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Occurrence and transmission efficiencies of *Borrelia burgdorferi ospC* types in avian and mammalian wildlife

Holly B. Vuong [a,b,*], Charles D. Canham [b], Dina M. Fonseca [a,c], Dustin Brisson [d], Peter J. Morin [a], Peter E. Smouse [a], Richard S. Ostfeld [b]

[a] Rutgers University, Ecology and Evolution Graduate Program, Cook Campus, ENR 1st Floor, New Brunswick, NJ 08901, USA
[b] Cary Institute of Ecosystem Studies, 2801 Sharon Turnpike, Millbrook, NY 12545, USA
[c] Rutgers University, Center for Vector Biology, 180 Jones Ave., New Brunswick, NJ 08901, USA
[d] University of Pennsylvania, Department of Biology, 209 Leidy Laboratories, Philadelphia, PA 19104, USA

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ABSTRACT

Borrelia burgdorferi s.s., the bacterium that causes Lyme disease in North America, circulates among a suite of vertebrate hosts and their tick vector. The bacterium can be differentiated at the outer surface protein C (*ospC*) locus into 25 genotypes. Wildlife hosts can be infected with a suite of *ospC* types but knowledge on the transmission efficiencies of these naturally infected hosts to ticks is still lacking. To evaluate the occupancy and detection of *ospC* types in wildlife hosts, we adapted a likelihood-based species patch occupancy model to test for the occurrence probabilities (ψ – "occupancy") and transmission efficiencies (ε – "detection") of each *ospC* type. We detected differences in *ospC* occurrence and transmission efficiencies from the null models with HIS (human invasive strains) types A and K having the highest occurrence estimates, but both HIS and non-HIS types having high transmission efficiencies. We also examined *ospC* frequency patterns with respect to strains known to be invasive in humans across the host species and phylogenetic groups. We found that shrews and to a lesser extent, birds, were important host groups supporting relatively greater frequencies of HIS to non-HIS types. This novel method of simultaneously assessing occurrence and transmission of *ospC* types provides a powerful tool in assessing disease risk at the genotypic level in naturally infected wildlife hosts and offers the opportunity to examine disease risk at the community level.

1. Introduction

Lyme disease in North America is caused by infection with the spirochete bacterium, *Borrelia burgdorferi s.s.* (Burgdorfer et al., 1982). This bacterium circulates within vertebrate host species, vectored primarily by the black-legged tick, *Ixodes scapularis*. Within local tick populations, the *B. burgdorferi* population is genetically heterogeneous, consisting of a group of distinct genotypes (Wang et al., 1999; Qiu et al., 2002; Gatewood et al., 2009; Barbour and Travinsky, 2010; Hamer et al., 2011; Brisson et al., 2012; Margos et al., 2012). These genotypes are differentiated by genetic differences at the highly variable antigenic site of the outer surface protein C (*ospC*) locus (Ohnishi et al., 2001; Liang et al., 2004). *B. burgdorferi s.s.* exhibits 25 alleles (types), of which 17 are known to occur in the northeastern United States (Qiu et al., 2002; Barbour and Travinsky, 2010).

Previous studies had detected differential infection frequencies of vertebrate hosts by particular *ospC* types (Brisson and Dykhuizen, 2004; Hanincova et al., 2006). Although *B. burgdorferi s.s.* varies in its reservoir-competence levels over a large suite of host species (Battaly and Fish, 1993; Rand et al., 1998; Giardina et al., 2000; Richter et al., 2000; LoGiudice et al., 2003; Ginsberg et al., 2005; Brisson et al., 2008; Taragel'ova et al., 2008; Keesing et al., 2009), the role of host species in supporting genotypic variation of the bacterium is not well understood. Here, we utilize the *ospC* locus as a marker for genetic diversity (Brisson et al., 2011) to determine the presence and frequencies of *ospC* genotypes in the vertebrate hosts.

