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Variable exposure and immunological response to Lyme disease *Borrelia* among North Atlantic seabird species

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Colonial seabirds often breed in large aggregations. These individuals can be exposed to parasitism by the tick *Ixodes uriae*, but little is known about the circulation of pathogens carried by this ectoparasite, including Lyme disease *Borrelia*. Here we investigated the prevalence of antibodies (Ab) against *Borrelia burgdorferi sensu lato* in seabird species sampled at eight locations across the North Atlantic. Using enzyme-linked immunosorbent assay tests, we found that the prevalence of anti-*Borrelia* Ab in adult seabirds was 39.6% on average (over 444 individuals), but that it varied among colonies and species. Common guillemots showed higher seroprevalence (77.1%$\pm$5.9) than black-legged kittiwakes (18.6%$\pm$6.7) and Atlantic puffins (22.6%$\pm$6.3). Immunoblot-banding patterns of positive individuals, reflecting the variability of *Borrelia* antigens against which Ab were produced, also differed among locations and species, and did not tightly match the prevalence of *Borrelia* phylogroups previously identified in ticks collected from the same host individuals. These results represent the first report of the widespread prevalence of Ab against *Borrelia* within an assemblage of seabird species and demonstrate that *Borrelia* is an integrated aspect in the interaction between seabirds and ticks. More detailed studies on the dynamics of *Borrelia* within and among seabird species at different spatial scales will now be required to better understand the implications of this interaction for seabird ecology and the epidemiology of Lyme disease.

Keywords: immuno-ecology; bird-borne disease; immunoblot; enzyme-linked immunosorbent assay; *Borrelia burgdorferi sensu lato*

1. INTRODUCTION

Recent outbreaks of avian influenza (Normile 2006; Olsen *et al.* 2006) and West Nile viruses (Rappole & Hubalek 2003; Gerhardt 2006) have highlighted the role that birds can play in the ecology of zoonotic diseases. They have also underlined the complexity of natural cycles of bird-borne (re-)emerging diseases; a multidisciplinary approach—ecology, epidemiology, host–parasite coevolution—is required in order to identify the underlying causes and control their spread (Daszak *et al.* 2000; Galvani 2003). Owing to their frequent infection by the members of the bacterial complex *Borrelia burgdorferi sensu lato*, there is a growing interest in the role played by birds as reservoir hosts to Lyme borreliosis (LB), the most common vector-borne zoonosis in temperate regions of the Northern Hemisphere. Indeed, Tsao *et al.* (2004) suggested that non-mouse hosts strongly participate in the natural cycle of LB and a number of studies have examined the reservoir competence of natural bird populations to Lyme disease bacteria (Kurtenbach *et al.* 1998; Richter *et al.* 2000; Gryczynska *et al.* 2002; Kaiser *et al.* 2002; Ginsberg *et al.* 2005). Several recent surveys have also investigated migratory land birds as potential long-distance dispersers of ticks and bacteria (Ishiguro *et al.* 2005; Comstedt *et al.* 2006; Poupon *et al.* 2006). Birds are thus not only confined to being a reservoir for LB but might also be key hosts affecting the spatial distribution and genetic structure of *B. burgdorferi s.l.*

Host diversity and host-associated selection driven by the immune system are likely to greatly affect the spatio-temporal structure of bird-borne zoonotic agents such as LB spirochetes (Kurtenbach *et al.* 2002). For example, high vertebrate species diversity in local patches has been suggested to decrease tick infection rates by *Borrelia* via a 'dilution effect' (Ostfeld & Keesing 2000; LoGiudice *et al.* 2003). Under this model, different vertebrate species have different reservoir capacities for *Borrelia* spp. Species with low reservoir competence act as hosts to the local tick vector and thus dilute the high reservoir competence of other local host species. Given the vast number of vertebrate species that may act as reservoirs to LB spirochetes (e.g. Pal & Fikrig 2003), a complete understanding of the epidemiology of this disease requires knowledge about the relative role of different local species in the maintenance, spread and divergence of this pathogen in natural populations.

More than a decade ago, Olsen *et al.* (1993, 1995) suggested a role for seabirds in a global transmission cycle of LB. Although most human cases of LB are connected to the terrestrial transmission cycle, infections through exposure to *Ixodes uriae*, a seabird tick, have been described (Gylfe *et al.* 1999). In addition, current

