *B. garinii* and arethe main source of infection of this genospecies to ticks and ultimately to humans.

HOW LONG DO THE BIRDS REMAIN INFECTIOUS?

Experiments of Kurtenbach et al. demonstrated that pheasants can be infected and that their infectivity for ticks may persist as long as 10 weeks. (57). In North America, four birds American robins infected with *B. burgdorferi* s.s. by lab nymphs *I. dammini* were used for determination of their subsequent infectivity for larvae (35). After the larvae molted, the resulting nymphs were examined by dark-field microscopy. The birds were able to infect 88% of laboratory-raised ticks (xenodiagnostic larvae) for at least 3 weeks. Infectivity waned by 2 months and disappeared by 6 months. The researchers also determined that American robins tolerated reinfection by tick-borne spirochetes. Six months after the birds were initially infected, each of the four American robins was exposed to the bites of eight infected nymphs. All four robins regained infectivity for ticks within the next 2 weeks. It was revealed that the birds remained tolerant to reinfection and became infectious again. The authors concluded that probably American robins tolerated reinfection in the nature as well, because they forage in ground, and are frequently infested by vector ticks. Several years later (2005), the reservoir competence of American robins in North America was proven again (58). In relation to the reactivation of *Borrelia* infection in birds an experiment was conducted under stressful conditions for birds by simulating their migration (59). It turned out that the redwing (*Turdus iliacus*) was able to carry Lyme disease as a latent infection for several months. As a result of migratory restlessness, the infection could be reactivated and passed on to tick vectors. Ticks can feed on birds with reactivated infections anywhere through the migration flight, to infect themselves and to pass the disease on to other organisms.

STUDIES IN BULGARIA

Bulgarian studies on the role of birds as disseminators of Ixodid ticks are very few and were conducted more than 40 years ago. There are no researches on the detection of *Borrelia* spp. in ticks feeding on birds or in tissues of birds. Data is available on the coastal strip of the Bourgas region for the early seventies (60). Ixodidae specimens collected from birds showed a prevalence of *Haemaphysalis punctata* larval and nymphal ticks. The most frequently infested bird species were the pheasants, *Corvus frugilegus* (rook) and *Perdix perdix* (grey partridge). During the Crimean-Congo hemorrhagic fever outbreak investigation in Pazardzhik region, mostly immature stages of *Hyalomma plumbeum* and *Haemaphysalis punctata* were found on birds (61). The common blackbirds, the grey partridges, *Upupa epops* (Eurasian hoopoe), *Motacilla fl. feldegg* (Black-headed yellow wagtail), *Athene noctua* (Little Owl), *Pica pica* (Eurasian magpie), *Garrulus glandarius* (Eurasian jay), and *Charadrius dubius* (Little ringed plover) were indicated as hosts. *I. ricinus*, reported years later as a European vector of Lyme disease, was not established as an avian ectoparasite back then. However *Haemaphysalis punctata* was established, which may play a role of a secondary vector of Lyme disease in Bulgaria (62).

CONCLUSION

The surveillance of infected questing ticks and reservoir hosts of *B. burgdorferi* s.l is the main part of the LB surveillance. The observation of spatial and temporal dynamics of pathogens in tick and vertebrate animal populations was defined as an essential part for preliminary assessment of LB incidence in humans and disease burden (63). The most frequent disseminated manifestation of Lyme disease in Europe is the neuroborreliosis. In Norway, for example, extensive data of annual disease incidence from 1995 to 2017 showed that 69% of disseminated Lyme borreliosis cases were neuroborreliosis (64). The bird-associated species *B. garinii* are considered to be their causal agent. Studies carried out during the period 2003-2008 found that *B. garinii* is dominant among ticks from migratory birds in Norway (65, 66). These results emphasize again the key role of birds in the epidemiology of LB.

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