Ecology, 88(12), 2007, pp. 3183–3191

INTERANNUAL DYNAMICS OF ANTIBODY LEVELS IN NATURALLY INFECTED LONG-LIVED COLONIAL BIRDS

VINCENT STASZEWSKI,[1,4,5] KAREN D. MCCOY,[2] TORKILD TVERAA,[3] AND THIERRY BOULINIER[4]

[1]*Laboratoire d'Ecologie, UMR 7625 CNRS-UPMC-ENS, Paris, France*
[2]*Génétique et Evolution des Maladies Infectieuses, CNRS/IRD - UMR 2724, Montpellier, France*
[3]*Division of Arctic Ecology, NINA, Tromsø, Norway*
[4]*Centre d'Ecologie Fonctionnelle et Evolutive, CNRS-UMR 5175, 1919 Route de Mende, F 34293 Montpellier, France*

Abstract. Little is known about the long-term persistence of specific antibodies (Ab) in natural host–parasite systems despite its potential epidemiological and ecological importance. In long-lived species, knowledge of the dynamics of individual immunological profiles can be important not only for interpreting serology results, but also for assessing transmission dynamics and the potential selective pressures acting on parasites. The aim of this paper was to investigate temporal variation in levels of specific Ab against the bacterium *Borrelia burgdorferi* sensu lato in adults of a long-lived colonial seabird, the Black-legged Kittiwake *Rissa tridactyla*. In wild populations, adults are naturally exposed each breeding season to a *Borrelia* vector, the tick *Ixodes uriae*. Breeding birds were captured during four consecutive breeding seasons, and parasite infestation quantified. Using enzyme-linked immunosorbent assay (ELISA) and immunoblots, we found that the immunological profiles of anti-*Borrelia* Ab were highly repeatable among years, reflecting the interannual persistence of Ab levels. We nevertheless also observed that year-to-year changes of Ab levels were related to exposure to ticks in the previous year. The long-term persistence of Ab levels may be an important mechanism of individual protection against future exposure to the microparasite. It will also affect the availability of susceptible hosts, and thus the transmission dynamics of the bacterium. These results illustrate the need to consider the dynamics of the immune response in order to better understand the evolutionary ecology of host–parasite interactions in natural populations.

Key words: Black-legged Kittiwake; Borrelia burgdorferi *sensu lato; colonial seabirds; eco-epidemiology; host–parasite interactions; immunoglobulins;* Ixodes uriae; *maternal antibodies;* Rissa tridactyla.

INTRODUCTION

The quantification of immunocompetence has attracted much attention in the context of sexual selection and life history trade-offs (Sheldon and Verhulst 1996, Norris and Evans 2000), but common approaches using exposure to artificial antigens have been criticized because they do not address how the immune response would have affected the outcome of natural host–parasite interactions (Hudson et al. 2002, Staszewski and Boulinier 2004, Viney et al. 2005). In particular, the protective role of antibodies (Ab) against specific natural parasites is rarely considered, nor the temporal dynamics of Ab under natural parasite exposure. For the most part, knowledge of immunological profiles during infection come from studies of human or model laboratory animals (Frank 2002). Very little is known about the dynamics of the immune response in natural populations of wild vertebrates, even when it concerns human pathogens of major epidemiological and ecological importance (Grenfell and Dobson 1995, Bunikis et al. 2004, Gibbs et al. 2005).

Because the risk of parasite exposure often follows a seasonal cycle (Altizer et al. 2006) and can vary geographically, the persistence of Ab levels could be a mechanism by which hosts rapidly and efficiently combat a predictable re-exposure to a pathogen. In the case of migratory species that are relatively faithful to their breeding site, individual immunity at an interannual scale could be particularly important with regard to life history decisions such as where and when to breed (Boulinier et al. 2001), but also to understand the propagation of parasites at different spatial scales (Comstedt et al. 2006). The profile of circulating Ab at the time of breeding also will directly affect the maternal transmission of Ab to offspring, which could procure passive protection to young against local parasites (Gasparini et al. 2001, Grindstaff et al. 2003) and affect the rate at which susceptible hosts enter the population (Frank 2002).

Colonial breeding bird species provide ideal models to investigate the interannual dynamics of immunological

Manuscript received 18 January 2007; revised 2 April 2007; accepted 20 April 2007. Corresponding Editor: K. Wilson.
[5] Address for correspondence: Evolutionary Research, Avian Research Group, Department of Biological and Environmental Science, P.O. Box 35, FIN-40014, University of Jyväskylä, Jyväskylä, Finland. E-mail: vistasze@cc.jyu.fi

