Journal of Animal Ecology

British Ecological Society

Journal of Animal Ecology 2012, **81**, 986–995 doi: 10.1111/j.1365-2656.2012.01979.x

Exposure of black-legged kittiwakes to Lyme disease spirochetes: dynamics of the immune status of adult hosts and effects on their survival

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Summary

1. Despite a growing interest in wildlife disease ecology, there is a surprising lack of knowledge about the exposure dynamics of individual animals to naturally circulating infectious agents and the impact of such agents on host life-history traits.

2. The exploration of these questions requires detailed longitudinal data on individual animals that can be captured multiple times during their life but also requires being able to account for several sources of uncertainty, notably the partial observation or recapture of individuals at each sampling occasion.

3. We use a multi-year dataset to (i) assess the potential effect of exposure to the tick-borne agent of Lyme disease, *Borrelia burgdorferi sensu lato* (*Bbsl*), on adult apparent survival for one of its natural long-lived hosts, the Black-legged kittiwake (*Rissa tridactyla*), and (ii) investigate the temporal dynamics of individual immunological status in kittiwakes to infer the rate of new exposure and the persistence of the immune response. Using a multi-event modelling approach, potential uncertainties arising from partial observations were explicitly taken into account.

4. The potential impact of *Bbsl* on kittiwake survival was also evaluated via an experimental approach: the apparent survival of a group of breeding birds treated with an antibiotic was compared with that of a control group.

5. No impact of exposure to *Bbsl* was detected on adult survival in kittiwakes, in either observational or experimental data.

6. An annual seroconversion rate (from negative to positive) of 1·5% was estimated, but once an individual became seropositive, it remained so with a probability of 1, suggesting that detectable levels of anti-*Bbsl* antibodies persist for multiple years.

7. These results, in combination with knowledge on patterns of exposure to the tick vector of *Bbsl*, provide important information for understanding the spatio-temporal nature of the interaction between this host and several of its parasites. Furthermore, our analyses highlight the utility of capture–mark–recapture approaches handling state uncertainty for disease ecology studies.

Key-words: antibody persistence, immuno-ecology, Lyme disease bacteria, misclassification, *Rissa tridactyla*, seroconversion dynamics, state uncertainty

Introduction

Understanding the processes that regulate pathogen circulation in natural host populations is required to better predict the dynamics of infectious diseases and the risks they may pose to human and animal populations (Grenfell & Dobson 1995; Daszak, Cunningham & Hyatt 2000). Numerous emerging or re-emerging infectious diseases of zoonotic origin have been identified in wild vertebrate populations over the past decades (Dobson & Foufopoulos 2001), increasing the interest in studying the ecology of wildlife pathogens (Daszak, Cunningham & Hyatt 2000, Jones *et al.*

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2008). However, there is still a surprising lack of knowledge about the exposure dynamics of individual animals to naturally circulating infectious agents and the impact of such agents on key host life-history traits such as adult survival (Benskin *et al*. 2009; Hoye *et al*. 2011). A better understanding of these ecological processes, as well as the development of methods to properly investigate such questions in natural populations, is thus of great interest for eco-epidemiology (Grenfell & Dobson 1995; Keeling & Rohani 2008). It is also of direct relevance for evolutionary ecology, as host-parasite systems represent privileged models for investigating the evolutionary mechanisms involved in interspecific interactions (e.g. sexual selection: Zuk 1992; local adaptation: Gandon & Michalakis 2002). However, determining the effects of pathogenic infections on wild hosts and the spatio-temporal variability in their exposure to disease agents represents some specific methodological challenges. Estimating key eco-epidemiological parameters, such as the incidence rate (i.e. rate of new exposure), or comparing key demographic parameters, such as adult survival, between exposed and non-exposed individuals, requires repeated sampling of these individuals to gather information on both their infection history and their fate at multiple points throughout their lifetime. Moreover, estimating these parameters from longitudinal data requires the use of formal statistical approaches to account for different sources of uncertainty (McClintock *et al*. 2010). For instance, to estimate differences in survival while accounting for imperfect detection of individually identifiable animals, capture–mark–recapture (CMR) modelling approaches (Williams, Nichols & Conroy 2002 and references therein) have been used in disease ecology (Brown, Brown & Rannala 1995; Telfer *et al*. 2002; Muths *et al*. 2003; Faustino *et al*. 2004; Lachish, Jones & McCallum 2007; Monticelli *et al*. 2008; Rossi *et al*. 2011). In addition, another type of uncertainty that is often neglected in such studies, but needs more systematic consideration, is the assignment of individual infectious states (Conn & Cooch 2009; McClintock *et al*. 2010; Lachish *et al*. 2011). This uncertainty can take two forms: (i) the state of an observed individual can simply be unobservable, and thus ignored, at a given capture occasion (i.e. partial observation) or (ii) one can erroneously assign a state to an individual (i.e. misclassification). Multi-event CMR modelling (Pradel 2005) represents one promising approach to handle this state uncertainty issue in disease studies based on longitudinal data.