Recent studies of associations between hosts and *B. burgdorferi* genotypes are limited by their focus on subsets of the hosts occurring at any single site, e.g., mammals or birds, but not both (Brisson and Dykhuizen, 2004; Alghaferi et al., 2005; Anderson and Norris, 2006; Hanincova et al., 2006; Ogden et al., 2008; but see MacQueen et al. (2012) for an exception). Although there is some information on the transmission rates of *B. burgdorferi* from host to tick, based

* Corresponding author. Present address: CSIRO Black Mountain, Plant Industry, GPO Box 1600, Canberra, ACT 2601, Australia. Tel.: +61 02 6246 5106.
E-mail addresses: Holly.vuong@csiro.au (H.B. Vuong), Canhamc@caryinstitute.org (C.D. Canham), Dinafons@rci.rutgers.edu (D.M. Fonseca), Dbrisson@sas.upenn.edu (D. Brisson), Pjmorin@rci.rutgers.edu (P.J. Morin), Smouse@aesop.rutgers.edu (P.E. Smouse), Ostfeldr@caryinstitute.org (R.S. Ostfeld).

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on needle and tick infections of mouse models in experimental inoculation studies, (Hofmeister et al., 1999; Hanincova et al., 2008; Baum et al., 2012), there is less information on transmission rates of strains from naturally infected wildlife host individuals to ticks. Understanding transmission and occurrence patterns is important since all *ospC* genotypes can infect humans, but the probability of the bacterium invading humans following a tick bite varies by genotype (Seinost et al., 1999; Dykhuizen et al., 2008; Wormser et al., 2008). Additionally, *ospC* genotypes appear to vary in their Lyme disease severity (Strle et al., 2011; Hanincova et al., 2013). In humans, most diagnosed cases involve only five of the seventeen *ospC* types, specifically A, B, I, K, and N (Seinost et al., 1999; Dykhuizen et al., 2008). For this study, we label these five types human invasive strains (HIS). Hence, understanding the relative occurrence and differential transmission efficiencies of *B. burgdorferi* genotypes can offer important insights to Lyme disease risk at the finer, genotypic scale.

In this study, we addressed the following two questions here: (1) What are the probabilities of occurrence of *ospC* types in hosts and the transmission efficiencies of the various *ospC* types from infected hosts to ticks? (2) How do the relative frequencies of HIS types and non-HIS types differ among phylogenetically distinct but frequently co-occurring host groups (e.g. shrews vs. rodents vs. birds)? Due to different ecological, behavioral and physiological traits among the groups, these traits could influence host–tick interactions, infection probability, and the potential to spread the bacterium at different geographic scales. Thus, examining *ospC* variation among these basic groups provides a good foundation for future investigations on *ospC* genotypic variation at the host community level.

2. Methods

2.1. Maximum likelihood model

To obtain probabilities of occurrence and transmission efficiencies from infected hosts to ticks, we used a likelihood-based occupancy approach (MacKenzie et al., 2002), which utilizes field data on naturally occurring *B. burgdorferi* infection in various hosts and the ticks that feed upon them. The principle of this approach is based on well-known ecological species occupancy models, which seeks to estimate the occurrence of species in habitats that may be difficult to survey, and in which detection is uncertain (MacKenzie et al., 2002; McCallum, 2013). Our model is a corollary to such models; here the aim is to detect *ospC* types (“species”) within target hosts (“habitat”) through the use of multiple larval ticks feeding on those host species (with the ticks serving as the “detection” method). The method requires multiple attempts at “detection” (ticks feeding) per “habitat” (host), and uses the numbers of true and false negatives and positives (transmission to one or more of the ticks feeding on a given host) to provide maximum likelihood estimates of both the occurrence rate of any particular *ospC* genotype in a given host species and the probability of transmission (“detection”) of that *ospC* genotype from that host to the ticks feeding on that host.