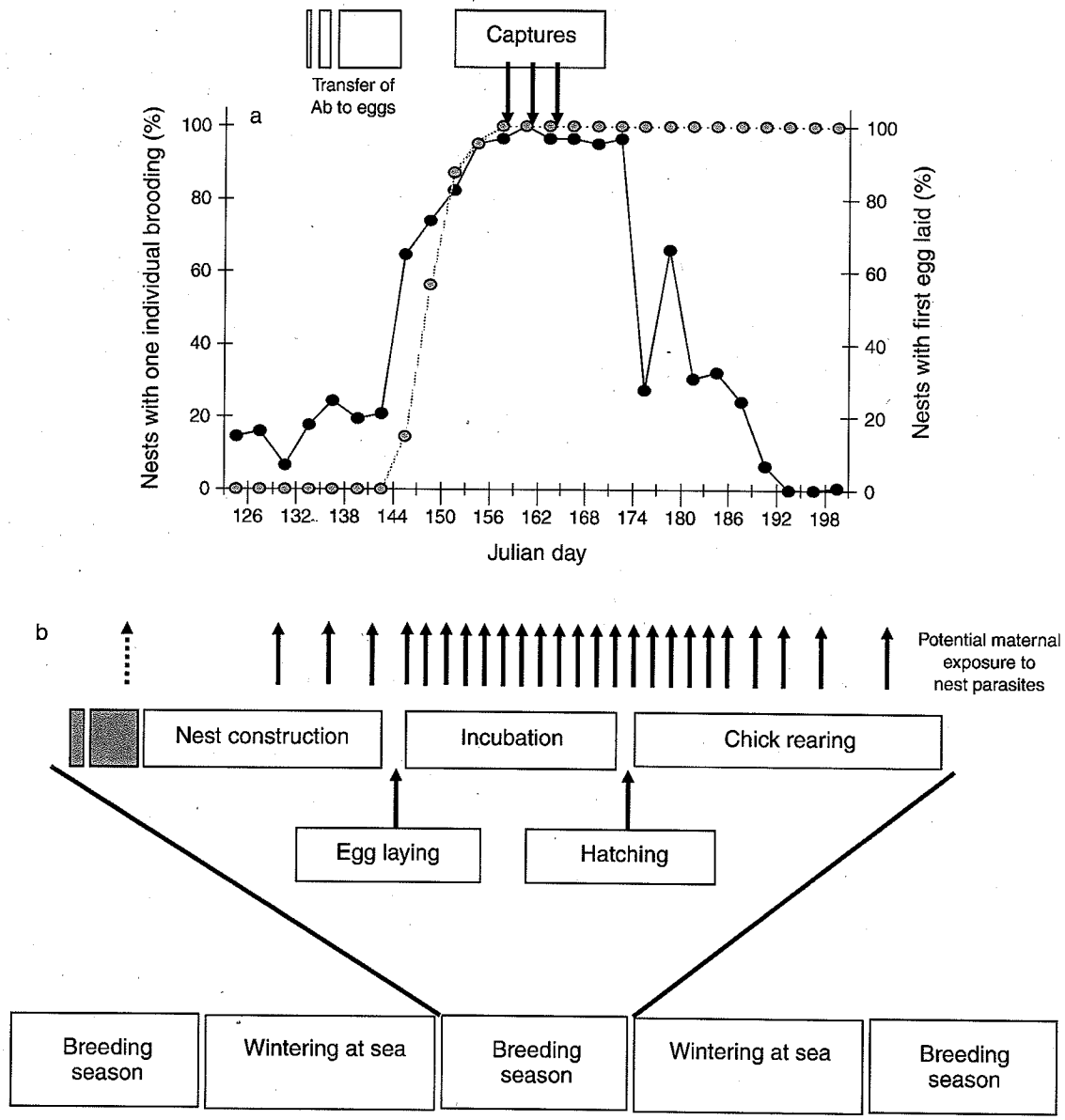

FIG 1. Time frame of the interaction between the parasite and the host immune system for the Black-legged Kittiwake *Rissa tridactyla*. (a) Black circles represent the percentage of nests with a brooding individual among nests where at least one egg will be laid during the season, and gray circles represent the percentage of nests with a first egg laid (data are from 2005, but the same pattern is observed every year). Julian day 1 is 1 January. Here, brooding means sitting on a nest. Adult birds were captured during incubation. (b) Because maternal exposure to nest parasites (general pattern indicated by solid arrows) is probably negligible before egg laying, the transfer of maternal antibodies (Ab) to the eggs occurs before peak exposure to nest parasites. The immunological memory targeted against nest parasites and acquired during incubation and chick rearing in one breeding season may therefore be key for the transfer of adequate Ab to the chicks in the subsequent season. At the left, the gray boxes and dotted arrow indicate potential, but rare, exposure to parasites for birds that begin nest construction ahead of the main period.

profiles as they are often exposed to high densities of nest parasites and individual birds can be readily recaptured during successive breeding attempts (e.g., Boulinier et al. 1997). Because exposure to nest parasites is likely to occur mainly after egg laying, the persistence of antibodies targeted against nest parasites or the microparasites they vector and acquired during a previous breeding season might be necessary for the transfer of an adequate amount of Ab to the chicks of these species (Fig. 1). Long-term persistence of specific IgY (the avian equivalent of IgG in mammals) can thus be expected and might be due to antigen-dependent or antigen-independent mechanisms (Gatto et al. 2006). The three-level host–parasite system constituted by seabirds, the tick *Ixodes uriae*, and Lyme disease bacterium *Borrelia burgdorferi* sensu lato, is especially

suitable to address these issues because immunological tools are available to track specific anti-*Borrelia* Ab (Connolly and Benach 2005). Lyme borreliosis is the most common vector-borne zoonosis in temperate regions of the Northern Hemisphere and the presence of its agent in seabird populations has been shown in both hemispheres (Olsen et al. 1995). The investigation of Ab dynamics in seabirds therefore may also shed some light on how this bacterium is maintained and spread in this marine enzootic cycle.

The aim of this paper was thus to investigate interannual variation in the level of specific Ab against a naturally prevalent microparasite in adults of a long-lived colonial seabird, the Black-legged Kittiwake *Rissa tridactyla*. We investigated the persistence of anti-*Borrelia* Ab by examining the level of plasma Ab in breeding kittiwakes captured over four successive years. Because exposure to the tick vector is temporally autocorrelated at a given breeding site (McCoy et al. 1999, Gasparini et al. 2001) and because kittiwakes are relatively faithful to their nests from one season to the next (Boulinier et al. 2001), we expected exposure to *Borrelia*, and consequently IgY Ab levels, to be correlated between years. To test this prediction, we investigated the repeatability of individual anti-*Borrelia* Ab profiles using immunological assays (enzyme-linked immunosorbent assay [ELISA] and immunoblots). We also examined whether year-to-year changes in Ab levels were related to past or current exposure to the tick vector. We discuss our results with respect to their consequences for the ecological dynamics of host–parasite interactions along with their possible epidemiological implications.

Methods

Study area and biological model

Fieldwork was conducted over four breeding seasons (2003–2006) on Hornøya, an island in northern Norway (70°22′ N, 31°10′ E) where more than 10 000 pairs of kittiwakes breed (Anker-Nilssen et al. 2000). Kittiwakes, like many colonial seabirds, are long-lived and show strong interannual breeding site fidelity (Boulinier et al. 2001). This can result in the repeated exposure of individuals over the course of their lifetime to parasites present in the breeding area, such as nest-dwelling ectoparasites and the microparasites they vector (Gasparini et al. 2001). Indeed, it has previously been found that kittiwake exposure to ectoparasites, such as the tick *Ixodes uriae*, is repeatable between breeding seasons and is spatially structured within and among colonies (McCoy et al. 1999, Gasparini et al. 2001).

The tick *Ixodes uriae* parasitizes several seabird species and has a life cycle lasting 2–4 years, depending on host phenology and local climatic conditions (Barton et al. 1996). This tick has been shown to vector several microparasites, including the bacterium responsible for Lyme borreliosis, *Borrelia burgdorferi* sensu lato (Olsen et al. 1995). This tick takes its annual blood meal on the bird for a continuous period of 3–12 days depending on the life stage (Barton et al. 1996, McCoy et al. 2002). Outside the breeding season, the birds are at sea. During this time, ticks live in the cracks of the cliff surrounding the empty breeding nests. Local levels of infestation in the breeding areas can be assessed by counting ticks on nestlings.

