

Global ecology and epidemiology of *Borrelia garinii* spirochetes

Pär Comstedt, PhD[†], Tobias Jakobsson, MD and
Sven Bergström, Professor*

Department of Molecular Biology, Umeå University, Umeå, Sweden

Lyme borreliosis (LB) is a tick-transmitted infectious disease caused by *Borrelia burgdorferi* sensu lato (s. l.). In Europe, three different *Borrelia* species are the main causative agents of LB: *B. burgdorferi* sensu stricto (s.s.), *Borrelia afzelii*, and *Borrelia garinii*. The latter depends heavily on birds as its main reservoir hosts. In fact, birds can act both as biological carriers of *Borrelia* and transporters of infected ticks. The seasonal migration of many bird species not only aid in the spread of *B. garinii* to new foci but also influence the high level of diversity found within this species. *B. garinii* have been isolated not only from terrestrial birds in Europe, but also from seabirds worldwide, and homology between isolates in these two different infection cycles suggests an overlap and exchange of strains. In addition, it has been shown that birds can maintain and spread *B. garinii* genotypes associated with LB in humans. This review article discusses the importance of birds in the ecology and epidemiology of *B. garinii* spirochetes.

Keywords: *Borrelia garinii*; Lyme borreliosis; birds; reservoir hosts; ticks; *Ixodes uriae*; *Ixodes ricinus*

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Lyme borreliosis (LB) is the most common vector-borne disease in the northern hemisphere. The multisystem illness is due to infection by *Borrelia burgdorferi* sensu lato (s.l.) bacteria, spread by different *Ixodes* ticks. In Northern America, only *B. burgdorferi* sensu stricto (s.s.) is regularly isolated from human patients. However, on the Eurasian continent, the situation is more complex because three different species cause the disease, namely *Borrelia afzelii*, *B. garinii*, and *B. burgdorferi* (s.s.) (1). In addition, these species are spread by different vectors, *Ixodes ricinus* in Europe and *I. persulcatus* in Asia. The habitats of these two tick species overlap in Eastern Europe (2). Also, the reservoir hosts for the three species are different. *B. afzelii* depend on various rodents as their main reservoir host, whereas *B. garinii* mainly depend on birds. *B. burgdorferi* s.s., on the other hand, appear to be more of a generalist and can use both birds and rodents as its reservoir host (3).

Borrelia garinii has not only been isolated from terrestrial birds but also from seabirds (4–6). The relationship between seabirds and seabird-oriented ticks was recently described in a review by Dietrich et al. (7). As a

consequence of seasonal bird migration, *B. garinii* is widely spread around the globe in a marine infection cycle (8–10). Possibly, bird migration can be one explanation to why *B. garinii* is the most heterogeneous of all LB-causing species studied so far (11–13).

In Europe, *B. garinii* is second only to *B. afzelii* as causative agent of LB and even though this species is dominating among patients with neuroborreliosis, it is not thoroughly studied (14). Explanatory factors for this could be the complicated ecology involving different reservoir hosts, the wide geographical distribution, and the heterogeneity among its genotypes. This is further discussed in subsequent sections of this review.

The role of birds in the epidemiology of different pathogens

Birds are known to play an important role in the epidemiology of many pathogens also infective to humans; their relatively long lifespan and the fact that they can fly aid in dispersing infections worldwide. Additionally, birds of diverse species tend to congregate at migration stopover sites and thereby allow for horizontal transfer of pathogens. In some cases, the

[†]Present address: Intercell AG, Campus Vienna Biocenter 3, A-1030 Vienna, Austria.

bird itself is a relatively poor host but, spreading pathogens to new distant foci where it can infect a more suitable reservoir host may be of great importance (15). There are three main mechanisms by which birds can be involved in spreading pathogens (16). Firstly, as biological carriers meaning that the bird is infected and the pathogen multiplies in the avian body. Some of the more well-known pathogens that have been isolated from wild migrating birds include: West Nile virus, Influenza A virus, *Vibrio cholerae*, *Campylobacter jejuni*, and *Salmonella typhimurium* (17–20). The two latter species can also contaminate water through the feces of birds. Secondly, birds can act as both external and internal carriers without pathogen amplification. Fungal spores from different species, including *Candida albicans* and *Aspergillus fumigatus* simply stick to feathers or other avian body parts as a means of transportation (21, 22). Thirdly, birds can act as transporters of infected ectoparasites. Medically, the most important ectoparasites on birds are ticks, i.e. *Ixodes* spp. and *Argas* spp. Tick-Borne Encephalitis virus has been found in ticks feeding on birds of many different species (22, 23). These birds often originate from Russia or Eastern Europe. Also, isolates of *Anaplasma (Ehrlichia) phagocytophila* identical to clinical strains causing human granulocytic anaplasmosis (ehrlichiosis) have been isolated from ticks feeding on wild birds (24). There are also reports of *Rickettsia sibirica*, *Coxiella burnetii*, and *Babesia microti* in ticks parasitizing on birds (25–27). In the case of *Borrelia* spirochetes and *B. garinii* in particular, birds can function both as biological carriers with and without amplification in the host and as transporters of attached ticks infected with *Borrelia* spirochetes for long-range dispersion of both the vector and the pathogen.