Bird–parasite systems can be especially convenient for addressing wildlife ecology issues because: (i) individual birds can be captured, marked and recaptured or re-sighted as part of long-term monitoring programmes conducted at some specific sites, notably where they breed (e.g. colonies, nest boxes; Loye & Zuk 1991; Brown & Brown 1996; Staszewski *et al*. 2007) or feed (feeders; Dhondt *et al*. 2001) and (ii) birds are thought to play a significant role in the circulation of disease agents of major relevance to humans (e.g. Influenza viruses, West Nile Virus, Lyme disease bacteria; see Reed *et al*. 2003; Olsen *et al*. 2006; Benskin *et al*. 2009). The tick-borne agent of Lyme disease (LD), *Borrelia burgdorferi sensu lato* (thereafter, *Bbsl*), is a pathogen of particular interest because it is known to occur in different independent ecosystems in which birds are key elements for its circulation and maintenance. LD is the most prevalent vector-borne disease in North America and Europe (O'Connell *et al*. 1998; Gray *et al*. 2002), and it is known to be caused by several bacteria of the *Bbsl* group, notably *B. burgdorferi s.s.*, *B. garinii* and *B. afzelii*. Birds have been recognized to be involved in the terrestrial cycle of *Bbsl* (Anderson *et al*. 1986; Kurtenbach *et al*. 1998), but also in the lesser-known marine cycle, involving seabirds and the tick *Ixodes uriae* (Olsen *et al*. 1993, 1995a; Gylfe *et al*. 1999; Duneau *et al*. 2008; Gómez-Díaz *et al*. 2010, 2011). Birds likely play a prominent role in the spatial dynamics of LD as dispersers of *Bbsl*-infected ticks (Olsen, Jaenson & Bergström 1995b; Nicholls & Callister 1996; Poupon *et al*. 2006; Gómez-Díaz *et al*. 2011), but may also represent significant reservoirs of this pathogen (Kurtenbach *et al*. 1998; Gylfe *et al*. 1999; Richter *et al*. 2000; Gómez-Díaz *et al*. 2010).

Clinical manifestations of LD have been described in humans and domestic animals (Lissman *et al*. 1984, Magnarelli *et al*. 1990; Parker & White 1992; Barbour & Fish 1993), but little is known about wild hosts. The various symptoms of LD (arthritis, neurological symptoms, skin disorders) are known to roughly correlate with the infecting bacterium species, but such clinical associations are not absolute (van Dam *et al*. 1993; Baranton *et al*. 2001; Tilly, Rosa & Stewart 2008). The manifestations of the disease can also be persistent and reappear chronically in infected hosts (e.g. Steere, Schoen & Taylor 1987; Logigian, Kaplan & Steere 1990; Steere, Coburn & Glickstein 2004). Furthermore, strong variability in the form of the disease among individuals is generally reported. In humans, for instance, many seropositive individuals show no symptoms, whereas infection in other individuals can be life threatening (Steere, Coburn & Glickstein 2004). Moreover, other types of Borreliosis, such as the one caused by *B. anserina*, are known to cause specific symptoms in domestic birds (Lisbôa *et al*. 2009). One can thus expect some negative effect of *Bbsl* in other host species, including wild animals, although this may be limited to a small proportion of exposed individuals.

A better understanding of key ecological aspects of the interaction between *Bbsl* and its natural hosts is thus needed, particularly concerning the potential negative impacts of *Bbsl* infection (Burgess, French & Gendron-Fitzpatrick 1990; Olsen, Gylfe & Bergström 1996; Schwanz *et al*. 2011) and the environmental context in which individuals are exposed. In the terrestrial cycle of *Bbsl*, most studied reservoir species are short-lived or difficult to recapture (Barbour & Fish 1993; Mather 1993), which complicates the task of detecting a potential chronic effect of infection on survival or investigating the temporal dynamic of the immune status of hosts. Conversely, the marine cycle mainly involves long-lived seabird species with colonial breeding habits (Furness & Monaghan 1987), facilitating the longitudinal survey of infected and non-infected individuals required to investigate these questions. For such long-lived species, any negative

effect on adult survival would have strong influence on population growth rate (Lebreton & Clobert 1991) and is thus particularly important to explore. The Black-legged kittiwake (*Rissa tridactyla*) is one long-lived host of *Bbsl* in the marine cycle (Gasparini *et al*. 2001; Staszewski *et al*. 2007; Gómez-Díaz *et al*. 2010). This colonial seabird, which reproduces on sea cliffs, is an appropriate biological model because it can easily be captured on the nest and displays high breeding site fidelity (Danchin, Boulinier & Massot 1998), permitting repeated blood sampling over time. *Borrelia burgdorferi s.l.* is transmitted to kittiwakes via the tick vector *Ixodes uriae*, a hard tick that lives in the substrate of seabird-breeding colonies throughout the circumpolar regions of both hemispheres (McCoy *et al*. 2005). This tick only takes a single, long blood meal per year during each of its three life-history stages (McCoy *et al*. 2002) and thus spends most of its life in the environment surrounding the host-breeding site. Therefore, tick bites, and thus the probability of *Bbsl* transmission, occur only during the breeding period and at the nest site. The detection of specific antibodies in kittiwake plasma can be used to determine which individuals have been exposed to *Bbsl* (Staszewski *et al*. 2007).

