Supplemental Materials

Methods

Study sites, tick, and host collection

We collected *Peromyscus leucopus* mice at three sites on Block Island, Rhode Island (BI): North Island (NI): 41°12'36.4"N, 71°34.18.8"W; East Island (EI): 41°09'47.6"N, 71°33'58.1"W; and Rodman's Hollow (RH): 41°09'25.2"N, 71°35'22.9"W and three sites in Connecticut (CT): Hilltop (HT): 41°22'27.0"N, 72°46'40.6"W; Lakeside (LS): 41°21'49.5"N, 72°46'35.8"W; and Old Lyme (OL): 41°22'32.0"N, 72°20'38.4"W over a three year period (2014-2016). At each location a trapping grid was established. Traps were placed every 10 m within the grid (HT: 12x11 grid = 132 traps; LS: 12x12 = 144 traps; OL: 8x15 = 120 traps; NI: 15x4 = 58 traps; EI: 10x6 = 60 traps; RH: 12x10 = 120 traps). Sherman live traps (7.62 cm × 8.89 cm × 22.86 cm; H.B. Sherman Traps, Inc. Tallahassee, FL) were baited with peanut butter, oats, and sunflower seeds and deployed for three consecutive trap nights per trapping session. Trapping occurred at each location on a biweekly basis from May to August 2014-2016, for a total of seven trapping sessions. We collected morphological traits (age, sex, weight, body measurements, etc.) from each animal, removed feeding ticks from the ears and body (preserved in 100% ethanol), administered a uniquely numbered ear tag, removed a small 3 mm ear punch biopsy (preserved in 100% ethanol), and collected a small blood sample (via submandibular vein) on a Whatman non-indicating FTA classic card (Fisher Scientific, Pittsburgh, PA). In 2014, blood samples from two BI sites (NI and EI) were lost and therefore not included in any analyses. Processed mice were released at the site of capture. Tissue and blood samples were only collected once during a trapping session, but attached ticks were removed every time an animal was captured. All animal procedures were in accordance with guidelines approved by the Columbia University Institutional Animal Care and Use Committee (IACUC no. AC-AAAL3656).

We collected host-seeking *I. scapularis* nymphs by dragging a 1 m^2 white corduroy cloth between the traps within each grid, stopping every 10 m to remove all attached ticks. Nymphs were stored in 100% ethanol. Tick phenology characterized in terms of mouse burden was used to derive parameters for the

start of overwintered tick emergence (the day when burden was first non-zero), the time until peak burden, and the magnitude of the peak burden. Parameter estimates varied between years and between study sites. We combined data from our study with estimates from the literature (Supp Tables S1, S2).

DNA extraction and infection assessment

We extracted DNA from the ear punch biopsy using the QIAcube HT DNA extraction system following the manufacturer's protocol (Qiagen, Valencia, CA) and DNA concentration was measured using a spectrophotometer (Denovix Inc, New Castle County, Delaware). DNA from the ear biopsy was then screened in duplicate for the presence of *Borrelia burgdorferi* (*Bb*) using a quantitative PCR (qPCR) protocol specific for a unique 69 bp segment of the 16S rRNA gene; forward primer: 5' GGC GGC ACA CTT AAC ACG TTA G 3', reverse primer: 5' GCT GTA AAC GAT GCA CAC TTG GT 3', probe: 6FAM-TTC GGT ACT AAC TTT TAG TTA A-MGBNFQ [1]. Samples were screened with a 7500 real-time PCR system (Applied Biosystems®, ThermoFisher Scientific, Waltham, WA) using TaqMan Fast Advanced chemistry (ThermoFisher Scientific, Waltham, WA) and cycling conditions of: 95°C for 20 s, followed by 40 cycles of 95°C for 3 s and 60°C for 30 s.

We extracted DNA from the dried blood samples on Whatman FTA cards (Fisher Scientific, Pittsburgh, PA) using the QIAcube HT DNA extraction system (Qiagen, Valencia, CA) and DNA concentration was analyzed using a spectrophotometer (Denovix Inc, New Castle County, Delaware). DNA from the blood samples was screened in duplicate for the presence of *Babesia microti* (*Bm*) using a qPCR protocol designed specifically for detecting a 104 bp section of the 18S rRNA gene (forward primer: 5' AAC AGG CAT TCG CCT TGA AT 3', reverse primer: 5' CCA ACT GCT CCT ATT AAC CAT TAC TCT 3', probe: 6FAM-CTA CAG CAT GGA ATA ATG A-MGBNFQ) [2]. These qPCR assays are highly sensitive and specific for *Bb* and *Bm*, therefore we are confident our infection prevalence data is accurate.