The influence of ticks and host reservoir competence on *B. garinii* ecology

In adult form, only the female tick seeks a blood meal and the mating often takes place on the blood host itself, sometimes even while the female tick is feeding. The female tick then lays eggs that hatch into larvae and the life cycle restart (28). *B. burgdorferi* (s. l.) spirochetes are found among different species of *Ixodes* ticks and a wide range of vertebrate hosts. In general, to maintain a large pool of infected reservoir animals, young individuals need to be infected and be able to stay infected for a long period of time. Similarly, the tick transmission cycle is dependent on tick larvae becoming infected when feeding on an infected host. After having completed the molting and the ticks feed again as nymphs, they transmit the infection to a new host. It is important to emphasize that infected larval or nymphal ticks remain infected after having molted to the next developing stage, even though bacterial

numbers may have decreased dramatically (29). Abiotic factors such as temperature and humidity influence the *Ixodes* tick life cycle and therefore also infecting spirochete numbers (28). Lower temperatures slow down the life cycle, and a decrease in ambient temperature from 24°C to 15°C has shown to extend the time needed for molting of certain Ixodidae ticks by an average of 30 days in laboratory settings. This in turn means that the incubation time for the spirochetes infecting the ticks will be extended. On the other hand, ambient temperatures in the range of 27–38°C have also been shown to negatively influence the spirochete numbers in infected *Ixodes* ticks and render them non-infective to mice (30, 31). The relative humidity can also have an indirect impact on the choice of blood host because it influences the height at which the tick quest in the herbage and therefore possibly the size of the animal (32). The competence of a particular animal to function as reservoir host is thought, at least in part, to depend on the complement system, indicating that not all animal species are equally susceptible to a *B. garinii* infection (3). *In vitro* studies and sampling of wild animals have shown that *B. garinii* Outer surface protein A (OspA) serotype 3, 5, 6, and 7 type of strains are resistant to normal bird sera but are sensitive to sera from different rodents. These four serotypes are therefore thought to rely on different birds as reservoir hosts. On the other hand, *B. garinii* OspA serotype 4 strains show the complete opposite pattern of serum resistance and in nature are also not found in birds but different species of rodents (3). Based on the ecological characteristics together with multilocus sequence analysis, it has also been suggested that *B. garinii* OspA serotype 4 strains should be raised to species status and be named *Borrelia bavariensis* sp. nov. (12).

Borrelia and birds

Anderson and Magnarelli (33) isolated *B. burgdorferi* (s.s.) from *Ixodes scapularis* collected from different species of birds. Later, they also isolated spirochetes from the livers of passerine birds, finally corroborating that birds are indeed important reservoir host for LB and as such important for the ecology and epidemiology of *B. burgdorferi* (s.s.) (34). Since then, a number of studies focusing on wild birds have expanded the knowledge regarding birds as reservoirs for *Borrelia* (4, 6, 35, 36).

Today we know that birds spread and maintain *Borrelia* spirochetes very similar to clinical isolates and therefore, we believe that the role of birds in the ecology and epidemiology of LB is greater than previously understood (4, 8). For a long time, birds were ruled out as potential hosts for *B. burgdorferi* (s. l.) spirochetes, mainly because of their high body temperature. As the optimal growth temperature for many

LB-causing strains had been determined to 34–37°C and since birds have an elevated body temperature compared to many mammals, they were not considered to be likely reservoir hosts (37, 38). However, unlike other species, some *B. garinii* isolates are reported to grow at temperatures of up to 41°C; this has also been suggested to be an adaptation to the avian host (39). Possibly, this is one reason why *B. garinii* is the species most frequently infecting birds in Europe and Asia (4, 6, 40).