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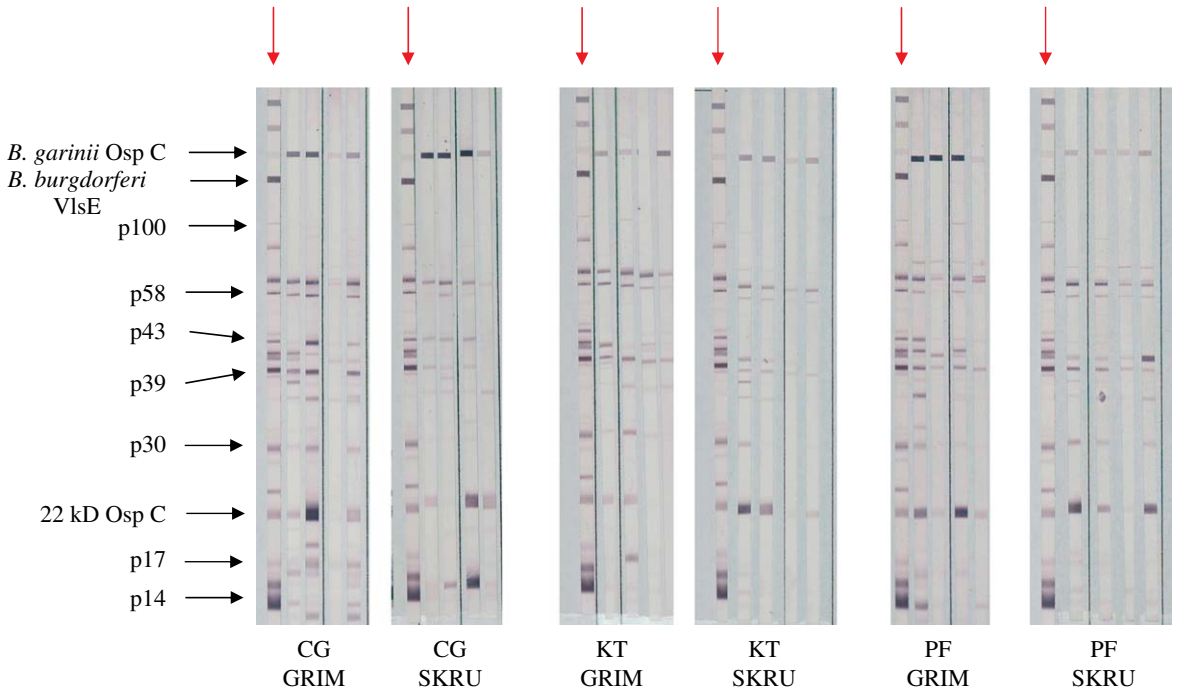

Figure 1. Immunoblot analyses of Ab to *B. burgdorferi s. l.* antigens in plasma sampled from the three studied seabirds species (CG, common guillemots; KT, black-legged kittiwakes; PF, Atlantic puffins) in two Icelandic colonies (GRIM, Grimsey; SKRU, Skrudur). Serum from a human patient with LB was used as a positive control (red arrows). The location of the selected *B. burgdorferi s. l.* antigens (*Borrelia garinii* Osp C, *B. b.* VlsE, p100, p58, p43, p39, p30, 22 kD Osp C, p17, p14) was determined using the positive control and the banding map provided with the kit.

information suggests an interaction between the two transmission cycles, although the nature and frequency of these exchanges have yet to be determined. Several studies reported the circulation of *Borrelia* spp. in different seabird species and in the tick *I. uriae*, the only known vector of *Borrelia* spp. in the marine cycle (Olsen *et al.* 1993, 1995; Bunikis *et al.* 1996; Gauthier-Clerc *et al.* 1999; Gylfe *et al.* 1999; Gasparini *et al.* 2001; Smith *et al.* 2006). *Ixodes uriae* is a common nest-dwelling ectoparasite of seabirds. During each of its three active stages, this tick takes a single, long blood meal (4–10 days; McCoy *et al.* 2002) on its bird host when the transmission of *Borrelia* (and other pathogenic agents) can occur. Although the ecology of *I. uriae* and its impact on bird populations start to be relatively well described (e.g. Boulinier *et al.* 2001; McCoy *et al.* 2002), little is known about the circulation of *Borrelia* spp. among tick populations and consequently the proportion of seabirds exposed to *Borrelia* among and within colonies. To better understand the mechanisms driving *B. burgdorferi s. l.* variability and the potential epidemiological role played by different seabird species, we analysed the prevalence of antibodies (Ab) against LB *Borrelia* spp. in three common North Atlantic seabird species in eight different colonies. Population genetic studies of the tick vector collected from different colonies and numerous seabird hosts have suggested that tick populations are structured in space and among host species within mixed colonies, forming distinct host races (McCoy *et al.* 2001, 2005). This structure could have important implications for the circulation of *Borrelia* spp. In particular, a recent study has shown that infected *I. uriae* are widely distributed across the North Atlantic, but that the prevalence and diversity of LB *Borrelia* spp. vary among tick host races both within and among colonies (Duneau *et al.* 2008). The potential role of seabirds in the maintenance of these patterns is unknown, but could be partially linked to interspecific variability in host competence to LB spirochetes in general or to certain species within the complex (Pal & Fikrig 2003). Here, we compare spatial and host-associated differences in seroprevalence and immunoblot-banding patterns to examine these aspects. We discuss our results with respect to their consequences for our understanding of the dynamics of host–parasite interactions and their epidemiological implications for the maintenance and diversification of LB spirochetes.