Blood sampling and tick infestation levels

Breeding birds were caught on their nests in a series of subcolonies during the incubation period, late May to mid June (Fig. 1). At first capture, individuals were ringed with a metal ring and a combination of five color rings that allowed us to identify birds from a distance before recapture in subsequent years. At each capture, a blood sample of 0.5 mL was taken from the left ulnar vein using a sterile syringe rinsed with heparin. Blood samples were stored in 1.5-mL tubes and kept cool until centrifugation a few hours later. Plasma samples were separated and kept frozen at −20°C until immunological assays. For this study, we captured 56 breeding birds in 2003, 102 in 2004, 86 in 2005, and 57 in 2006. The number of birds recaptured from previous year was 13 in 2004, 20 in 2005, and 19 in 2006. Except in Fig. 3, only birds captured at least twice (i.e., in a minimum of two consecutive years) are taken into account in the analyses.

On Hornøya, ticks are difficult to find on adults during incubation, so the number of ticks feeding on chicks is used as an index of local infestation (Gasparini et al. 2001). To assess the local level of infestation by the tick, the number of feeding ticks was counted by a thorough examination, consisting of a visual inspection while palpating the skin (McCoy et al. 2002). Because some nests encountered reproductive failures before infestation levels could be quantified, we used the mean number of ticks per chick in the five closest nests as an index of local exposure, as spatial autocorrelation of parasite exposure has previously been found in this system (McCoy et al. 1999, Gasparini et al. 2001).

Immunological assays

Anti-*Borrelia* Ab titers in the plasma were determined using a blocking enzyme-linked immunosorbent assay (Enzygnost Borreliosis ELISA Kit, Dabe Behring, Marburg, Germany). 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 (Sigma A-9046 Ab, Sigma-Aldrich, St. Louis, Missouri, USA). Anti-*Borrelia* Ab levels are expressed as the optical density (OD) of the resulting solution (wavelength of 492 nm in a spectrophotometer). The OD provides us with a relative measure of specific Ab concentration in the plasma samples. The repeatability of measurements on the same sample from two different ELISA-plates was high (96.8%, $F_{17,18} = 84.4$).

We used immunoblots (Western blots) to confirm that high OD values meant that the individuals had been

exposed to antigens of *Borrelia burgdorferi* sensu lato (Wilske 2003). The immunoblots also enabled us to characterize the immunological profile of the *Borrelia* antigens against which Ab were produced (Hauser et al. 1998, Pachner et al. 2002) and, consequently, to investigate the repeatability of banding patterns between years (10 seropositive individuals sampled in 2005 and 2006). The immunoblot assays were performed using a commercial kit (Western Blot Lyme IgG + VlsE, Meridian Bioscience Europe, Nice, France). These tests were based on the detection of Ab against the following *Borrelia* antigens: p14, p17, 22kD OspC, p30, p39, p 43, p58, p100, *B. garinii* OspC, *B. burgdorferi* s.l. VlsE (Fig. 2). 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-Aldrich, St. Louis, Missouri, USA). Plasma samples were used at a dilution of 1:100 and were incubated for 45 minutes at room temperature. We analyzed the gels using the public domain ImageJ image program (U.S. National Institutes of Health; *available online*).[6]

Statistical analyses

To investigate the effect of year, capture date, and subcolony on Ab levels, we used a mixed model (SAS Proc Mixed; SAS Institute 1999) with three fixed factors (year, capture date, and subcolony) and an individual random effect to account for repeated measures on the same birds in consecutive years. To test for an overall effect of tick exposure on the between-year change of individual Ab levels, we compared the mean yearly change in Ab levels to the mean tick infestation level the year before ($n = 3$ years). To further investigate the factors affecting the interannual variation of anti-*Borrelia* Ab, we used individual Ab levels in year t (2004–2006) as a dependent variable and, as fixed effects, the year of recapture, as well as local infestation levels in year t, local infestation levels in year $t - 1$ (2003–2005), and the individual Ab levels in year $t - 1$ (and their interactions, which were removed if not significant). For the six individuals captured in more than two consecutive years, we kept only the first two capture events.

To determine the repeatability of banding patterns obtained with the immunoblots strips, we used similarity coefficients. Because the absence of bands should not contribute to similarity in the Ab profiles, we used coefficients that exclude negative co-occurrences from the similarity measure (Fromin et al. 2002). The Jaccard coefficient and Sorensen-Dice coefficient were used for the presence–absence analysis and the Steinhaus coefficient was used to take into consideration the intensity of each band. We determined the repeatability of profiles by comparing the mean similarity coefficient for individuals between the two years with the average pairwise similarity of all potential profile pairs. All computations were performed with R and SAS (R Development Core Team 2005; SAS Institute 1999).

[6] ⟨http://rsb.info.nih.gov/ij/⟩

Results

Mean Ab levels of recaptured birds varied among years and individuals (year $F_{3,55} = 9.80$, $P < 0.0001$; OD $= 0.23 \pm 0.05$ [individual $\beta \pm$ SE], $P < 0.0001$; Fig. 3). There was no effect of capture date (after accounting for a subcolony effect) on mean Ab level (capture date $F_{1,57} = 1.30$, $P = 0.25$; subcolony $F_{11,57} = 4.04$, $P = 0.0002$). Overall changes in mean Ab levels were correlated with tick prevalence in the previous year; a heavy tick infestation the year before was associated with a mean increase in Ab level (Fig. 4). As predicted, there was a significant effect of the individual Ab level in year $t - 1$ (2003–2005) (Fig. 5a; OD $= 0.72 \pm 0.048$ [$\beta \pm$ SE], $n = 47$, $P < 0.0001$), of local exposure to ticks in year $t - 1$ (2003–2005) (Fig. 5b; OD $= 0.04 \pm 0.01$ [$\beta \pm$ SE], $n = 47$, $P = 0.0006$), and of year ($F_{2,42} = 4.79$, $P = 0.013$) on the individual Ab level in year t (2004–2006). Interestingly, the effect of local exposure to ticks in year t was not significant (OD $= -0.0014 \pm 0.0014$ [$\beta \pm$ SE], $n = 41$, $P = 0.33$). The slope of the relationship between individual Ab levels in year t and parasite exposure in year $t - 1$ were not significantly different among years ($F_{2,40} = 2.59$, $P = 0.087$). This may be due to low infestation levels in 2004 and 2005 (Fig. 5b).