Terrestrial birds as reservoir hosts

Some experimental studies suggest that birds are relatively poor reservoir hosts for *B. burgdorferi* (s. l.) Piesman et al. showed that 1-week-old chickens infected with *B. burgdorferi* (s.s.) were only able to transmit the spirochetes to new larvae during the first 3 weeks post infection. In addition, the reservoir competence also seemed to decline dramatically with age. When 3-week-old chickens were challenged with *B. burgdorferi* s.s., only a fraction of feeding ticks were positive for spirochetes 1 week post challenge (41). Other studies show that birds indeed have the potential to serve as competent reservoir hosts. When Isogai et al. infected Japanese quails (*Coturnix japonica*) with *B. garinii*, they were able to reisolate spirochetes from lung and kidney tissue 1 month post infection and from skin samples up to 2 months post infection. (42). Also, Canary finches (*Serinus canaria*) have been assessed for reservoir competence when challenged with *B. burgdorferi* (s.s.) Spirochetes could be detected in blood 2 weeks after challenge using microscopy. Furthermore, *Borrelia* DNA could be detected by polymerase chain reaction (PCR) up to 3 months post infection in several internal organs, suggesting a persistent infection (43).

Apparently, *B. burgdorferi* (s.s.) can infect both rodents and birds (3). However, differences in reservoir competence have been reported for some ground-foraging bird species in North America, meaning all are not equally competent in transmitting *B. burgdorferi* (s.s.) to naïve ticks. For example, in one field experiment using xenodiagnosis, American robins (*Turdus migratorius*) were suggested to be competent reservoir hosts. On the other hand, Song sparrows (*Melospiza melodia*) and Gray cat birds (*Dumetella carolinensis*) were shown to have intermediate and low reservoir competence respectively (44). Some of the data are also supported by others performing experimental infections (45). For instance, the reservoir competence for American robins has been shown to be comparable to that of some rodents, measured by the capacity to infect feeding larvae. Even though the infection in the birds disappeared after 6 months, they remained susceptible to reinfection. In addition, ticks that acquired an infection by feeding on birds could subsequently infect mice, showing

the potential influence of birds on the ecology of *B. burgdorferi* (s.s.) in North America (45).

Reservoir competence of passerine birds in Europe

In the first large-scale study focusing on migrating terrestrial birds, the importance of passerine birds as reservoirs for *B. burgdorferi* (s. l.) spirochetes and as carriers of infected ticks was shown (4), where 13,123 migrating passerine birds, comprising 38 different species, were captured (at Ottenby bird observatory in Southern Sweden) and examined for infected ticks. The birds were divided into two groups, ground foragers and non-ground foragers. In total, 1,120 *I. ricinus* larvae and nymphs were collected and screened for the presence of *B. burgdorferi* (s. l.) spirochetes by quantitative PCR (qPCR). Aggregation of infestation risk was indicated by the finding that once a bird was infested with one tick, the likelihood of infestation with two or more ticks was higher than would be expected. Not surprisingly, the risk was greater for the ground-foraging birds than for the non-ground-foraging birds (5.4% vs 1.4%). The same pattern was also seen when retrospectively analyzing data from more than 15,000 birds captured in 1991 (6). In total, 14% of the ticks were positive for *B. burgdorferi* (s. l.) spirochetes and almost twice as many nymphs than larvae (19.3% vs 10.1%) (4). This is probably explained by successive accumulation of spirochetes during feeding both as larvae and nymphs. The finding that also larvae were infected, again points to the fact that birds act as reservoir hosts. To exclude the possibility of transovarial transmission, birds infested with larvae were studied by comparing the proportion of birds with infected larvae among birds infested with one larva and the proportion of birds with one or more infected larvae among multiple infested birds. A higher prevalence of infection among ticks from multiple infested birds (21.4%) compared to single infested birds (5.5%) was found, showing that birds indeed were the source of infection. Also, a correlation between the proportion of birds infested with larvae and nymphs and the proportion of birds infested with infected ticks showed that birds were the reservoir hosts of the spirochetes. In addition, 3% of the ground-foraging birds were infested with infected ticks, compared to 0.6% of the non-ground-foraging birds. The percentage of birds with infected larvae among the larvae-infested birds was higher among ground-foraging species (34.0%) than among other bird species (7.8%). This, again, suggests a more central role in the ecology of *B. burgdorferi* (s. l.) for the former group of birds. The birds' reservoir competence, determined as the efficiency of spirochete transmission to larvae with regard to spirochete numbers and infection prevalence, was also evaluated. When analyzing