2. MATERIAL AND METHODS

(a) *Blood sampling*

Samples were collected from eight colonies across the North Atlantic. Approximately 30 adult birds of three seabird species (the black-legged kittiwake (KT) *Rissa tridactyla*, the Atlantic puffin (PF) *Fratercula arctica* and the common guillemot (CG) *Uria aalge*) were captured and sampled for blood at each location where possible. In Iceland, all three species were sampled in 2003 on the islands of Skrudur (64°54′ N, 13°38′ W) and Grimsey (66°33′ N, 18°00′ W; figures 1 and 2). In this country, we also sampled birds from the colonies of Hrolfsklettur (65°23′ N, 22°54′ W) and Latrabjarg (65°29′ N, 24°32′ W), but the species sampled at each location was variable (figure 2). Likewise, birds were sampled from three colonies in Scotland in 2001 (Fair Isle (59°32′ N, 01°39′ W), Sumburgh Head (59°51′ N, 01°16′ W) and Gruney (60°39′ N, 01°18′ W)), and one in Norway (Hornøya (70°22′ N, 31°10′ E)). At capture, a blood sample of 0.5 ml was taken from the left ulnar vein using a

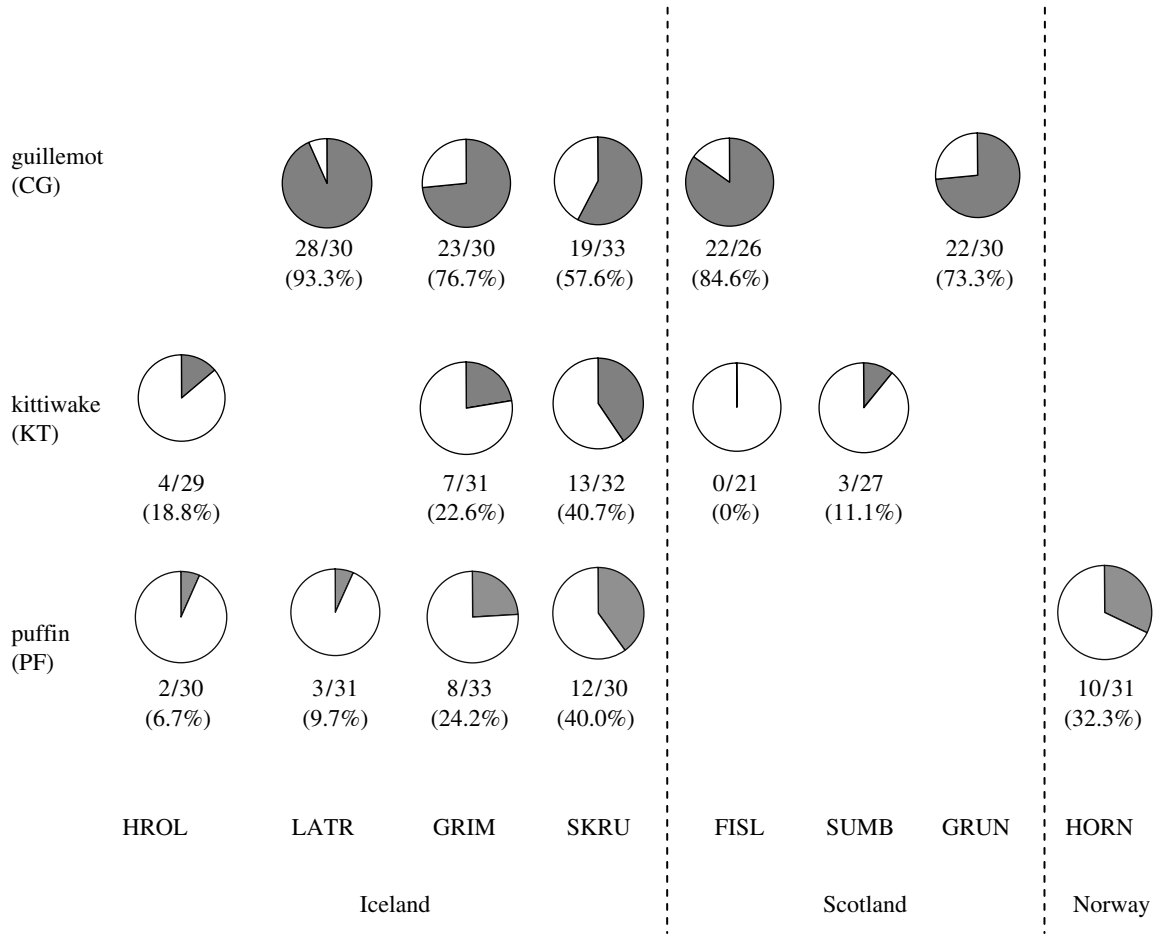

Figure 2. Prevalence of adult birds with anti-*Borrelia* Ab, according to species and colony (Hrolfskrettur (HROL), Latrabjarg (LATR), Grimsey (GRIM), Skrudur (SKRU), Fair Isle (FISL), Sumburgh Head (SUMB), Gruney (GRUN), and Hornøya (HORN)). Grey sections indicate the prevalence of seropositive individuals. The number and prevalence of positive individuals are indicated below each pie chart.

sterile syringe rinsed with heparin. Blood samples were stored in 1.5 ml tubes and centrifuged a few hours later. Plasma samples were then separated, kept cool until the sampling session was completed and then frozen at −20°C until immunological assays were performed.