In this study, we assessed the potential impact of infection by *Bbsl* on survival in kittiwakes and investigated the dynamics of their serological status (presence or absence of specific antibodies against *Bbsl* and seroconversion between negative and positive states). Both observational and experimental approaches were used. Using capture–recapture data from 7 years of observation, we tested whether there was an association between the immunological status of individuals against *Bbsl* and their annual survival rate. As data consisted of a mixture of physical captures (permitting current serological state assignment) and visual detections (preventing the determination of the current individual serological state), a multi-event modelling approach (Pradel 2005) was used to explicitly integrate uncertainty linked to partial observation. In addition to providing robust estimates of apparent survival (i.e. probability of surviving and not permanently emigrating from the study site), this approach allowed us to estimate rates of seroconversion. In the experimental approach, we compared the return rates of a group of marked individuals treated with an antibiotic to that of a control group. The results are discussed in relation to exposure patterns to the tick vector.

Materials and methods

STUDY AREA AND DATA COLLECTION

The study was conducted on Hornøya, an island in Northern Norway (70°23′N, 31°10′E), where approximately 21 000 pairs of kittiwakes breed (Anker-Nilssen *et al*. 2000). The study population consists of a sample of birds individually colour ringed as breeders on several spatially distinct cliffs (plots) for which data are also available on the degree of infestation by the tick *Ixodes uriae* (Gasparini *et al*. 2001). The presence of marked kittiwakes is monitored during the breeding season each year by re-sighting surveys conducted on each plot every 3 days. In addition, about 50–100 birds are physically captured or re-captured each year and, starting in 2003, a blood sample for use in immunological analyses is systematically collected at each capture (Staszewski *et al*. 2007). Blood samples are obtained from the left ulnar vein with a sterile syringe flushed with heparin (Staszewski *et al*. 2007). After centrifugation, the plasma is separated from the blood cells and stored at −20 °C until immunological analysis. Throughout the breeding season, the detection probability of individuals can vary according to the reproductive fate of the birds, but is high overall (almost 1·0), attributed to the number of sampling occasions (Chambert *et al*. 2011).

IMMUNOLOGICAL ASSAYS AND DETERMINATION OF SEROLOGICAL STATE

As the detection of *Borrelia* DNA in bird blood is technically difficult, we used the presence of anti-*Bbsl* antibodies, obtained using enzyme-linked immunosorbent assay (ELISA) analyses, as a proxy of infectious state. A major issue arising from the use of such a proxy is that the presence of anti-*Bbsl* antibodies (Ab) does not necessarily mean that the individual is currently infected or has recently been exposed. Indeed, as the presence of anti-*Bbsl* antibodies is expected to be persistent (Staszewski *et al*. 2007), a seropositive status could reflect exposure to the bacteria several years before. However, as it is known that *Bbsl* can cause chronic infections in humans and can be responsible for persistent symptoms long after initial infection (Steere, Coburn & Glickstein 2004), we could expect seropositive individuals to display survival costs, regardless of the time since exposure. Indeed, the reactivation of *Bbsl* infection has been described in passerine birds subjected to stress (Gylfe *et al*. 2000), suggesting long-term infections. Given the high breeding site fidelity displayed by kittiwakes (Danchin, Boulinier & Massot 1998, Boulinier *et al*. 2008) and the relative immobility of the tick vector (McCoy *et al*. 2005), it is likely that individuals infected once (i.e. seropositive individuals) could become re-infected by *Bbsl* (Staszewski *et al*. 2007).