spirochete load and infection prevalence for larvae collected from the same bird, these parameters were found to positively correlate for 56 larvae from 25 birds. However, an experimental infection where larvae would be allowed to molt to nymphs and subsequently were allowed to feed on new hosts would fully determine the role of birds as reservoir hosts as proposed by Richter et al. (45). Due to the size of the sample material, this was unfortunately not possible. Furthermore, in order to know if the two groups of birds (ground-foraging and non-ground-foraging birds) differed in reservoir competence, larvae from individual birds were studied. There were no differences between the two groups regarding the mean value of spirochete numbers in the larvae: 135 (95% CI 21–862) and 23 (95% CI 2–318), respectively. Also, there was no difference in infection prevalence between the two groups of larvae: 61% (95% CI 46–77%) and 77% (95% CI 50–100%), respectively. Together, this suggests that the two groups of birds are equally competent for infecting larvae and their importance as reservoir hosts is rather decided by their foraging behavior. The *Borrelia* species were determined for 88 of the 160 positive samples. The overall dominance of *B. garinii* and total lack of *B. afzelii* among larvae was in agreement with other studies focusing on host association of *B. burgdorferi* (s. l.) spirochetes (3). This finding yet again indicates the important role of birds in the ecology of *B. garinii*.

To study the clinical relevance of the *B. garinii* spirochetes spread by passerine birds, 47 isolates from ticks infesting birds and 11 clinical isolates of the same species from erythema migrans (EM) patients (46) were compared, using the amplicons generated from the *rrs* (16S)–*rrl* (23S) intergenic spacer (IGS) (8, 47). Two groups were defined with nine and two sub groups

respectively (1:1–1:9 and 2:1–2:2) as shown in Table 1. Six subgroups (1:1, 1:3, 1:4, 1:9, 2:1, and 2:2) comprising 29 (62%) of the bird tick isolates were also found in the biopsies from EM patients. In addition, larvae were infected with three variants (1:1, 1:3, and 2:1) that also cause disease in humans. Passerine birds are therefore reservoir hosts of clinically important *B. garinii* spirochetes.

Dubska et al. examined the prevalence of various *Borrelia* spp. in *I. ricinus* ticks collected from passerine birds in Czech Republic. In agreement with our findings, great tits (*Parus major*), Eurasian blackbirds (*Turdus merula*), and song thrushes (*Turdus philomelos*) were found capable of transmitting *B. garinii* to feeding ticks. Furthermore, the two latter bird species were shown not only to be competent reservoir hosts but also suggested to play a central role in the ecology of *B. garinii* in Europe (48, 49).

In conclusion, migratory passerine birds can have dual roles in the maintenance of *B. burgdorferi* (s. l.) spirochetes, both as long-range carriers of infected ticks and as reservoir hosts infecting larvae. Thus, their contribution to *B. burgdorferi* (s. l.) ecology and epidemiology is of great importance.

The marine infection cycle

Olsen et al. (5) were the first to isolate *Borrelia* spirochetes from *Ixodes uriae*. The ticks were collected from razorbills (*Alca torda*) on an isolated island in the Baltic Sea where no mammals were present. As seabirds were the only blood host available for the ticks, a marine enzootic infection cycle was suggested, where *B. garinii* circulating among colonial seabirds depend on *I. uriae* as the only vector (5). The distribution of this marine infection cycle was then studied since

Table 1. Grouping of *Borrelia garinii* isolates from ticks infesting passerine birds and clinical samples [erythema migrans (EM)] based on the *rrs* (16S)–*rrl* (23S) intergenic spacer (IGS)

Group	Sub Group	<i>B. garinii</i> isolates from ticks infesting passerine birds	<i>B. garinii</i> from clinical samples
1	1	A31 A99 C85¹ C86¹ C87¹ C89¹ C90¹ D46	Bio56059 Bio56061
		D48 D88 E09 F28 F88 K29 L58	
	2	D83 F89	
	3	C24 D49 E07	Bio56016 Bio56081
	4	C28 C55 G09 K96	Bio56014 Bio56045
	5	C78 E06 E08 E13 K60 K92	
	6	D40 D47 K70 K73	
	7	C51 K20 F87 L78	
	8	L05 L07	
	9	K22	Bio56002
2	1	B02 D12² D13² K89	Bio30058 Bio56056 Bio56101
	2	A35 B69	Bio56077

Bold letters: Isolates from *I. ricinus* larvae.

^{1,2}Larvae collected from the same bird.