(**b**) ***Serological analyses***

Anti-*Borrelia* Ab levels in the plasma were determined using a sandwich enzyme-linked immunosorbent assay (ELISA; kit Elilyme G/M, Diagast). Because this kit was manufactured for human use and was designed to recognize mammalian Ab, we replaced the anti-IgG Ab of the kit by an anti-chicken IgY Ab conjugated with peroxidase (Sigma A-9046, Sigma-Aldrich). Samples were diluted to 1 : 100 with the dilution buffer provided in the kit, the adequate dilution being determined by preliminary testing. One hundred microlitres of the diluted sample was then distributed into each well and incubated for 45 min at 37°C. After this period, the plates were washed three times with TBE buffer. One hundred microlitres of peroxidase-conjugated rabbit anti-chicken IgG (dilution 1 : 750 in TBE buffer) was added and left for 1h 30 min at room temperature. After washing, 100 µl of peroxidase substrate (*o*-phenylenediamine dihydrochloride, 0.4 mg ml^{-1}, Sigma) was added and left for 15 min at room temperature. The colorimetric reaction was stopped using 50 µl of hydrochloric acid (HCl 1 M). Anti-*Borrelia* Ab levels are expressed as the optic density (OD) of the resulting solution (absorbance at 492 nm, read using an automatic ELISA reader; Victor3, Perkin Elmer). The OD provided us with a relative measure of specific Ab concentration in the plasma samples. The repeatability, calculated according to Lessells & Boag (1987), of measurements on the same sample from two different ELISA plates was high (94.1%, $n=41$; $F_{40,41}=16.43$).

We used immunoblots (Western blots) to determine the positive threshold of the ELISA tests for each seabird species and to analyse the repertoire of *Borrelia* antigens to which individuals responded (figure 1). We performed immunoblots on samples from all three species on Grimsey and Skrudur. Tests were performed on individuals starting with the highest OD values and continuing down with lower values until negative samples were obtained. Next, we selected five samples from among the 10 lowest ELISA OD values to confirm the negative status of the individuals. This was done for all species except the common guillemot. For this species, the prevalence of positive individuals according to immunoblots was high even for low ELISA values; we consequently tested all guillemot individuals from Grimsey and Skrudur. Using the information from these two colonies, we then calculated the positive ELISA threshold for each species as the mean OD minus one standard deviation of all individuals shown positive by immunoblot tests. An individual was then considered positive if its OD was above this threshold. We used the results of the immunoblot test as a gold standard to evaluate the sensitivity (i.e. the probability that the test was positive given that the

Table 1. Immunoblot band frequencies according to species and colony. (We also indicate *Borrelia* phylogroup frequencies identified in ticks collected from different host species in the same colonies (Duneau *et al.* 2008).)

	species	CG		KT		PF	
bands (antigen)	site	GRIM	SKRU	GRIM	SKRU	GRIM	SKRU
B. garinii Osp C[a]		0.93	1.00	1.00	1.00	1.00	1.00
B. burgdorferi VlsE[b]		0.00	0.10	0.00	0.20	0.00	0.00
p100[c]		0.00	0.00	0.00	0.00	0.11	0.00
p58[d]		0.73	0.40	1.00	0.90	0.33	0.91
p43[e]		0.67	0.60	0.00	0.00	0.56	0.18
p39[f]		0.60	0.70	1.00	0.90	1.00	1.00
p30[a]		0.47	0.10	0.67	0.20	0.11	0.73
22 kD Osp C[a]		0.60	0.70	1.00	0.90	0.78	0.82
p17[a]		0.33	0.00	0.67	0.20	0.33	0.55
p14[g]		0.93	0.70	0.33	0.10	0.56	0.64
number of seropositive individuals		15/30	10/33	3/31	10/32	9/33	11/30
Borrelia phylogroup	group 1	0.37	0.16	0.25	0.40	0.00	0.14
frequencies	group 2	0.62	0.83	0.74	0.60	1.00	0.85

[a]Outer surface membrane protein; [b]Surface lipoprotein; [c]Protoplasmic cylinder or flagellum associated; [d]Function unknown; [e]Associated with infectivity; [f]Membrane associated; [g]Internal flagellin fragment.

bird actually had Ab, as shown by a positive immunoblot result) and the specificity (i.e. the probability that the test was negative given that the bird had no Ab) of the ELISA tests.

Immunoblot assays were performed using a commercial kit (Western Blot Lyme IgG+VlsE, Meridian Bioscience). These tests were based on the detection of Ab against the following *Borrelia* antigens: p14, p17, 22 kD Osp C, p30, p39, p 43, p58, p100, *Borrelia garinii* Osp C, *B. burgdorferi s. l.* VlsE (table 1). As this kit was also manufactured for human use, we replaced the anti-IgG of the kit by anti-chicken IgY Ab conjugated with alkaline phosphatase (Sigma A-9171, Sigma-Aldrich). Plasma samples were used at a dilution of 1 : 100 and tests were incubated for 45 min at room temperature. The positive control provided by the kit was used to determine band positions and as a reference for band intensity. The repeatability of measurements on the same sample from two different immunoblots was high (94.5%, $n=100$ (10 antigens for 10 samples); $F_{99,100}=35.05$).