Antibody levels used in the present study are expressed as the optical density (OD; wavelength of 492 nm in a spectrophotometer) of the solution resulting from a specific ELISA (Enzygnost Borreliosis ELISA Kit; Dade Behring, Marburg, Germany). Because this kit was manufactured for human use and designed to recognize mammalian Ab, we replaced anti-human IgG Ab by an anti-chicken IgY Ab (see Staszewski *et al*. 2007 for a detailed protocol of the immunological assays; the binding of anti-chicken IgY to kittiwake IgY was verified via Western blots, Lobato *et al*. 2011). Measures of Ab levels were taken from 315 individually marked kittiwakes surveyed from 2003 to 2009. The distribution of OD values was bimodal (Fig. 1), with high OD values corresponding to seropositive plasma samples and low OD values to seronegative samples. To confirm that samples with high OD values were indeed seropositive, we performed immunoblots (Western Blot Lyme IgG + VlsE; Meridian Bioscience, Inc, Cincinnati, Ohio, USA) on 20 of these plasma samples to detect the presence of antibodies against specific antigens of *Bbsl* (Fig. S1; see Staszewski *et al*. 2007 and Staszewski, McCoy & Boulinier 2008 for methodological details). Using the positivity threshold obtained from these analyses, the following criterion was used to assign the serological state of a plasma sample (i.e. an individual in a given year): negative if OD $<$ 0·5, intermediate if 0·5 $<$ OD $<$ 0·7 and positive if OD $>$ 0·7. The negative threshold (OD = 0·5) was 2 SD below the mean of seropositives, and the positive threshold (OD = 0·7) was 5 SD above the mean of seronegatives. We chose to define an intermediate state to avoid false negatives and false positives. The number of intermediate cases was low, representing only 7% of the entire

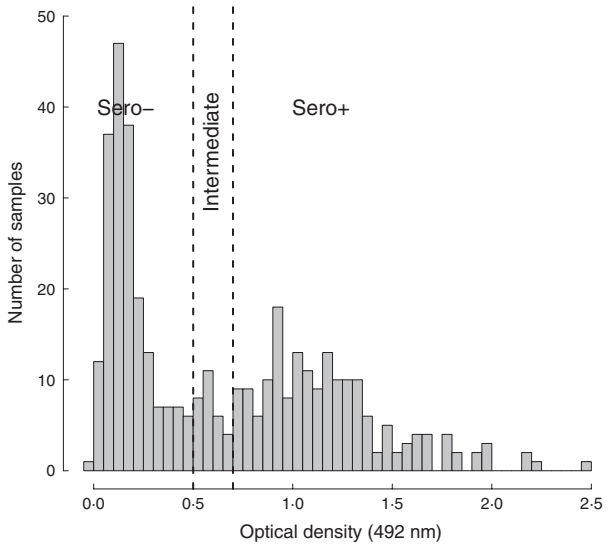

Fig. 1. Distribution of optical density measures of *Bbsl* antibodies in kittiwakes. The distribution is bimodal, with low values corresponding to seronegative plasma samples and high values to seropositive plasma samples. We could not determine the serological state with certainty in the intermediate class. Dotted lines represent the threshold (0·5 and 0·7) used to separate the three serological states.

sample, against 44% and 49% for seronegatives and seropositives, respectively.

CAPTURE–MARK–RECAPTURE MODELLING

We used CMR data from the period 2003 to 2009 to estimate annual survival rates, seroconversion rates and to test whether survival was correlated with immunological status. As physical captures occurred at most once per season, only one serological measure was used per individual per year. Re-sighting data collected over multiple occasions within a same breeding season were therefore pooled into a single occasion per year to summarize re-sighting data to the same temporal scale (see Chambert *et al*. 2011 for a detailed analysis of within-year re-sighting data).

Because the immunological status of some re-sighted individuals was not known some years (i.e. when an individual has been visually detected but not physically captured), we analysed this dataset under the general framework of multi-event models (Pradel 2005; see also Conn & Cooch 2009 and Lachish *et al*. 2011 for comparable modelling approaches). In this modelling approach, the same observational event (e.g. a bird is visually detected) can correspond to different states (e.g. the observed bird can be seropositive or seronegative), which allows one to account for uncertainty in the state (partial observation of the serological state). In our dataset, the capture histories could include five different events: '0' when a bird was not seen a given year, '1' when a bird was seen but not physically captured, 'N' when it was captured and determined to be seronegative, 'I' when it was captured and determined to be in the 'intermediate' state and 'P' when it was captured and determined to be seropositive. Those events correspond to four states: N (seronegative), I (intermediate), P (seropositive) and dead.

The program E-SURGE (Choquet, Rouan & Pradel 2009a) was used to build and evaluate the relative support of multi-event models (Appendix S1 in Supporting Information). We decomposed overall state transitions in two components to obtain separate estimates for survival (S; assuming that survival only depends on the departure state and not on the arrival state) and seroconversion parameters (Ψ, i.e. 'transition conditional on survival'). As our interest was particularly drawn to survival, the selection of relevant covariates was conducted last for this parameter, after having selected the best structure for initial-state, events (i.e. recapture and re-sighting) and conditional transition probabilities while keeping a 'state and year' interactive effect for survival. No formal Goodness-of-fit (GOF) test currently exists for multi-event models. Therefore, as usually done in the context of multistate analyses, we assessed the fit of the Jolly-Move (JMV) model (Pradel, Wintrebert & Gimenez 2003), assuming that survival, recapture and transition probabilities vary with year and state. Because there was no evidence of lack of fit for the JMV model (χ^2 = 55·73, d.f. = 64, P = 0·760), we started the model selection procedure using the corresponding general multi-event model that included: (i) the effects of state and year on the initial-state parameter, the event probability and on survival; and (ii) the effects of departure and arrival states and year on the transition parameter. The program U-CARE (Choquet *et al*. 2009b) was used for this purpose. The relative support of competing models was assessed using an information-theoretic approach based on the Akaike Information Criterion (AIC) (Burnham & Anderson 2002) adjusted for sample size (AICc) and possible overdispersion (QAICc) and on relative AIC weight (w).