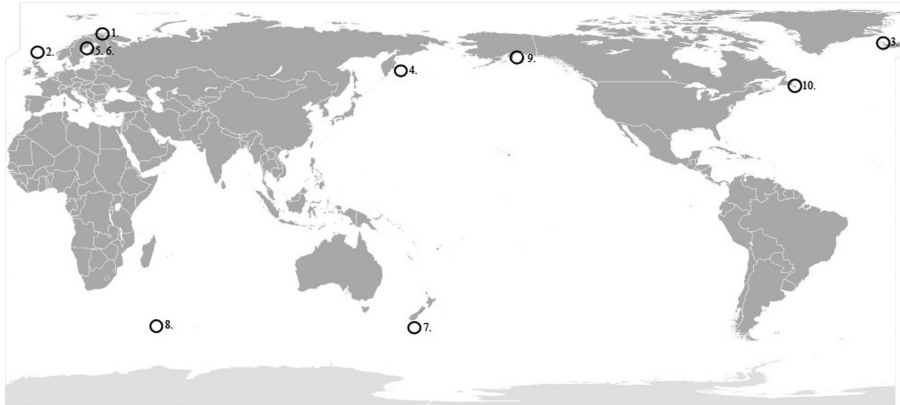


Fig. 1. Global distribution of *Borrelia garinii* in the marine infection cycle. Seabird colonies where *B. garinii* spirochetes are known to be present are indicated with a circle. The seabird colonies are: 1. Hornöya Island, Norway (9); 2. Faroe Island, Denmark (61); 3. Flatey Island, Iceland (10); 4. Commander Island, Russia (8); 5. Malgrundet Island, Sweden (62); 6. Bonden Island, Sweden (5); 7. Campbell Island, New Zealand (10); 8. Crozet Islands (10); 9. Egg and St. Lazaria Islands, USA (10); 10. Gull Island, Canada (53).

large seabird colonies are present globally. *I. uriae* were collected from seabirds at nine different locations worldwide. *B. garinii* spirochetes with identical flagellin B (*flaB*) genes were found in ticks from both the southern hemisphere (Campbell Island, New Zealand, and Crozet Islands) and the northern hemisphere (Egg and St. Lazaria Islands, USA) suggesting that seabirds can act as long-distance carriers of the infection (Fig. 1; 10). As the ticks only feed for a few days and the birds' migration causes them to spend longer periods of time above or in open water, the spirochetes are probably transported as a latent infection in the birds, rather than by infected ticks. A phylogenetic analysis of *I. uriae* from both the southern and the northern hemisphere also suggests separate reproduction cycles of the ticks (50). It has been shown that a latent *B. burgdorferi* (s.s.) infection can be reactivated when the birds are subjected to stress as in the case of seasonal migration (51). This could further aid in the global dispersal of *B. burgdorferi* (s.l.) infected ticks. *B. garinii* is

by far the most prevalent species isolated from seabirds and *I. uriae*, even though occasionally also *B. lusitaniae* and *B. burgdorferi* (s.s.) have been found (52). As discussed earlier, *B. garinii* is one of the most prevalent species found among *I. ricinus* and *I. persulcatus* in Europe and Asia. However, the marine infection cycle increases the habitat to include also the North Pacific Ocean (Egg and St. Lazaria Islands outside Alaska, USA) and the North Atlantic Ocean (Gull Island, Canada). This suggests *B. garinii* is present on both the West and the East coasts of North America (10, 53).

Surprisingly, *B. garinii* have also been isolated from *I. uriae* found in seabird colonies located in extreme climate zones such as the Arctic and the subAntarctic regions as well as the Bering Sea littoral (8–10). Seabird colonies where *B. garinii* infected *I. uriae* or birds have been found are indicated in Fig. 1. When comparing seabird-associated *B. garinii* strains from Arctic Norway (Hornöya Island) with other isolates collected from seabird colonies on Faroe Islands, identical IGS were



Fig. 2. *Ixodes uriae* ticks in different developmental stages can be found under almost each stone in the tufted puffin colony on Commander Islands, Russia. The tufted puffins nest in burrows in the ground. Photo: Ingvar Eliasson.

found (9). Also, close to identical IGS were found when comparing the Arctic *B. garinii* isolates to those isolated from migrating passerine birds and LB patients in southern Sweden. This supports the theory of birds as global carriers and disseminators of *B. garinii*, also with potential impact on human health. This will be discussed more in detail later in this review article.