We analysed immunoblot strips using the public domain ImageJ image program (US National Institutes of Health; http://rsb.info.nih/ij/) and obtained a value for the intensity of each band present. The intensity of the band reflects: (i) the incubation time and technical parameters of the immunoblot (controlled for by the systematic use of a human positive control as a reference), (ii) the concentration of the anti-chicken antibody (used in excess to avoid interspecific variation due to a differential affinity for the antibody; see §3), and (iii) the concentration of Ab specific to the antigen (band) considered. A sample was declared positive if its immunoblot revealed at least three bands more intense than the p41 band of the positive control, as recommended by the kit manufacturer (modified MIQ 12 2000 interpretative criteria; Wilske *et al.* 2000). Heterogeneity among *Borrelia* strains and the occurrence of cross-reacting Ab can complicate the comparability and standardization of assay systems (Hauser *et al.* 1997). However, given the criteria we used to declare a sample positive (i.e. minimum of three bands) and the fact that the same immunoblot kit was used for all species, we believe that the immunoblot results obtained are conservative and can be used for comparative purposes.

(c) Borrelia *phylogroup identification*

In order to relate the presence of plasma Ab to *Borrelia* infection of the local tick vectors, captured birds were sampled for ticks. Approximately 30 ticks from as many host birds were sampled in each colony for each species. Ticks were tested for *Borrelia* spp. infection using a nested PCR procedure for the amplification of a 309 bp region of the *FlaB* gene. Positive amplifications were used to determine the prevalence of *Borrelia*-infected ticks and the subsequent sequences were analysed using a phylogenetic approach to identify the different LB *Borrelia* spp. present in these areas (see details in Duneau *et al.* 2008). In general, we could not use the number of ticks collected from adult birds as an index of the local exposure to ticks, because levels of infestation of adult birds are highly variable and we are only able to detect a limited part of the tick population, mostly engorging females that have escaped preening.

(d) *Statistical analyses*

To investigate whether the prevalence of seropositive birds was affected by species or colony, we used logistic regression (proc catmod, SAS institute) with species and colony as independent variables. We tested the interaction colony× species for the colonies of Grimsey and Skrudur where information on all the three species was available. We then tested for a species effect in each colony separately and a colony effect for each species.

Differences in immunoblot patterns among species and colonies were examined using a principal component analysis (PCA) on the band intensity scores. After analysis of the Scree diagram (Quinn & Keough 2002), we kept only the first two principal components that explained 33.0 and 15.0% of the total variation. We then used a generalized linear model (proc GLM, SAS) with colony, species and the interaction as explanatory variables and the value of the first two PCA principal components as dependent variables. To determine whether immunoblot-banding patterns could be explained by the genetic diversity of *Borrelia* spp., we analysed the correlation between the mean values of each PCA axis and the frequencies of each phylogroup in each colony and species. We also performed the same analysis using immunoblot band frequencies.

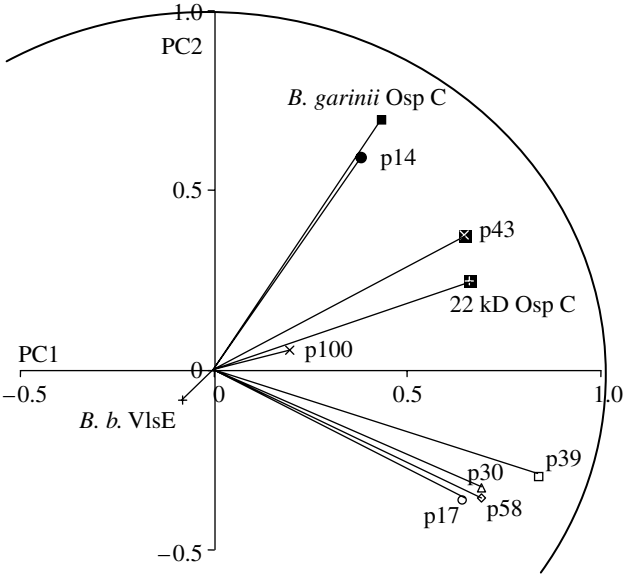

Figure 3. Correlation circle of the immunoblot-banding patterns of positive individuals obtained by a PCA of band intensity. The *x*- and *y*-axes correspond to the first two components (respectively 33.0 and 15.0% of the variance), the coordinates of each band (antigen) reflect their correlation with each axis. Lines join the different bands to the origin and the angle between two bands expresses the strength of the correlation between them (small angle= strong correlation). The length of each line to the origin symbolizes the degree to which a particular band is represented by the first two principal components (long= strong influence).