DESIGN OF THE EXPERIMENTAL APPROACH

In 2009, 84 marked individuals breeding on surveyed plots were captured for an experiment involving the use of a single subcutaneous injection of a suspension of an antibiotic. The main aim of the treatment was to reduce ongoing infections by *Bbsl* at the time of injection to detect a possible beneficial effect on bird survival. The birds were captured and treated during late incubation. Owing to field constraints, only one injection could be given, and thus, it was not expected that the treatment would fully clear Bbsl infection, particularly as these infections are known to require long-term treatment in some individuals (Steere, Coburn & Glickstein 2004). The antibiotic chosen nevertheless consisted of a long-acting preparation of amoxicillin (Clamoxyl LA, Pfizer, Paris, France, 150 mg mL^{-1} of amoxicillin) that should have led to an efficient, but potentially temporary, reduction in circulating bacteria. Forty-three of the captured birds were randomly chosen and injected with 0·1 mL of the antibiotic suspension (treated group), whereas the 41 others received a subcutaneous injection of 0·1 mL of physiological solution (control group); practically, birds were alternatively injected with the antibiotic vs. the sham. Anti-Borrelia antibody levels of experimental individuals were measured using a specific enzymoimmunoassay (Borrelia IgG + VlsE ELISA, RE57201; Meridian Bioscience, Inc, Cincinnati, Ohio, USA), substituting the secondary anti-human IgG by an anti-chicken IgY Ab.

Of the 84 individuals included in the experiment, 23 were seropositive at the time of the injection (2009), 12 in the treated group and 11 in the control group. Using re-sighting data collected over the 2010 breeding season, the proportion of individuals from the treated vs. control group that returned to the colony were compared. Multiple re-sighting occasions during the 2010 season ($n \geq 25$ different days) enabled us to ensure that the detection probability was very high in this year and that any bird that returned was likely detected (Chambert *et al*. 2011). We first focused on the subsample of individuals that were seropositive in 2009 because the goal of the experiment was to evaluate the effect of an experimental treatment against *Bbsl* on annual survival in birds known to have been previously exposed

to the bacterium. We also compared return rates for the entire sample of birds in the experiment (i.e. both seropositive and seronegative individuals), as well as for the subsample of seronegative individuals only, to account for potential additional effects of the antibiotic treatment, in particular, the protection of naïve individuals against *Bbsl* infection (i.e. some individuals may have been first exposed to *Bbsl* at the time of treatment).

Results

OBSERVATIONAL APPROACH

The results from the model selection did not support the hypothesis of a correlation between survival and serological state (Table 1; see also Table S2 in Supporting Information, for full results of the model selection procedure). Indeed, the model with most support from the data ($w = 0{\cdot}60$) excluded the effect of serological state on survival. Models including an effect of state on survival received less support from the data (Table 1: ΔAICc $= 2{\cdot}08$, $w = 0{\cdot}21$ for the model with an additive effect of state and year; ΔAICc $= 7{\cdot}96$, $w = 0{\cdot}01$ for the model with a state effect only; and ΔAICc $= 8{\cdot}30$, $w = 0{\cdot}01$, for the model with an interactive effect of state and year). Moreover, estimates from these latter models did not support any consistent effect of seropositivity on survival: (i) in the additive model, survival estimates were slightly higher for the seropositive state than for the seronegative state (Fig. 2a); (ii) in the interactive model, the relative position of survival estimates varied between the two states across years (Fig. 2b). Differences in survival between the two states were thus neither statistically nor biologically significant.

We found significant variation in survival among years (Table 2, Fig. 2), as illustrated by the fact that all top-ranked models include a year effect on the survival parameter. The detection probability of marked individuals also varied among years, but was high every year (above 0·9), with a mean of 0·93 (Table S3 in Supporting Information).