The immunological profiles of individuals were highly repeatable, as indicated by the comparison of immunoblot banding patterns for the same individuals in 2005 and 2006 (Fig. 2). The repeatability was high both in terms of presence–absence (comparison of similarity index with average similarity index: $n = 10$ pairs; Mann-Whitney U test: Sorensen-Dice Pr $> Z = 0.0047$, Jaccard Pr $> Z = 0.0047$) and in terms of band intensity (Steinhaus coefficient Pr $> Z = 0.0016$). The most prevalent immunoblot bands corresponded to highly specific antigens of *Borrelia burgdorferi* s.l.: p58, *B. garinii* OspC and p39 (Hauser et al. 1998). The comparison of the immunoblots with Ab levels showed that individuals with high OD values for the ELISA (>0.62) had IgY Ab against at least three of the diagnostic *Borrelia* antigens, the criterion for positive diagnosis in humans (Wilske 2003).

Discussion

In spite of the numerous ecological and epidemiological studies of host–parasite interactions, long-term investigations of the dynamics of immunity in natural populations are still scarce (Grenfell and Dobson 1995, Mills et al. 1999, Bunikis et al. 2004). In most studies involving the quantification of circulating Ab in natural systems, the dynamics of Ab levels among years is not explored (Hudson et al. 2002), although the occurrence of a long-term persistence of antibodies after controlled antigen exposure is well known (for a review, see Gatto et al. 2006). Knowledge about the interannual dynamics

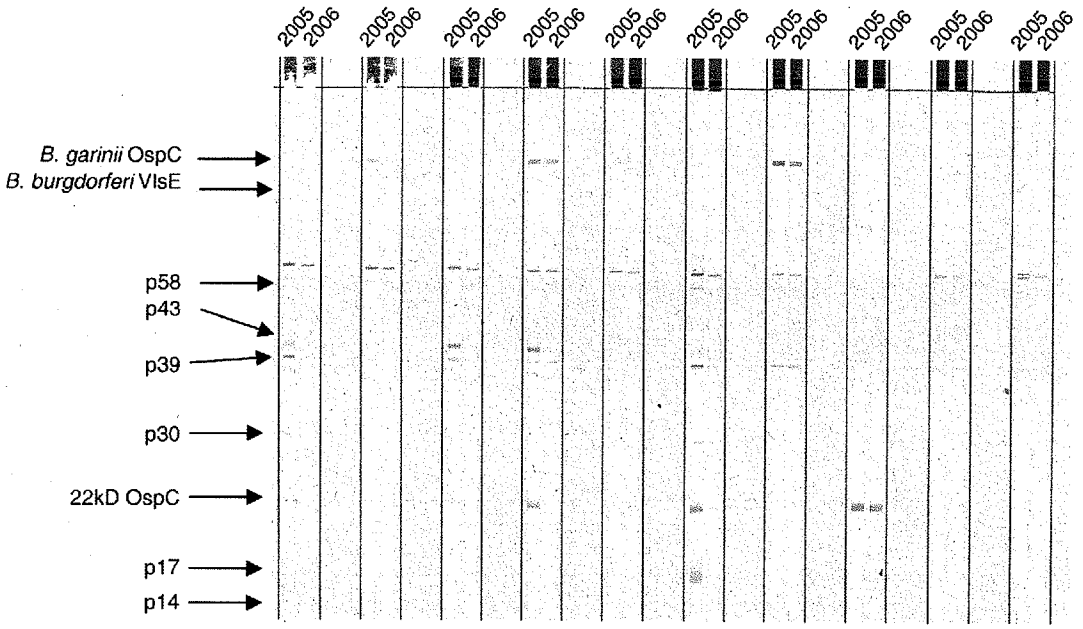

FIG. 2. Immunoblot analyses of antibodies to *Borrelia burgdorferi* sensu lato antigens in plasma sampled from birds captured in 2005 and recaptured in 2006. Serum from a human patient with Lyme borreliosis was used as a positive control. The location of the selected *B. burgdorferi* s.l. antigens (*B. garinii* OspC, B. b. VlsE, p58, p43, p39, p30, 22Kd OspC, p17, p14) was determined using the positive control and banding map provided with the kit (Western Blot Lyme IgG + VlsE, Meridian Bioscience Europe, Nice, France).

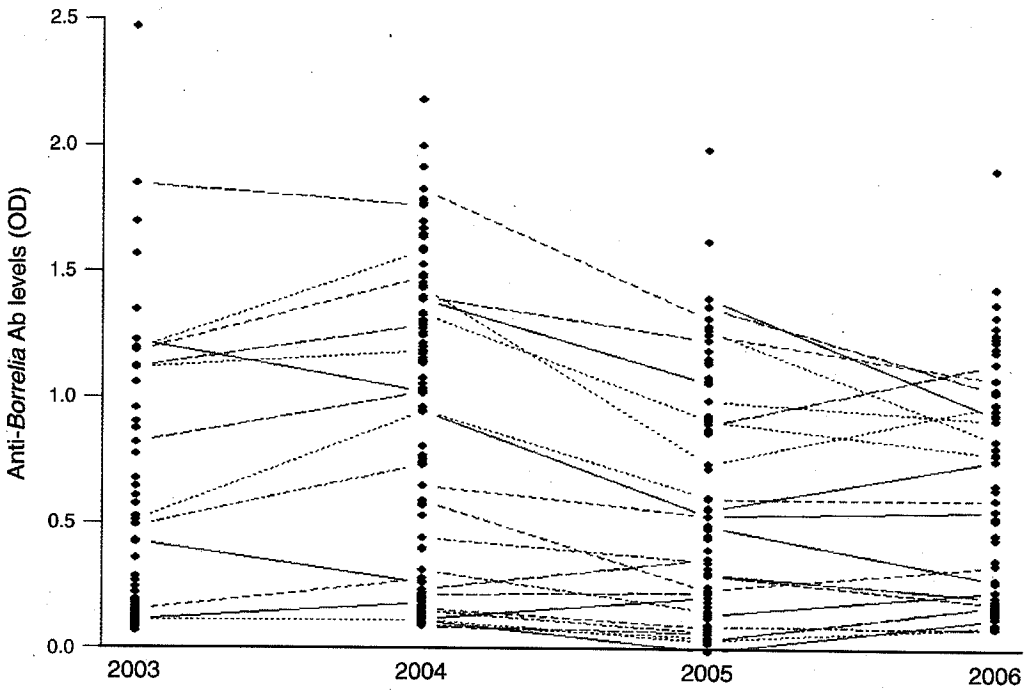

FIG. 3. Anti-*Borrelia* antibody levels expressed as optical density (OD), by year. All captured individual kittiwakes are shown; different line types connect two consecutive values for one individual. Optical density was calculated as $\log_{10}(1/\text{transmittance})$ at 492 nm in a spectrophotometer; low OD values indicate low antibody titer. Optical density values multiplied by 10 give transmission loss in decibels; e.g., an OD value of 0.5 is 5 dB.