Liquid nitrogen was used to pulverize the exoskeleton of host-seeking nymphs, which were then incubated overnight in lysis buffer and proteinase K. After incubation, DNA was extracted using the aforementioned procedures for mouse ear punch biopsy and dried blood samples. qPCR was also

completed using the same protocols as for the mouse samples. qPCR standards were constructed by separately cloning the aforementioned targeted regions of *Bb* and *Bm* into pUC57-Kan plasmids (GENEWIZ, Inc., South Plainfield, NJ). A dilution series (10^6-1 copy number dilutions) for each pathogen was developed by combining a single uninfected *I. scapularis* nymph (acquired from the CDC) and a known amount of plasmid DNA followed by DNA extraction [1, 2]. Average cycle threshold (CT) and quantity values were collected from each run and mean infection prevalence (number of infected individuals/total number of individuals) was calculated for each location.

Estimation of transition intensities between mouse infection states

We used a multi-state Markov (MSM) model for mark-recapture data to estimate the transition intensities between different *P. leucopus* infection states: uninfected (0), *Bb* infected (1), *Bm* infected (2), or coinfected with both pathogens (12). The infection state of a mouse was recorded each time it was caught, and each year contained seven field sessions. Some mice were sampled in multiple sessions (recaptures), and some were sampled only once (single captures) in a year, but individual mice were never observed or collected between years. For each year, the dataset was composed of a 7-point time series for each mouse $Y_{i,t}$ where $i = 1..N$ is the mouse identifier, $t = 1..7$ is the session, and $Y_{i,t} \epsilon \{-1, 0, 1, 2, 12\}$ is the infection state of mouse i in session t, with -1 indicating that the mouse was not observed in that session.

We used the Marked program [3] with a multistrata Cormack-Jolly-Seber model for the MSM analysis. The model framework is composed of parameters representing the probability (S) of survival between two sample sessions, the probability (p) of being observed at a given sample session and the probabilities (ψ_{ij} for $i, j \epsilon \{0,1,2,12\}$) of transition from state i to state j between two consecutive field sessions. Since our data is relatively limited, we use the simplest possible framework in which all parameters are independent of time and age, while S and p are also independent of infection state. The probability of any given sequence of state observations can be computed as a product of these parameters [4]. We used the Mark program, via the RMark interface [5], to calculate maximum likelihood estimates

of *S*, *p*, and the 16 ψ_{ij} parameters for the combined state observation sequences of all mice in all years for each field location (BI and CT). Our code is available in the GitHub repository at https://github.com/cowparsley/borrelia-babesia-eco-epi. Our data files can be downloaded from Dryad at https://doi.org/10.5061/dryad.573n5tbd3.

Mechanistic mathematical model

We developed a mechanistic eco-epidemiological model to examine how interactions between *Bb* and *Bm*, combined with vertical transmission of *Bm*, drive the epidemiological dynamics of both pathogens. The model integrates two components: an ecological model of tick and host population dynamics without infection, and an epidemiological model of infection dynamics in the populations represented by the ecological model. The model formulation aims to capture the complex seasonality and epidemiological dynamics of the system in a simple framework using a semi-discrete-time approach. Each year is divided into two projection intervals, `summer' (day 90 to day 240, roughly April to September) and `winter' (day 241 to day 90, roughly October to March). In the summer interval the complete eco-epidemiological dynamics are modelled as continuous-time processes. In the winter interval the only ongoing processes are mortality and disease recovery. These are modelled by using the probabilities of survival and recovery over the entire winter interval to update the population sizes between the end of one summer interval and the beginning of the next.

Ecological model of tick and host population dynamics

In the ecological model, we consider the population dynamics of *P. leucopus* mice and *I. scapularis* ticks. In this model, we let $T = 1, 2 \ldots$ be a discrete variable indicating the year and t be a continuous variable indicating the time in days since the beginning of any given year. We let $90 \leq t \leq 240$ so that t spans the summer interval. We have variables for the density of mice ($M^T(t)$), the density of tick eggs ($E^T(t)$), questing larvae ($L^T(t)$), engorged larvae ($W^T(t)$), and questing nymphs ($N^T(t)$) on day t of year T. Engorged nymph and adult populations were not included in the model because they have a negligible role in the epidemiological dynamics.