In the most supported models, transitions probabilities varied by departure and arrival states only (Table 1). Estimates of transition probabilities from the top-ranked model (Table 2, Table S3) revealed that (i) the probability of transition from a seropositive state to a seronegative state was null (i.e. once seropositive, kittiwakes remained in this state) and (ii) the annual probability of becoming seropositive was relatively small (0·12, SE = 0·05). Indeed, in the dataset only seven of 114 seronegative birds seroconverted during the

Table 1. Summary of multi-event model selection. Only the six most supported models are shown (Table S2, for full results of the model selection procedure). All other models, representing various structures for the other parameters, received no support from the data (ΔAICc > 18). Parameters of the models are (i) initial-state probability; (ii) survival; (iii) transition probability conditional on survival; and (iv) event probability conditional on state. Sources of variation tested on survival and other parameters are immunological state and year. Additive effects are denoted by a plus symbol (+) and interactive effects by a star (*)

Model parameterization[a]					
Survival	Transition[b]	Num. Par.	QAICc	△AAICc	AICc Weight
Year	State	37	2747·78	0·00	0·60
State + Year	State	39	2749·86	2·08	0·21
Year	State + Year	40	2750·52	2·74	0·15
State + Year	State + Year	42	2754·46	6·68	0·02
State	State + Year	37	2755·74	7·96	0·01
State * Year	State + Year	52	2756·08	8·30	0·01

[a]The structure of event probabilities and initial-state parameters are the same for these six models. Event probabilities (i.e. recapture and visual detection) vary by state and across years, but the overall rate of detection does not depend on state. The initial-state probability varies across years, but is the same for the two relevant states (seronegative and seropositive), as there is approximately the same proportion of individuals in each state in our sample. [b]For the transition parameter, a state effect means dependence on both departure and arrival states.

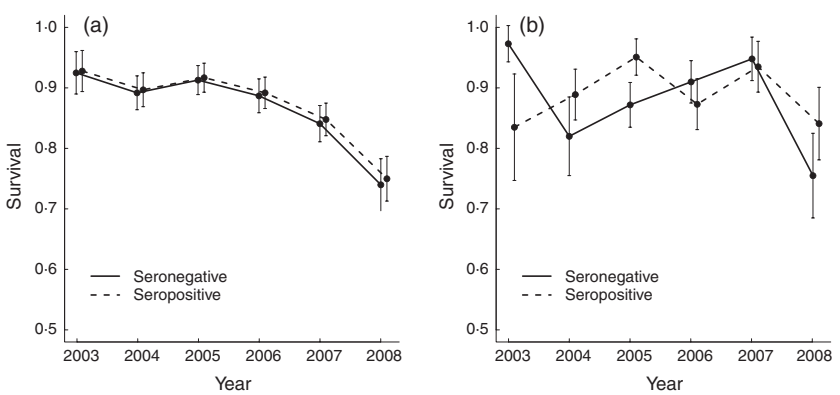

Fig. 2. Annual variation of survival estimates from (a) the model with an additive effect of year and state (second-ranked model) and (b) the model with an interactive effect of year and state (sixth-ranked model). Only the two relevant serological states, seropositive (dashed line) and seronegative (full line), are represented on the graphs. Error bars represent standard errors.

Table 2. Estimates and standard errors (SE) of annual survival and seroconversion rates from the top-ranked model

Parameter	Year interval	Estimate	SE
Survival	2003–2004	0·95	0·02
Survival	2004–2005	0·90	0·02
Survival	2005–2006	0·92	0·02
Survival	2006–2007	0·89	0·02
Survival	2007–2008	0·85	0·02
Survival	2008–2009	0·75	0·03
State transition			
Positive to Negative	–	0·00	0·00
Negative to Positive	–	0·12	0·05

The two types of seroconversion (transition from positive to negative and from negative to positive) are shown. See Table S3, for estimates of all model parameters.

study period. Given these results, which demonstrate the inter-annual stability in the immunological status of adult kittiwakes (see also Staszewski *et al*. 2007), we conducted some complementary single-state analyses, where the individual serological state was fixed (except for the seven observed cases of seroconversion; see Appendix S2 in Supporting Information for details). These analyses were performed in order to re-test the potential association between immunological status and survival, assuming that states were known with certainty. Full details of the methodology and results of these complementary analyses are provided in Appendix S2. We modelled the effect of immunological status first as a constant qualitative variable (i.e. group effect) and then as a quantitative individual covariate, using the OD value as a measure of the relative amount of circulating Ab. Finally, to check for potential confounding factors linked to spatial structuring, we tested whether there was a difference in individual apparent survival among breeding cliffs that displayed contrasting levels of *Bbsl* seroprevalence. These complementary results led to the same conclusion as the multi-event analyses, that is, no statistical association between an individual's immunological status and its survival (Appendix S2).

EXPERIMENTAL APPROACH

Among the 12 treated individuals that were seropositive in 2009, 9 (75%) were re-sighted in 2010 on the colony, whereas eight of the 11 (80%) control individuals that were seropositive in 2009 were re-sighted in 2010. There was therefore no difference in the return rate of treated vs. control seropositive individuals (chi-square test: $\chi^2 = 0{\cdot}015$, d.f. = 1 and $P = 0{\cdot}90$). Similarly, no significant difference in the return rate of treated vs. control birds was found when we considered the entire sample of seropositive and seronegative birds ($\chi^2 = 0{\cdot}855$, d.f. = 1 and $P = 0{\cdot}36$), nor when we considered seronegative individuals only ($\chi^2 = 1{\cdot}12$, d.f. = 1, $P = 0{\cdot}29$). Therefore, the antibiotic treatment applied did not seem to enhance (or decrease) survival for any individual regardless of its immunological status.