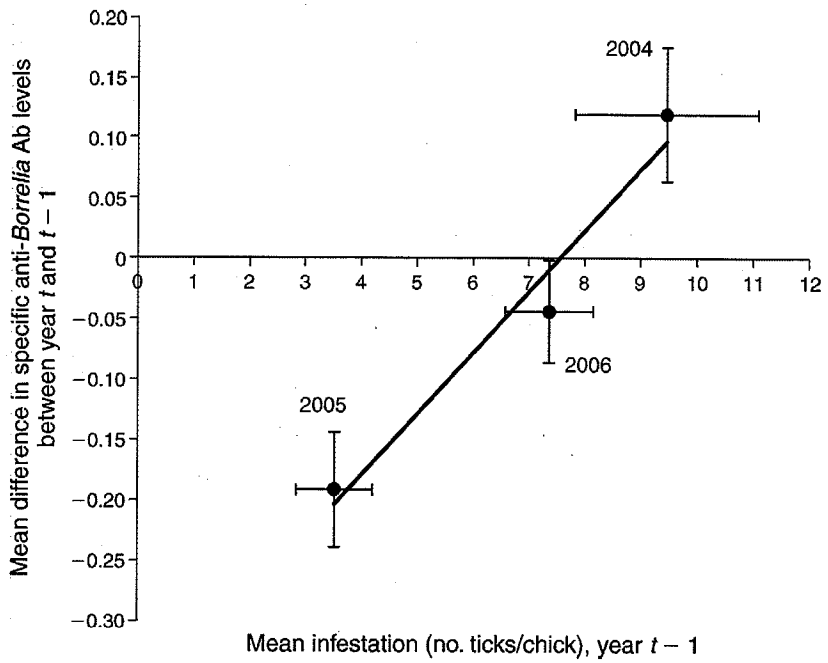

FIG. 4. Relationship between the mean interannual change in Ab levels (measured as the difference in OD between years) and mean infestation levels by ticks in the previous year (for all chicks measured in the year). Each point represents the mean ± SE for a year (2004–2006).

of immunity is nevertheless required to understand the strategies followed by long-lived species and because host immunity can affect the spatial and temporal dynamics of disease agents in natural populations. Studies of natural populations suggest potential differences in the serological dynamics of species with different life spans (long- vs. short-lived) and among different host–parasite systems, but few data are currently available, and most studies involve mammals. For example, Feuer et al. (1999) observed a good repeatability of anti-Sin Nombre Virus levels in deer mice when individuals were recaptured at a one-month interval. The maintenance of antibodies for the expected life span (approximately one year) of rodents has been observed for specific anti-Hantavirus Ab (Mills et al. 1999, Kuenzi et al. 2005) and for anti-*Borrelia* Ab (Hofmeister et al. 1999, Bunkis et al. 2004). Although studies of longer-lived species also tend to show relatively high repeatabilities in specific Ab levels, some variation exists. Seventeen percent of rabbits infected by rabbit hemorrhagic disease virus revealed declining titers over a three-year period (Henning et al. 2006). Semidomesticated reindeer also showed relatively high individual repeatabilities in specific Ab levels after repeated exposures to warble flies (Asbakk et al. 2005), but the persistence of Ab levels declined in older animals. In contrast, a survey of macaque populations naturally infected by West Nile virus illustrated high individual variation in Ab persistence, ranging from complete stability to major interseasonal variation (Hukkanen et al. 2006). In the present study, we found that the immunological profiles of individual birds were highly repeatable between years for specific anti-*Borrelia* Ab, but that when changes in Ab levels occurred between years, they tended to be linked to exposure to the ectoparasite vector the year before.

In the considered system, where birds are only exposed to the *Borrelia* vector during the breeding season (Fig. 1), there are two main hypotheses that may explain the interannual repeatability of Ab levels. First, there may be a persistence of Ab levels independent of external antigen exposure (i.e., without actual re-exposure to *Borrelia burgdorferi* sensu lato). In this case, the maintenance of high levels of anti-*Borrelia* Ab could be due either to antigen-independent activation of memory B cells (Bernasconi et al. 2002) or to antigen persistence (persistent infections and/or immune complexes). Whether persisting "internal" antigens are required to maintain serological memory remains debated (Zinkernagel and Hengartner 2006). In the case of *B. burgdorferi* s.l., all of these mechanisms could be involved. There appears to be a general agreement that the spirochete can persist in humans and laboratory animals for months or years in refuge niches like the spleen or joints (Straubinger et al. 1997). Studies investigating the reservoir competence of *B. burgdorferi* s.l. in bird species have reported the persistence of bacteria for several months (Isogai et al. 1994, Olsen et al. 1996) and the reactivation of bacteremia under stressful conditions (experimentally induced migratory restlessness) after months of latent infection (Gylfe et al. 2000). Likewise, in humans and laboratory animals,

after antibiotic treatment that presumably cleared *B. burgdorferi* s.l. from the organism, anti-*Borrelia* specific Ab have been observed to persist for more than 400 days (Straubinger et al. 1997, Glatz et al. 2006).