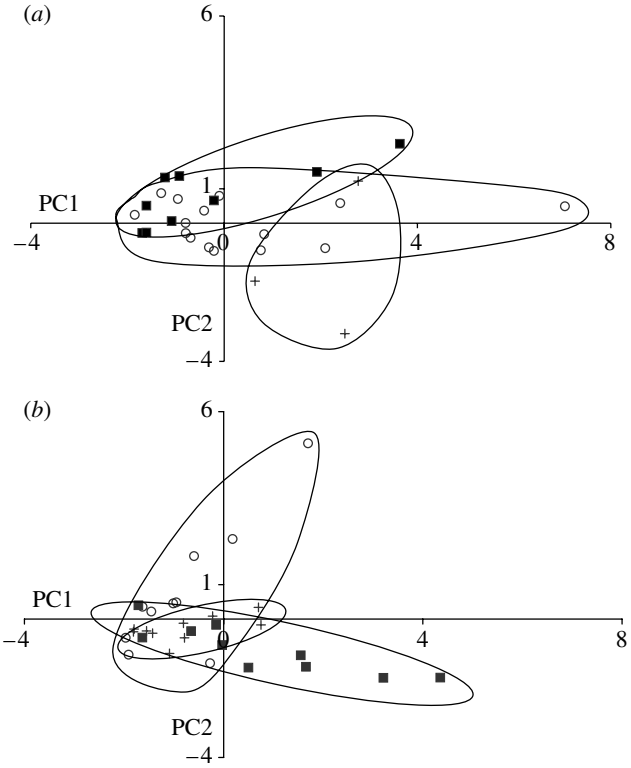

Figure 4. Species-specific immunoblot-banding patterns for the colonies of (*a*) Grimsey and (*b*) Skrudur (circles, CG; pluses, KT; squares, PF). The *x*-axis represents the first component and the *y*-axis, the second, and each point corresponds to an immunoblot-positive individual. This figure illustrates the significant effect of the species×colony interaction on the antigen response patterns.

3. RESULTS

(a) *Prevalence*

Positive thresholds for the ELISA tests (expressed as optical density; see §2) of the different species were set as 0.73 ($n=25$) for common guillemots (CG), 0.88 ($n=13$) for kittiwakes (KT) and 1.17 ($n=19$) for puffins (PF). Sensitivity and specificity were high for KT and PF (sensitivity/specificity: KT 92.3%/95.0%; PF 84.2%/92.8%), but the low number of negative immunoblots for CG (5/63) inevitably induced lower specificity for this species (sensitivity/specificity: 84.0%/60.0%).

The prevalence of Ab against *Borrelia* in adult seabirds was on average higher for CG compared with KT and PF (mean±s.e.: CG 77.1%±5.9, KT 18.6%±6.7, PF 22.6%±6.4; figure 2). There was nevertheless a significant effect of the interaction between colony and species on the prevalence of seropositive individuals ($\chi^2=6.07$, d.f.=2, $p=0.048$); prevalence differed significantly among species sampled on Grimsey, but not on Skrudur (Grimsey: $\chi^2=20.6$, d.f.=2, $p<0.0001$; Skrudur: $\chi^2=2.54$, d.f.=2, $p=0.27$). Prevalence also differed among colonies for CG and PF (CG: $\chi^2=10.7$, d.f.=4, $p=0.029$; PF: $\chi^2=12.1$, d.f.=4, $p=0.016$), but was marginally non-significant for KT ($\chi^2=8.93$, d.f.=4, $p=0.062$).

(b) *Immunoblot-banding patterns and* Borrelia *phylogroups*

The first principal component of the immunoblot-banding patterns reflects band intensity; all bands contribute to this axis approximately to the same extent as reflected by their *x*-values in figure 3. In other words, birds that responded strongly to one antigen had a tendency to respond strongly to the others. The second PCA axis reflects associations among certain antigens; p58, p39, p30, p17 formed one group, a second group was constituted by p14 and *B. gar* Osp C and a third by 22 kD Osp C and p43 (figure 3). The correlation among bands (especially p58, p39, p30 and p17) is also observable in terms of immunoblot band frequencies for each species and colony (table 1). The contribution of two bands, *B. b.* VlsE and p100, to the first two axes was low as reflected by the short length of the corresponding line on the correlation circle and can be attributed to the low frequency of birds with Ab against these antigens (figure 3; table 1). Differences in band frequencies (table 1) and results of the PCA illustrate the effects of colony and species on immunoblot-banding patterns (colony×species interaction on the first PCA component: $F_{2,51}=4.23$, $p=0.02$; on the second PCA component: $F_{2,51}=8.06$, $p=0.0009$; figures 1 and 4).

Phylogroup diversity of *Borrelia* detected in ticks was characterized by two main clades associated with two known *Borrelia* species, *B. garinii* (group 2) and *Borrelia lusitaniae* (group 1; phylogeny not shown; see Duneau *et al.* 2008). The majority of isolates were from group 2 (table 1). Most immunoblot-banding patterns could not be explained by variation in *Borrelia* phylogroups (Pearson's correlation coefficient; $-0.62<r<0.73$; $0.09<p<0.97$). As only two main clades could be distinguished using the *FlaB* gene, this result is not surprising. We would have required a strong effect to find statistical significance for more bands.