Aside from the marine infection cycle, other enzootic cycles involving avian hosts and specialized tick species have been studied. The tick *Ixodes dentatus* has been described as vector for *Borrelia* spp. It is to a large extent ornitophilous and has been proposed a potential factor in maintenance and dispersal of *Borrelia* spp. (54). Also, *B. garinii* and *B. burgdorferi* (s.s.) have been found in *Ixodes lividus*, which is almost exclusively associated with Sand Martins (*Riparia riparia*) (55).

The seabird tick *I. uriae*

Ixodes uriae is also known as the ‘seabird tick’ (Fig. 2). It is strictly localized to seabird colonies where it completes the entire life cycle by feeding on various seabirds (56, 31). It is wide ranging in the choice of seabird host and has been reported to feed on more than 50 different colonial seabird species (56, 57). *I. uriae* can be found all around the world, both on the southern and the northern hemisphere (58). It is also present in very demanding climates not typically associated with ticks, such as the Arctic and subAntarctic (9, 10, 58). This tick can withstand temperatures as low as -30°C , which is necessary for survival in these often very harsh environments. In milder climates, the tick has a 3-year lifespan and feeds once per year. However, in colder climates (Arctic and Barents Sea littoral), the tick’s life cycle can be as long as 7 years (31, 59). *I. uriae* are often very abundant in these seabird colonies and to keep the appropriate humidity, the ticks tend to stay together in clusters (60). Therefore, virtually under every stone and in every crack, it is possible to find great numbers of ticks in all developmental stages and both sexes (Fig. 3) and as a consequence, many birds become heavily infested.



Fig. 3. *Ixodes uriae* ticks collected from a seabird colony on Commander Islands, Russia. 1. engorged larva; 2. unfed nymph; 3. engorged nymph; 4. unfed adult female; 5. engorged adult female; 6. unfed adult male. Bar equals 10 mm. Photo: Pär Comstedt.

Prevalence and densities of *B. garinii* in ticks and seabirds

Many seabird colonies are very crowded, and spread of infection among birds should therefore be easily facilitated. Different studies suggest that as many as 30% of the nymphs and adults in seabird colonies are positive for *B. garinii* (8, 10). A rather homogeneous *B. garinii* population could be expected in these colonies since high numbers of ticks and birds thrive on often relatively limited areas. Surprisingly, great heterogeneity among the *B. garinii* spirochetes has been observed in different studies, suggesting a frequent contact with other distant geographical locations (8, 10). Today, colonies worldwide are known to harbor *B. garinii*-infected *I. uriae* or seabirds. Some of the *B. garinii* isolates from different colonies also share high homology, based on sequencing of *flaB* or IGS, even though the seabird colonies are separated by thousands of kilometers (Fig. 1; Table 2).

When DNA extracts from 299 *I. uriae* collected from a colony with tufted puffins (*Fratercula cirrhata*) on the Commander Islands, Russia (Fig. 1; Fig. 3), were screened by qPCR for presence of *B. burgdorferi* (s. l.) spirochetes, 99 (33.1%) were found positive. By partial sequencing of the IGS amplicon, all positive samples were identified as *B. garinii*. The infection prevalence was equally distributed among the different development stages of ticks. As expected, the bacterial densities dramatically increased after each blood meal. When nymphal ticks had completed their feeding, mean bacterial numbers per specimen had increased from 16 (95% CI 5–49) to 893 (95% CI 144–5,546). A similar increase from 209 (95% CI 54–807) to 2,489 (95% CI 535–11,561) mean spirochetes per adult tick could also be observed. The molting process had a negative influence on bacterial numbers, and therefore an oscillating pattern of the spirochete population appeared when different development stages of the tick were compared (Fig. 4; 8). Interestingly, even though the infection prevalence was high among the ticks in the colony and the tufted puffins are by far the most dominating seabird species present, none of 86 blood samples collected from adult birds of this species were positive for *B. burgdorferi* (s. l.) spirochetes, as shown by qPCR analysis (8). As discussed earlier, some studies suggest that birds are poor reservoir hosts for *B. burgdorferi* (s. l.) spirochetes, whereas others show that *Borrelia* infection in birds can persist for months (51, 41). *B. garinii* have been isolated earlier from Atlantic puffins (*Fratercula arctica*), a close relative to the tufted puffin (61). The relatively high prevalence of infected ticks of all developmental stages and the total absence of infected adult tufted puffins in the colony on Commander Islands suggest that ticks may constitute the actual reservoir of *B. garinii* (8). Possibly, newly hatched birds staying in their burrows for weeks could be transiently infected and subsequently infect new ticks. In this way,

Table 2. *Borrelia garinii* rrs (16S)-rrl (23S) intergenic spacer (IGS) sequence identity within and between strain collections of different geographical and biological origins