The second nonexclusive hypothesis to explain the high repeatability of individual Ab levels between years is a repeated exposure to *B. burgdorferi* s.l. The probability of exposure to the bacterium is directly linked to the local abundance of its vector, the tick *I. uriae*, which is autocorrelated between years (McCoy et al. 1999, Gasparini et al. 2001), and to the prevalence of *B. burgdorferi* s.l. in the ticks. In accordance with this hypothesis, we found that changes in individual Ab levels were correlated with tick infestation levels in the previous year; after a year of high overall tick infestation there was an increase in individual Ab levels (Fig. 4). If the maintenance of Ab levels observed between years $t-1$ and t was due to early re-exposure in year t, we would have expected a relationship between Ab levels and current tick exposure (in year t). Given the life history of the tick and of its seabird host, and the interannual patterns of change in Ab levels that we recorded, it is likely that both a long-term persistence of Ab and an effect of re-exposure to the parasites are important for explaining the interannual dynamics of the immune response (Fig. 1).

In rodents naturally exposed to *B. burgdorferi* s.l., Bunikis et al. (2004) found a high number of persistently seropositive individuals over 80 days, but in this case, as in ours, they could not make any conclusions about the putative causal link between natural exposure to the bacterium and patterns of change in Ab levels. Experimental manipulations of parasite exposure are thus required to explore how important annual reinfection is for the maintenance of high Ab levels. In our system, the manipulation of tick burdens (treatment of the nests with acaricides such as pyrethrynoids) and/or *Borrelia* infection (antibiotic treatment) could be used to investigate the consequences of parasite exposure on Ab persistence over time. If specific Ab levels decrease between years in the deparasitized group compared to controls, an impact of seasonal re-exposure to nest parasites on the repeatability of specific Ab levels would be supported. Similarly, the comparison of ectoparasite-free and antibiotic-treated groups could help us disentangle the influence of persistent infection, annual re-exposure via *Borrelia* vectors, and Ag-independent maintenance factors on individual Ab levels. Clearly, the investigation of the proximal mechanisms involved in observed Ab persistence, although difficult to carry out in natural conditions, could open several new and exciting perspectives in immunoecology.

To investigate the dynamics of the immune response in more detail, we analyzed the similarity of immunological profiles using immunoblots in addition to measuring specific Ab levels. We observed a high repeatability in immunoblot banding patterns between the two years considered (2005–2006). These patterns

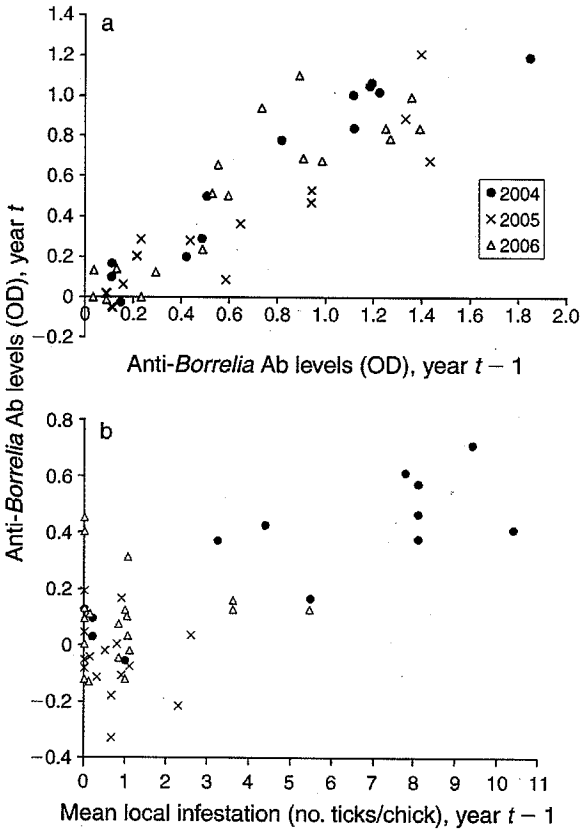

FIG. 5. Relationship between anti-*Borrelia* Ab levels in year t and (a) anti-*Borrelia* Ab levels in year $t-1$ and (b) mean local infestation by ticks in year $t-1$. Local infestation is measured in the five closest nests. In this graph, Ab levels in year t are corrected for other factors in the model (partial residuals of the model described in *Methods: Statistical analyses*).

can differ according to *B. burgdorferi* strains (Wilske 2003) and the immunogenetic characteristics of host individuals (Brown and Reiner 2000). The Ab repertoire has even been shown to vary during the course of infection, because antigen expression by the spirochete changes over time (Pachner et al. 2002, Bykowski et al. 2006). Interestingly, we did not observe variation in Ab repertoires at an interannual scale. The repeatability of immunoblot banding patterns suggests that the spectrum of *B. burgdorferi* s.l. proteins to which the individuals respond are constant between years, possibly because they are re-exposed to the same strains of *B. burgdorferi* s.l. or because the maintenance of Ab levels is linked to the persistence of the same repertoire of B-cells. Another possibility is that the sampled birds might also have reached a plateau in terms of their immunological profile; immunoblot patterns might only vary during the initial months of infection and the birds considered here were all at least three years old. The capture of young prospecting birds when they first return to the colonies at 2–3 years of age and some knowledge about their exposure to ticks/*Borrelia* when they were chicks would shed some light on to how

profiles change after first exposure. The further use of molecular techniques to determine the variability in the *B. burgdorferi* s.l. strains present in this system (K. D. McCoy, *unpublished data*) and long-term studies using kittiwakes or other avian species infected with different bacterial strains could also help us to determine the expected range of immunological responses.

The long-term dynamics of Ab levels could have important fitness consequences for colonial seabirds. The maintenance of Ab levels might confer an advantage in terms of immune response efficiency when re-exposed to nest parasites during subsequent breeding attempts. In an environment where parasites are heterogeneously distributed in space but autocorrelated in time, the costs and benefits of breeding site fidelity could thus be strongly affected by the immunological profile of individuals. Moreover, the long-term persistence of Ab may enable a female to transfer an adequate repertoire of Ab to her young despite physiological and ecological constraints, with adult exposure to nest parasites most likely occurring during egg incubation, after Ab transfer (Gasparini et al. 2001) (Fig. 1). Indeed, such a mechanism may allow females to use their past experience to prepare their young for the conditions of the local environment. Even though this pattern could simply be a by-product of maternal exposure to parasites and the tendency for mothers to transfer Ab as a function of circulating plasma levels, the transmission of maternal Ab could still confer passive protection to the chick or attenuate first exposure to the pathogen (Grindstaff et al. 2003). In the present study, we examined the immune response to *B. burgdorferi* s.l., but these mechanisms are also likely to be involved in the response against other parasites of nidicolous birds like ticks, fleas, or mites (Boulinier et al. 1997, Heeb et al. 1998) and their associated bacteria and viruses.