Discussion

LACK OF AN EFFECT OF *BBSL* EXPOSURE ON ADULT SURVIVAL

The results of the different analyses performed in this study converge to the same conclusion of no difference in the apparent survival of adult kittiwakes attributed to infection by *Bbsl*. However, this result should be considered with some caution. As previously discussed by Telfer *et al*. (2002), the impact of a parasite on host mortality can be underestimated when analysing longitudinal data. Indeed, because these data consist of a series of discrete point samples rather than a continuous monitoring of individuals, one cannot know exactly when an individual becomes infected, even if a seroconversion event is recorded. For example, if susceptible individuals die relatively rapidly after infection, their seroconversion is not likely to be observed and they remain recorded as 'uninfected' in the data. In this case, the only individuals appearing in the 'infected' state are those that actually survived infection. Survival is thus overestimated for the 'infected' state, whereas it is artificially decreased for the 'uninfected' state, leading to the underestimation of the negative effect of the parasite. This bias may even lead to the detection of an effect opposite to the real effect, that is, higher survival of the 'infected' state. Such an artefact could be present in our CMR analyses, but our confidence in these results is increased by the fact that the experiment led to the same conclusion. Moreover, the results from the complementary single-state spatial analysis (Appendix S2) failed to show any difference in survival among areas of high and low *Bbsl* seroprevalence, independently of an individual's immunological status.

Few studies have explicitly assessed the potential effect of infection by *Bbsl* on host survival, other than effects on humans and domestic animals for which the clinical manifestations are well known. To our knowledge, the only such study conducted *in natura* (Hofmeister *et al*. 1999) was carried out on a population of white-footed mice (*Peromyscus leucopus*), a major reservoir host species of *Bbsl* in North America (Mather *et al*. 1989). No effect on survival was found in this study, a result in accordance with recent experimental work that showed that *Bbsl* has no impact on the field activity of this species (Schwanz *et al*. 2011). Previous experimental studies have also indicated that *Bbsl* infection induces few observable clinical manifestations on susceptible bird species (Mallard ducks *Anas platyrynchos*: Burgess 1989; Japanese quails *Coturnix japonica*: Isogai *et al*. 1994; Canary finches *Serinus Canaria*: Olsen, Gylfe & Bergström 1996). The results of these different studies, in combination with our work here, suggest that *Bbsl* has no major effect on the survival of its natural reservoir host species. Future studies will now need to investigate whether infection could induce other major effects, such as fecundity costs, that would ultimately reduce fitness in infected individuals. Likewise, as *Bbsl* infection has been shown to be reactivated under stress in birds (Gylfe *et al*. 2000), studies that investigate costs under particularly stressful conditions are called for.

Bbsl exposure and host seroconversion rate

State transition estimates obtained from the multi-event CMR analysis indicated that the seropositive state was persistent for multiple years. Temporal persistence of individual levels of anti-*Bbsl* antibodies was previously found in the black-legged kittiwake using the same dataset (Staszewski *et al.* 2007), but the mechanism behind this persistence remains unknown. It could be due to: (i) a continuous production of antibodies by the immune system once an individual has been exposed; (ii) a chronic reactivation of infection in the host, inducing the regular production of antibodies; or (iii) the recurrent exposure of the bird to the infectious agent attributed to the high fidelity of this species to its nest and the strong spatial heterogeneity in exposure to the tick vector (Boulinier, Ives & Danchin 1996). We also found that seronegative individuals tended to remain seronegative. However, seven cases of seroconversion out of 114 initially seronegative individuals were recorded during the 5-year study period, corresponding to an incidence of about 1·5% each year. This relatively low rate of new infections in adult kittiwakes is certainly linked to the philopatric habits of this species. Indeed, most individuals showing no sign of exposure bred on patches with low tick densities and thus have a low infection probability. Some spatial variability in *Bbsl* prevalence in ticks among breeding cliffs has been recorded within the study site, with an overall infection prevalence of 11% and with most infected ticks co-occurring in areas of high seroprevalence in the host (Dietrich *et al.* 2008). The seven kittiwakes that seroconverted during the study period seem, however, to be a relatively random sample from the surveyed population. Indeed, none of these seven individuals was recorded to disperse between breeding cliffs, and no pattern could be detected concerning their breeding location. Four of these birds bred in cliffs with relatively low tick density and low *Bbsl* prevalence in ticks, whereas the three others bred in cliffs with relatively high tick density and high *Bbsl* prevalence in ticks.