		The marine infection cycle					The terrestrial infection cycle				
		Hornöya Island, Norway (n = 8)	Faroe Islands, Denmark (n = 4)	Flatey Island, Iceland (n = 1)	Commander Islands, Russia (n = 10)	Malgrundet Island, Sweden (n = 2)	Bonden Island, Sweden (n = 1)	Norrbyckär Island, Sweden (n = 1)	Migrating Passerine Birds, Sweden (n = 6)	LB Patients, Sweden (n = 10)	LB Patient, Germany (n = 1, Pbi)
The marine infection cycle	Hornöya Island, Norway (n = 8)	90.4% (488)– 95.1% (489)									
	Faroe Islands, Denmark (n = 4)	90.4% (488)– 100.0% (395)	99.2% (367)– 99.8% (594)								
	Flatey Island, Iceland (n = 1)	77.8% (351)– 79.3% (352)	77.8% (352)– 78.1% (352)	not applicable							
	Commander Islands, Russia (n = 10)	87.5% (447)– 96.9% (489)	85.1% (496)– 93.0% (359)	77.2% (364)– 80.9% (300)	88.0% (458)– 100.0% (505)						
	Malgrundet Island, Sweden (n = 2)	89.0% (418)– 99.7% (390)	97.1% (414)– 99.8% (421)	77.3% (352)– 78.1% (352)	88.8% (420)– 92.4% (395)	98.5% (387)– 98.5% (387)					
	Bonden Island, Sweden (n = 1)	90.5% (421)– 94.1% (421)	93.8% (433)– 96.0% (426)	78.2% (353)	89.9% (425)– 91.7% (395)	94.5% (415)– 95.9% (437)	not applicable				
The terrestrial infection cycle	Norrbyckär Island, Sweden (n = 1)	92.7% (343)– 95.3% (343)	95.3% (343)– 95.3% (343)	78.1% (352)	91.7% (348)– 94.2% (343)	94.8% (343)– 95.3% (343)	100.0% (343)	Not applicable			
	Migrating Passerine Birds, Sweden (n = 6)	91.0% (490)– 99.2% (478)	83.0% (612)– 94.8% (405)	77.8% (351)– 79.0% (352)	84.7% (497)– 94.9% (510)	89.0% (418)– 94.5% (400)	90.7% (430)– 92.6% (431)	92.7% (344)– 94.8% (343)	81.3% (646)– 98.0% (616)		
	LB Patients, Sweden (n = 10)	90.2% (379)– 99.2% (369)	80.8% (479)– 94.8% (405)	78.1% (351)– 78.6% (351)	83.3% (426)– 93.9% (510)	89.7% (312)– 94.5% (400)	90.4% (312)– 92.6% (431)	92.7% (344)– 94.8% (343)	77.8% (513)– 100.0% (624)	74.4% (910)– 100.0% (525)	
	LB Patient, Germany (n = 1, Pbi)	90.4% (489)– 92.8% (489)	82.9% (619)– 93.0% (372)	77.8% (360)	87.2% (553)– 94.7% (395)	90.4% (415)– 93.7% (441)	92.1% (468)	93.6% (343)	80.2% (651)– 93.2% (658)	77.5% (533)– 93.2% (660)	not applicable

Note: LB, Lyme borreliosis.

Jalview, pairwise local alignment showing minimum and maximum percent (%) identity between and within different *B. garinii* strain collections using IGS. Bold numbers indicate identities of >99% between two or more isolates representing different strain collections. Numbers of strains within each collection are indicated (n). Aligned numbers of bases are indicated in parenthesis (minimum cutoff of 300 bases). In case a collection only includes one isolate, the IGS identity within the collection could not be calculated. This is indicated as 'not applicable.' References: Hornöya Island, Norway (9); Faroe Island, Denmark (61); Flatey Island, Iceland (10); Commander Island, Russia (8); Malgrundet Island, Sweden (62); Norrbyckär Island, Sweden (5); Bonden Island, Sweden (5); Migrating Passerine Birds, Sweden (4); LB Patients, Sweden (46); LB Patient, Germany (65).