Because transmission efficiencies of *ospC* types from hosts to tick are assumed to be less than 1 (Brisson and Dykhuizen, 2004; Hanincova et al., 2006), “detection” probabilities are also (routinely) less than 1. Thus, absence of an *ospC* type from a given tick could be the result either of the absence of that type from that host or from failure of transmission of the *ospC* type from the host to the tick being sampled. This method provides simultaneous maximum likelihood estimates of both occupancy (ψ rates) and probabilities of successful transmission (ε rates) of those *ospC* types to ticks from different vertebrate host species. The approach is robust for even small numbers of replicate ticks per host, as long as the detection probabilities of the *ospC* types in the host are greater than approximately 0.3 (MacKenzie et al., 2002). The important features of the method are that it accounts for variation in both host and tick sample sizes, allows for sampling variation associated with both hosts and ticks (e.g., genetic, feeding success, intra-specific variation, etc.), and that both parameters (ψ and ε) are estimated from information on *ospC* types from the sampled ticks. The novel use of a patch-occupancy model for estimation of infection and transmission rates of *ospC* types drawn from different vertebrate hosts should provide a powerful approach for the elucidation of disease risk associated with *B. burgdorferi*, and can be extended to other vector-borne zoonotic diseases.

For each of the *ospC* genotypes, we compared two alternative models: (a) a null model that ignored the identity of the vertebrate host and estimated a separate probability of occurrence (ψ) for each *ospC* type, averaged over all host species, and an average transmission efficiency (ε) of that *ospC* type from the vertebrate hosts to ticks; and (b) a contrasting species-specific model of separate (ψ) rates for each host species and average (ε) for each *ospC* type. Only host species with at least one positive tick for a particular *ospC* type were included in the model, as the method cannot infer both ψ and ε from an absence of bacteria. We did not analyze *ospC* type J, because we only detected this genotype in a single host species, so that cross-species comparisons were not possible. Hence, only 16 of 17 recovered types were analyzed for the competing models.

Maximum likelihood estimates and two-unit support intervals (likelihood analogues of 95% confidence intervals) for the parameters of each model were obtained after 2500 iterations, using global optimization methods in the likelihood 1.5 package (Murphy, 2012) in R version 2.15.1 (R, 2012). The two models (null vs. species- & strain-specific) were compared using AIC, corrected for small sample size (AIC_c) (Burnham and Anderson, 2002). The comparison provides an explicit evaluation of the hypothesis that ψ and ε values for a given *ospC* type differ among species of vertebrate hosts, with the null hypothesis of no differences rejected if the more elaborate model had a lower AIC_c ($\Delta AIC_c > 2$). The magnitude of the difference in AIC_c between the two models provides a measure of the strength of evidence for the best model, after controlling for the different numbers of parameters in the two models (Burnham and Anderson, 2002).

Lastly, we examined for relative differences in HIS and non-HIS types among different species and their phylogenetic groups using contingency table analysis. All analyses were conducted using R version 2.15.1 (R, 2012).

2.2. Field methods

To assess the association of *ospC* types with reservoir hosts, animals carrying ticks were captured during the summers of 2008–2010 at the Cary Institute of Ecosystem Studies in Millbrook, NY, an area of endemic Lyme disease and high incidence rates (NYSDOH, 2013). Many of the species examined were captured as part of a larger study that examined host reservoir competency for various tick-borne pathogens (Keesing et al., 2009, 2012; Hersh et al., 2012). Mammals were live-trapped and birds were mist netted (IACUC #06-01 and 09-01) in mid to late summer each year, coinciding with the peak activity for larval ticks at our sites (Ostfeld et al., 1996). All animals were moved temporarily (<1 week) to the laboratory, where they were held in appropriately sized cages with wire mesh floors and pans of water or moistened paper towels beneath the floors. We collected fully fed larvae that dropped from the hosts and allowed them to molt into nymphs before flash-freezing them for DNA extraction and *B. burgdorferi* characterization (protocol following Keesing et al., 2009). As