Previous studies that have estimated *Bbsl* incidence from serological data in natural populations of white-footed mice (*Peromyscus leucopus*) found much higher rates of seroconversion than in our study (e.g. incidence of 20% per week, Bunikis *et al.* 2004; see also Hofmeister *et al.* 1999). This striking difference may highlight important differences between the terrestrial and the marine cycles of *Bbsl*. For instance, the ecological picture of *Bbsl* circulation is known to be particularly complex in its terrestrial cycle because it involves several genospecies of *Bbsl*, several tick vector species that are all non-nidicolous (i.e. that actively quest for new hosts), as well as a host species spectrum that is ecologically and taxonomically diverse (Gray *et al.* 2002). In contrast, the marine system may be more stable. The different seabird hosts possess ecological similarities (coloniality and strong site fidelity) that make them abundant and predictable host sources for ticks. Moreover, genetic studies have shown that *Ixodes uriae* has diverged into a series of distinct host-specific races (McCoy *et al.* 2001, 2005), which can be related to the isolation of *Bbsl* strains circulating among the different tick race-seabird species combinations within heterospecific colonies (Duneau *et al.* 2008) and to differential patterns of prevalence and infection intensity in each host-associated group (Gómez-Díaz *et al.* 2010). Finally, the eco-physiology and population ecology of ticks and hosts may also interact in subtle ways to drive the local dynamics of tick infection and host immunological status. For instance, it is interesting to note that a state-related (breeders vs. pre-breeders) acquired immunity effect on infection by a tick-borne virus was reported in another seabird species, the common guillemot *Uria aalge* (Nunn *et al.* 2006). These findings, in combination with ours, highlight the importance of considering different characteristics (spatial structure, age-effects, fitness costs, etc.) when undertaking eco-epidemiological studies on complex host–vector–parasite systems. Further investigations of these spatially structured systems could prove useful for deciphering the role of these characteristics on the circulation of vector-borne disease agents.

Methodological aspects: considering uncertainty

Uncertainty is becoming an issue of major importance in disease ecology (McClintock *et al.* 2010), as it was previously in population and community ecology (e.g. Pradel 2009, Williams, Nichols & Conroy 2002; Yoccoz, Nichols & Boulinier 2001). Beyond the biological interest of the results obtained here, the present study also aimed at showing the utility and necessity of systematically considering approaches that take uncertainty about state assignment into account in wildlife disease ecology studies, as previously outlined by Conn & Cooch (2009) and Lachish *et al.* (2011). While uncertainty arising from partial observation is robustly accounted for in the multi-event approach, the issue of misclassification, which is very likely to occur in disease ecology studies (McClintock *et al.* 2010), also poses difficulties (Pradel *et al.* 2008, Conn & Cooch 2009). The development of more efficient laboratory techniques is the primary remedy to decrease uncertainty linked to the assignation of infectious or serological states (i.e. occurrence of false negatives and false positives; McClintock *et al.* 2010). Recent statistical developments allowing one to estimate misclassification probabilities (Runge, Hines & Nichols 2007), such as generalized site occupancy modelling (Royle & Link 2006), also offer promising alternatives to deal with this issue. In this context, study designs based on multiple sampling occasions within a single season, such as the robust design, must be privileged when possible.

Conclusion

Using longitudinal data collected over 7 years and an experimental field study, we found that infection by the bacterium *Bbsl* does not seem to impact adult survival of black-legged kittiwakes. We also confirm that birds exposed to this

infectious agent display high level of circulating anti-*Bbsl* antibodies for several years. Finally, we show that the rate of new infections in adult kittiwakes in breeding colonies is relatively low and likely linked to the high breeding site fidelity of this seabird species combined with a heterogeneous distribution of the tick vector within colonies. In addition to these biological results, our analyses highlight the utility of CMR approaches for handling state uncertainty in disease ecology studies. Detailed knowledge about the circulation and potential effects of endemic microparasites in wildlife populations can be gained by combining epidemiological sampling with CMR population studies.

Acknowledgements

We thank Romain Garnier and Muriel Dietrich for useful discussions related to the topic of the paper. Permits to manipulate kittiwakes were obtained via the Norwegian Animal Research Authority and Stavanger Museum. E. Lobato was supported by Postdoctoral grants from Ministry of Science of Spain, reference 2008-0488. This is a direct contribution of the research program of the French Polar Institute (IPEV) *n*°033 on the ecology of dispersal and local interactions in arctic seabird-tick interactions. Support from the ANR EVEMATA (11-BSV7-003) project is acknowledged.

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Received 4 August 2011; accepted 4 February 2012
Handling Editor: Sheena Cotter

Supporting information

Additional Supporting Information may be found in the online version of this article.

Appendix S1. Detailed structure of the set of models used in the multi-event analysis.

Appendix S2. Single-state analyses.

Fig. S1. Results of Western Blot analyses to assess the relationship between the immunological status of a serum sample and its value of optical density obtained from an ELISA analysis.

Table S1. Structure of multi-event models that were used in the analysis.

Table S2. Detailed results of the model selection procedure of the multi-event analysis.

Table S3. Estimates of all identifiable parameters of the best model.

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