4. DISCUSSION

Serology, the detection of Ab against infectious agents, is a useful and sensitive tool because it enables one to determine both present and past exposures to pathogens. Moreover, serological studies allow us to take the vertebrate immune response into consideration when trying to understand the ecology of pathogen–host interactions (Frank 2002). In the *Borrelia*/tick/vertebrate host system, selection driven by the immune system of the different host species may play a key role in the spatio-temporal structure of Lyme disease (Kurtenbach *et al.* 2002). Here, we investigated the marine cycle of LB and explored the potential role played by three common North Atlantic seabird species in the ecology and epidemiology of *B. burgdorferi s.l.* using serological information from a large number of individuals. The qualitative results of the immunoblot tests allowed us to account for a differential affinity of the anti-immunoglobulin conjugate for the Ab of each species. We were then able to determine the percentage of seropositive individuals within different populations and species of the North Atlantic. We found that the prevalence of anti-*Borrelia* Ab in adult seabirds varied among colonies and among species within colonies. Overall, common guillemots showed higher seroprevalence than kittiwakes and puffins. We next examined the immunoblot-banding patterns in two colonies where all three seabird species were breeding sympatrically and found that the patterns also differed among locations and species. Finally, we tested for a correlation between immunoblot-banding frequencies, intensity and the presence of different *Borrelia* phylogroups previously determined in ticks sampled in these same two colonies. No strong relationship was found at the coarse scale considered.

(a) *Interspecific variability in ELISA thresholds*

Studies conducted on small mammals have highlighted interspecific variability in the prevalence of anti-*Borrelia* Ab. However, these studies used anti-mouse Ab to analyse Ab levels for all the species examined without considering the potential interspecific differences in the affinity of anti-mouse Ab for the Ab of each species. In addition, the same positive threshold value was used for all species, one based on the mean value of mouse controls (Kurtenbach *et al.* 1994; Pawelczyk & Sinski 2000; Vostal & Zakovska 2003; Stefancikova *et al.* 2004). This can be problematic because different affinities can bias comparisons of seroprevalence and these estimates are essential for evaluating the relative importance of host diversity for Lyme disease ecology (e.g. LoGiudice *et al.* 2003; Keesing *et al.* 2006).

In the present study, the use of a qualitative test (immunoblot) enabled us to define a separate positive threshold for each species for our quantitative test (ELISA), and consequently to obtain a more accurate estimate of seroprevalence in each population. Although potential variability in the affinity of anti-chicken IgY Ab for the Ab of the three seabird species could affect the global intensity of immunoblot bands, it should not alter the qualitative results of these tests (positive or negative) based on the presence/absence of bands. Indeed, the incubation of immunoblot strips was performed with a high concentration of the anti-IgY conjugate (5- to 10-fold the concentration used for the ELISA), and the positive/negative result of the immunoblot is not proportional to the quantity of the conjugate fixed by the Ab, as is the case for the ELISA test. This means that even if the Ab of certain species show a lower binding affinity, the excess conjugate should still reveal a positive result.

Overall, the sensitivity and specificity of the ELISA tests were reasonably good. However, we found that guillemots showed a somewhat lower specificity (60%) compared with the other two species. Although this could lead to an overestimate of seropositive individuals, this result is most probably linked to the low number of individuals with negative immunoblots (5/63 = 8%) in the two colonies where immunoblots were performed on all samples (Grimsey and Skrudur). Few negative individuals reduce the precision of estimates and thus can reduce the specificity of the test. However, the low number of negative tests from these populations also suggests that the risk of false positives is probably low and therefore that the significantly higher seroprevalence found in this species is not simply due to the low specificity of the test.

(b) *Interspecific variability in seroprevalence*

Seroprevalence levels were higher on average for the guillemots compared with the kittiwakes or puffins. This could be explained by the recurrent nature of host race formation in the tick vector *I. uriae* across its range, coupled with a tendency for different tick races to host *Borrelia* to different degrees (McCoy *et al.* 2001, 2005; Duneau *et al.* 2008). Likewise, the dynamics of exposure to ticks may differ according to the seabird species; some birds nest in high-density breeding areas (guillemots) compared with others (lower densities of individual burrows for puffins or nests for kittiwakes). Host-related variability in exposure to ticks may then affect exposure to *Borrelia* spp. In a previous study, the prevalence and diversity of *Borrelia* spirochetes were examined in ticks from the same seabird species at the same series of locations (Duneau *et al.* 2008). Interestingly, this study revealed a similar prevalence of LB *Borrelia* spp. in ticks sampled from the different seabird species (puffin ticks 28.9%, kittiwake ticks 27.3%, guillemot ticks 26.0% in the colonies of Skrudur and Grimsey). This contrasts with the higher seroprevalence level we found for guillemots compared with the other species and suggests either host-associated variability in tick exposure or re-exposure, or a differential capacity among species to mount and maintain an immune response. Indeed, using experimental infections, Kurtenbach *et al.* (1994) showed different transmission rates of *Borrelia* to different small mammal species via ticks. Likewise, Nunn *et al.* (2006) found that only guillemots had neutralizing Ab against Great Island virus, even though both guillemots and kittiwakes were infested by the infected ticks. Owing to the nesting characteristics of guillemots, this species may have evolved a more efficient capacity to control parasite attacks via its immune response (Pal & Fikrig 2003; Attie *et al.* 2007).