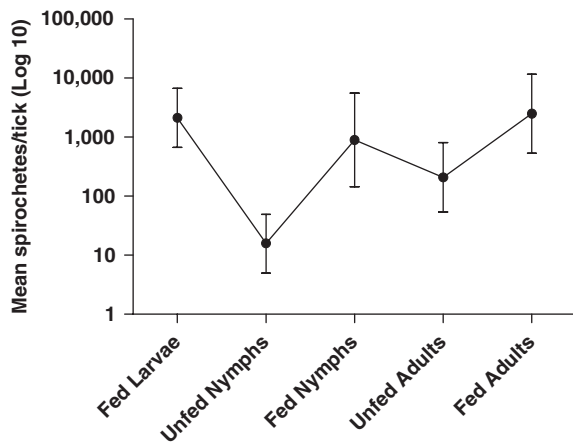


Fig. 4. The spirochete population found in different stages of *Ixodes uriae* collected within one seabird colony on Commander Islands, Russia. Mean numbers of spirochetes per tick increase dramatically after each feeding, but the subsequent molting has a negative effect on the cell count. Mean and asymmetric confidence intervals are antilogs of log₁₀-transformed data from positive samples.

the pool of infected ticks could be maintained even though the infection of the birds wanes over time. This could be clarified by studying blood samples and ticks collected from very young birds.

Interaction between the terrestrial and the marine infection cycle

Borrelia garinii spirochetes are found among rodents and passerine birds as well as among seabirds, suggesting that there has to be some overlap between the marine and the terrestrial infection cycles. The marine infection cycle is restricted to different seabirds and the open sea. *I. uriae* ticks are very wide ranging in their choice of seabird host, but the ticks are seldom found outside seabird colonies. Therefore, an interaction with the terrestrial infection cycle has to take place within the seabird colony. Rodents searching for food and resting migratory passerine birds are possible candidates mediating contact with *I. uriae* or *I. ricinus*. The frequency by which *I. uriae* feed on rodents and other birds is however not known. On some islands in the Baltic Sea, where the *I. uriae* and *I. ricinus* habitats overlap and seabirds as well as migrating passerine birds nest side by side, host cross-over may be possible. Furthermore, *B. garinii* isolates with identical IGS have been found in both *I. ricinus* and *I. uriae* collected from the Swedish islands Norrbyskär and Bonden, separated only by 25 km (62).

To further study the global ecology of this species, the IGS of 77 *B. garinii* isolates of diverse biological and geographical origins were compared by Comstedt

et al. (8), where 20 different genetic variants were found. The seabird-associated strains as a group, isolates from seabirds or *I. uriae*, represented 32% of the sample collection, but comprised 50% of the IGS types. This suggests that *B. garinii* associated with seabirds constitute a more diverse group of spirochetes than the terrestrial group. Indeed, the 20 IGS types grouped into six phylogenetic clusters, all including *B. garinii* circulating among seabirds. Five of the clusters also involved isolates from migrating passerine birds. Furthermore, *B. garinii* isolated from clinical samples were found in three of the clusters. Strains originating from one seabird colony on Commander Islands in far eastern Russia also did not group together. Instead, some of them were more closely related to *B. garinii* isolated from European LB patients or passerine birds. One of the most complex genetic variant contained *B. garinii* isolates from *I. ricinus*-infesting migrating birds in Sweden, questing *I. ricinus* from Lithuania, as well as both skin and cerebrospinal fluid isolates from LB patients. Other genetic variants identified represented *B. garinii* from *I. uriae* and *I. ricinus* as well as isolates from migrating passerine birds. Because the *B. garinii* isolates did not necessarily cluster according to geography, biological hosts, or tick vectors, this again supports the theory of a frequent exchange of strains between the marine and the terrestrial infection cycles (8). The range of IGS identity between as well as within different *B. garinii* strain collections is summarized in Table 2. Even though some collections are limited in size, the overall pattern suggests extensive sequence variation among the strains, but also occasionally high sequence identity (IGS identities above 99% between two or more strains representing different collections are indicated in bold numbers in Table 2).

In summary, the current view suggests two reasons why the marine infection cycle is not an immediate threat to humans. Firstly the geographical barrier, as the seabird colonies are often located in coastal areas or on islands where human population density is low, the risk of acquiring an infection is very limited. Secondly the biological barrier, implying that *I. uriae* prefer to feed on different seabirds rather than mammals including humans (56, 63, 64). We therefore conclude that the marine infection cycle instead influences the global spread and the heterogeneity among *B. garinii* strains. However, the exchange of strains with the terrestrial infection cycle as suggested in this review article overcomes the two described barriers, and new *B. garinii* strains are introduced in environments where also humans are at risk of acquiring the infection.

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***Sven Bergström**

Department of Molecular Biology
 Umeå University
 SE 90187 Umeå
 Sweden
 Email: Sven.Bergstrom@molbiol.umu.se