Our results on the long-term persistence of Ab also have important implications for the epidemiology of zoonotic diseases. In particular, host immunity can affect the spatial and temporal dynamics of disease agents by modifying the number of susceptible individuals in a population. It can also be a key element used to assess disease risk by tracking the exposure of individuals to a pathogen. For example, the use of species as disease "sentinels" requires meeting a series of prerequisite conditions (Komar 2001). Notably, in the case of long-lived wild species, baseline knowledge of the long-term dynamics of Ab is critical as it will help to determine the potential time frame of exposure to the antigen. In this respect, our study shows that immunity in kittiwakes, and possibly other seabirds, has the advantage of being traceable over a series of years. However, the long-term persistence of Ab to bacteria like *B. burgdorferi* s.l. may limit the possibility of identifying the time of exposure and thus the use of these species as sentinels. More generally, our results highlight the need for a better understanding of the factors affecting the long-term immunological response of individuals in natural conditions and its implications for disease transmission. Further exploration of the links between the dynamics of the host immune response and the evolutionary ecology of host and parasite species should thus be a priority in the growing field of immunoecology.

Acknowledgments

We thank R. T. Barrett, A. Dorie, C. Doutrelant, J. Gasparini, A. Grégoire, A. Simon, and A.-M. Thierry for help at various stages of this work and two anonymous referees for their constructive comments. Financial support for this research was received from the French Polar Institute (IPEV, programme number 333), the Centre National de la Recherche Scientifique (CNRS), and the Agence National de la Recherche (ANR). V. Staszewski was supported by the French Ministry of Research. We also thank Fylkesmannen i Finnmark and Kystverket (Norway) for allowing us to carry out this work on Hornøya. Permits to manipulate kittiwakes were granted by the Norwegian Animal Research Authority.

Literature Cited

Altizer, S., A. P. Dobson, P. Hosseini, P. J. Hudson, M. Pascual, and P. Rohani. 2006. Seasonality and the dynamics of infectious diseases. Ecology Letters 9:467–484.

Anker-Nilssen, T., V. Bakken, H. Strøm, A. N. Golovkin, V. V. Bianki, and I. P. Tatarinkova. 2000. The status of marine birds breeding in the Barents Sea region. Norsk Polar Institut, Tromsø, Norway.

Asbakk, K., A. Oksanen, M. Nieminen, R. E. Haugerud, and A. C. Nilssen. 2005. Dynamics of antibodies against hypodermin C in reindeer infested with the reindeer warble fly, *Hypoderma tarandi*. Veterinary Parasitology 129:323–332.

Barton, T. R., M. P. Harris, S. Wanless, and D. A. Elston. 1996. The activity periods and life cycle of the tick *Ixodes uriae* (Acari: Ixodidae) in relation to host breeding strategies. Parasitology 112:571–580.

Bernasconi, N. L., E. Traggiai, and A. Lanzavecchia. 2002. Maintenance of serological memory by polyclonal activation of human memory B cells. Nature 298:2199–2202.

Boulinier, T., K. D. McCoy, and G. Sorci. 2001. Dispersal and parasitism. Pages 169–179 *in* J. Clobert, E. Danchin, A. Dhondt, and J. D. Nichols, editors. Dispersal. Oxford University Press, Oxford, UK.

Boulinier, T., G. Sorci, J.-Y. Monnat, and E. Danchin. 1997. Parent–offspring regression suggests heritable susceptibility to ectoparasites in a natural population of kittiwake *Rissa tridactyla*. Journal of Evolutionary Biology 10:77–85.

Brown, C. R., and S. L. Reiner. 2000. Genes outside the major histocompatibility complex control resistance and susceptibility to experimental Lyme arthritis. Medical Microbiology and Immunology 189:85–90.

Bunikis, J., J. Tsao, C. J. Luke, M. G. Luna, D. Fish, and A. G. Barbour. 2004. *Borrelia burgdorferi* infection in a natural population of *Peromyscus leucopus* mice: a longitudinal study in an area where Lyme Borreliosis is highly endemic. The Journal of Infectious Diseases 189:1515–1523.

Bykowski, T., K. Babb, K. von Lackum, S. P. Riley, S. J. Norris, and B. Stevenson. 2006. Transcriptional regulation of the *Borrelia burgdorferi* antigenically variable VlsE surface protein. Journal of Bacteriology 188:4879–4889.

Comstedt, P., S. Bergström, B. Olsen, U. Garpmo, L. Marjavaara, H. Mejlon, A. G. Barbour, and J. Bunikis. 2006. Migratory passerine birds as reservoirs of Lyme borreliosis in Europe. Emerging Infectious Diseases 12: 1087–1095.

Connolly, S. E., and J. L. Benach. 2005. The versatile role of antibodies in *Borrelia* infections. Nature reviews 3:411–420.

Feuer, R., J. D. Boone, D. Netski, S. P. Morzunov, and S. C. St. Jeor. 1999. Temporal and spatial analysis of Sin Nombre virus quasispecies in naturally infected rodents. Journal of Virology 73:9544–9554.

Frank, S. A. 2002. Immunology and evolution of infectious disease. Princeton University Press, Princeton, New Jersey, USA.

Fromin, N., J. Hamelin, S. Tarnawski, D. Roesti, K. Jourdain-Miserez, N. Forestier, S. Teyssier-Cuvelle, F. Gillet, M. Aragno, and P. Rossi. 2002. Statistical analysis of denaturing gel electrophoresis (DGE) fingerprinting patterns. Environmental Microbiology 4:634–643.

Gasparini, J., K. D. McCoy, C. Haussy, T. Tveraa, and T. Boulinier. 2001. Induced maternal response to the Lyme disease spirochaete *Borrelia burgdorferi* sensu lato in a colonial seabird, the kittiwake *Rissa tridactyla*. Proceedings of the Royal Society of London B 268:647–650.

Gatto, D., S. Martin, J. Bessa, E. Pellicioli, P. Saudan, H. J. Hinton, and M. F. Bachmann. 2006. Regulation of memory antibody levels: the role of persisting antigen versus plasma cell life span. Journal of Immunology 178:67–76.

Gibbs, S. E. J., D. M. Hoffman, L. M. Stark, N. L. Marlenee, B. J. Blitvich, B. J. Beaty, and D. E. Stallknecht. 2005. Persistence of antibodies to West Nile Virus in naturally infected rock pigeons (*Columba livia*). Clinical and Diagnostic Laboratory Immunology 12:665–667.