Interannual environmental variation, for example in resource availability, could also differentially affect the different seabird species (Sandvik *et al.* 2005) and may alter the dynamics of disease cycles via effects on host immunity and parasitaemia (Gylfe *et al.* 2000). A longitudinal study of the dynamics of antibody levels against LB *Borrelia* spp. in breeding adult kittiwakes

nevertheless has shown that antibody levels and immunoblot profiles are highly persistent between successive years (Staszewski *et al.* 2007). Experimental manipulation of the immune system of the different hosts (e.g. immunosuppression) and controlled pathogen exposure could thus complement our understanding of the role of the immune system in the interspecific variability observed and the potential effect of environmental variability on this response.

(c) *Colony-associated variability in seroprevalence*

The spatial dimension of host–parasite interactions has been relatively neglected until recently (Thomas *et al.* 2005), probably because traditional parasitology has focused more on detailed and/or mechanistic studies. However, spatial variability is a key aspect required for predicting exposure to zoonotic diseases (Randolph *et al.* 2002). For example, in domestic animals such as goats and sheep, the proportion of seropositive individuals against *B. burgdorferi s.l.* is approximately 20% across various countries (see Travnicek *et al.* 2002 for review), but can range from 5 to 62% according to habitat type (mountains versus plains). Similarly, a variety of studies on small mammals have found that natural seropositivity against *B. burgdorferi s.l.* varies according to location (for example, from 0 to 20% among regions; Stefancikova *et al.* 2004). In our study, seroprevalence varied according to colony, ranging from 57.6 to 93.3% for guillemots, 0 to 41% for kittiwakes and 6.7 to 40.0% for puffins. These results show that LB spirochetes are widespread and that spatial variation in prevalence exists. In general, these patterns could be linked to heterogeneity in bird populations with respect to parasite exposure, susceptibility and/or parasite strain variability. In the case of *Borrelia* spp. in the marine system, the latter hypothesis is not supported based on our current findings as there was no clear indication of a link between the immunological profiles and the presence of different *Borrelia* phylogroups within populations. However, we are currently conducting more detailed genetic analyses on *B. garinii* isolates, this group being one of the most heterogeneous of all Lyme disease spirochetes; these data may help reveal a potential link between the strain variability and the observed variability in the immune response of the birds.

(d) *Species- and colony-associated variability in immunoblot patterns*

The immunoblot tests enabled us to partially characterize the immunological profile of the *Borrelia* antigens against which Ab were produced (Hauser *et al.* 1998; Pachner *et al.* 2002). Species- and colony-associated variability in seroprevalence was mirrored by immunoblot-banding patterns, where heterogeneity in the global intensity of the bands (first component) and in the antigens targeted by Ab (second component) differed among seabird species and colonies. The factors that might affect exposure to *Borrelia* spp. or the ability to mount an immune response have been discussed above to explain spatial and host heterogeneity in seroprevalence. The same factors are likely to affect the repertoire of antigens against which Ab are produced. This repertoire depends mainly on two factors: the history of past antigen exposure and the response elicited within the host to different antigens. The repertoire of antigens against which Ab have been produced and the factors likely to affect it are important to consider because they will determine the selective pressures imposed on parasites and thus will shape the changing patterns of *Borrelia* spp. within populations (Frank 2002). More detailed tests that consider a wider range of populations could enable us to test this hypothesis and to clarify the potential links between different specific Ab and variability in *Borrelia* spp. isolates.

Here we have shown that the prevalence and the repertoire of anti-*Borrelia* Ab in positive individuals vary among seabird species and locations within the North Atlantic. These results can have important implications for both the global epidemiology of LB and the evolution of these spirochetes. In particular, the host specificity of the tick vector and the different life-history characteristics of the diverse seabird species involved may result in different transmission patterns at different spatial scales. Similarly, these aspects could affect the selection patterns generating novel genetic variants of the pathogen that could then be transferred to terrestrial disease cycles. Understanding the role of birds and the marine LB cycle in the maintenance and periodic (re)-emergence of *Borrelia* spp. will ultimately rely on the effective combination of phylogenetic analyses of pathogen diversity with more ecological approaches that consider the potential role of different avian hosts.

All work with seabirds was made in accordance with standard animal care protocols and approved by the Ethical Committee of the French Polar Institute (CREEA Midi-Pyrénées). In the UK, Iceland and Norway, sampling was conducted under licences from the Home Office, the Icelandic Institute of Natural History and the Norwegian Animal Research Authority.

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