Glatz, M., M. Golestani, H. Kerl, and R. R. Müllegger. 2006. Clinical relevance of different IgG and IgM serum antibody responses to *Borrelia burgdorferi* after antibiotic therapy for *Erythema migrans*. Archives of Dermatology 142:862–868.

Grenfell, B. T., and A. P. Dobson. 1995. Ecology of infectious diseases in natural populations. Cambridge University Press, Cambridge, UK.

Grindstaff, J. L., E. D. Brodie, and E. D. Ketterson. 2003. Immune function across generations: integrating mechanism and evolutionary process in maternal antibody transmission. Proceedings of the Royal Society of London B 270:2309–2319.

Gylfe, A., S. Bergström, J. Lundstrom, and B. Olsen. 2000. Epidemiology: reactivation of *Borrelia* infection in birds. Nature 403:724–725.

Hauser, U., G. Lehnert, and B. Wilske. 1998. Diagnostic value of proteins of three *Borrelia* species (*Borrelia burgdoferi* sensu lato) and implications for development and use of recombinant antigens for serodiagnosis of Lyme Borreliosis in Europe. Clinical and Diagnostic Laboratory Immunology 5:456–462.

Heeb, P., I. Werner, M. Kolliker, and H. Richner. 1998. Benefits of induced host responses against an ectoparasite. Proceedings of the Royal Society of London B 265:51–56.

Henning, J., D. U. Pfeiffer, P. R. Davies, J. Meers, and R. S. Morris. 2006. Temporal dynamics of rabbit haemorrhagic disease virus infection in a low-density population of wild rabbits (*Oryctolagus cuniculus*) in New Zealand. Wildlife Research 33:293–303.

Hofmeister, E. K., B. A. Ellis, G. E. Glass, and J. E. Childs. 1999. Longitudinal study of infection with *Borrelia burgdorferi* in a population of *Peromyscus leucopus* at a Lyme disease-enzootic site in Maryland. American Journal of Tropical Medicine and Hygiene 60:598–609.

Hudson, P. J., A. Rizzoli, B. T. Grenfell, H. Heesterbeek, and A. P. Dobson. 2002. The ecology of wildlife diseases. Oxford University Press, Oxford, UK.

Hukkanen, R. R., H. D. Liggitt, S. T. Kelley, R. Grant, D. M. Anderson, R. A. Hall, R. B. Tesh, A. P. T. DaRosa, and H. Bielefeldt-Ohmann. 2006. West Nile and St. Louis encephalitis virus antibody seroconversion, prevalence, and persistence in naturally infected pig-tailed macaques (*Macaca nemestrina*). Clinical and Vaccine Immunology 13:711–714.

Isogai, E., S. Tanaka, I. S. Braga, C. Itakura, H. Isogia, K. Kimura, and N. Fujii. 1994. Experimental *Borrelia garinii* infection of Japanese quail. Infection and Immunity 62:3580–3582.

Komar, N. 2001. West Nile virus surveillance using sentinels birds. Annals of the New York Academy of Sciences 951:58–73.

Kuenzi, A. J., R. J. Douglass, C. W. Bond, C. H. Calisher, and J. N. Mills. 2005. Long-term dynamics of Sin Nombre viral RNA and antibody in deer mice in Montana. Journal of Wildlife Diseases 41:473–481.

McCoy, K. D., T. Boulinier, J. W. Chardine, E. Danchin, and Y. Michalakis. 1999. Dispersal and distribution of the tick *Ixodes uriae* within and among seabird host populations: the need for a population genetic approach. Journal of Parasitology 85:196–202.

McCoy, K. D., T. Boulinier, S. Schjoerring, and Y. Michalakis. 2002. Local adaptation of an ectoparasite *Ixodes uriae* to its seabird host. Evolutionary Ecology Research 4:441–456.

Mills, J. N., T. L. Yates, T. G. Ksiazek, C. J. Peters, and J. E. Childs. 1999. Long-term studies of hantavirus reservoir populations in the southwestern United States: rationale, potential, and methods. Emerging Infectious Diseases 5:95–101.

Norris, K., and M. R. Evans. 2000. Ecological immunology: life history trade-offs and immune defense in birds. Behavioural Ecology 11:19–26.

Olsen, B., D. C. Duffy, T. G. T. Jaenson, A. Gylfe, J. Bonnedahl, and S. Bergström. 1995. Transhemispheric exchange of Lyme disease spirochetes by seabirds. Journal of Clinical Microbiology 33:3270–3274.

Olsen, B., A. Gylfe, and S. Bergström. 1996. Canary finches (*Serinus canaria*) as an avian infection model for Lyme borreliosis. Microbial Pathogenesis 20:319–324.

Pachner, A. R., D. Dail, L. Li, L. Gurey, S. Feng, E. Hodzic, and S. W. Barthold. 2002. Humoral immune response associated with Lyme borreliosis in nonhuman primates: analysis by immunoblotting and enzyme-linked immunosorbent assay with sonicates or recombinant proteins. Clinical and Diagnostic Laboratory Immunology 9:1348–1355.

R Development Core Team. 2005. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

SAS Institute. 1999. SAS. Version 8. SAS Institute, Cary, North Carolina, USA.

Sheldon, B. C., and S. Verhulst. 1996. Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. Trends in Ecology and Evolution 11:317–321.

Staszewski, V., and T. Boulinier. 2004. Vaccination: a way to address questions in behavioral and population ecology? Trends in Parasitology 20:17–22.

Straubinger, R. K., B. A. Summers, Y. F. Chang, and M. J. Appel. 1997. Persistence of *Borrelia burgdorferi* in experimentally infected dogs after antibiotic treatment. Journal of Clinical Microbiology 35:111–116.

Viney, M. E., E. M. Riley, and K. L. Buchanan. 2005. Optimal immune responses: immunocompetence revisited. Trends in Ecology and Evolution 20:665–669.

Wilske, B. 2003. Diagnosis of Lyme borreliosis in Europe. Vector-Borne and Zoonotic Diseases 3:215–227.

Zinkernagel, R. M., and H. Hengartner. 2006. Protective "immunity" by pre-existent neutralizing antibody titers and preactivated T cells but not by so-called "immunological memory." Immunological Reviews 211:310–319.