In each year T, the mouse population dynamics over the summer interval are modelled by a logistic reproduction rate, with intrinsic growth rate (r), reproductive carrying capacity (K), and a constant mortality rate (μ). Reproduction and mortality are separated in the logistic model in order to incorporate vertical transmission of *Bm* (v). At the beginning of the summer interval in year T the mouse population size is $M^T(t = 90) = \omega_M M^{T-1}(t = 240)$ where ω_M is the winter survival probability.

In each year T, the tick population dynamics over the summer interval are modelled by the processes of emergence from overwintered states, questing, and diapause. At the beginning of each summer interval there are a constant number of overwintered eggs (Ω) together with overwintered unengorged and engorged larvae from the previous year. Larvae hatch from eggs at constant rate (η_L) beginning on day $t = \tau_L$. Emergent larvae join the questing larval population ($L^T(t)$) immediately. Overwintered unengorged larvae re-emerge and join the questing population at rate η_E from day $t = \tau_E$. All questing larvae contact mice, and enter the engorged class $W^T(t)$, at per capita rate $\lambda(M^T(t) + D)$ where D is the density of hosts that are not competent for either pathogen (e.g. white-tailed deer). Engorged larvae do not molt into nymphs until the following year. Overwintered engorged larvae emerge as nymphs at rate η_N from day $t = \tau_N$. Nymphs join the questing nymph population $N^T(t)$ immediately after emerging. Questing nymphs contact mice at per capita rate $\lambda(M^T(t) + D)$, become engorged, and are effectively removed from the system because adult states do not feature in the model. At the end of the summer interval all questing and engorged larvae enter a diapause state to overwinter and all questing nymphs die. While some questing nymphs may not find a host and will overwinter, the extent of overwintering in nymphs has not been quantified. Based on observations of larvae, the numbers of overwintering nymphs appear to be relatively small. Unlike larvae, emerging overwintered nymphs do not produce a distinct signal in the observed nymph population dynamics. Epidemiologically, all nymphs have the same role. Therefore, in the interests of parsimony, we omitted overwintering nymphs from the model. At the beginning of the summer interval in year T the overwintered unengorged larval population density is $\omega_L L^{T-1}(t = 240)$ and the overwintered engorged larval population density is $\omega_L W^{T-1}(t =$

240) where ω_L is the winter survival probability (Supp Table S2). Because hatching and emergence rates are constant, the population density of eggs, overwintered unengorged larvae, and overwintered engorged larvae at any time t of the summer interval after emergence has begun are, respectively, $\Omega e^{-\eta_L(t-\tau_E)}$, $L^{T-1}(240)e^{-\eta_E(t-\tau_E)}$, and $W^{T-1}(240)e^{-\eta_N(t-\tau_N)}$.

Epidemiological model of Bb and Bm infection dynamics

In the epidemiological model we consider the infection dynamics of *Bb* and *Bm* in populations of *P. leucopus* mice and *I. scapularis* ticks with dynamics driven by the ecological model. Each population is subdivided according to infection state, designated as uninfected (state 0), infected with *Bb* (1), infected with *Bm* (2), and coinfected with both pathogens (12). Thus, the mouse population in year T has partitions: uninfected M_0^T, *Bb* infected M_1^T, *Bm* infected M_2^T, and coinfected with both pathogens M_{12}^T. The total mouse population is represented by: $\bar{M}^T = M_0^T + M_1^T + M_2^T + M_{12}^T$. The tick population in year *T* is partitioned in a similar way. *Bb* and *Bm* are not transovarially transmitted in *I. scapularis* [6, 7]; therefore, questing larvae are always categorized as uninfected. The number of engorged larvae in each infection state are: W_0^T, W_1^T, W_2^T, and W_{12}^T while the number of questing nymphs in the environment in each infection state are: N_0^T, N_1^T, N_2^T, and N_{12}^T. Larvae and nymphs bite mice at rate $\lambda\bar{M}^T(t)$ as described above. Tick attachment to a host may result in pathogen transmission if either party is infected. The probability of transmission depends on the infection states of the transmitter and recipient (Supp Table S3). Based on previous research, we incorporated into the model three mechanisms hypothesized to influence transmission of *Bb* and *Bm*. Firstly, an existing *Bm* infection increases mouse susceptibility to *Bb* by a factor $\alpha > 1$. Secondly, coinfection increases the transmission probability of *Bm* from a mouse by a factor $\sigma > 1$ [8]. Thirdly, coinfection reduces the transmission probability of *Bb* from a mouse by a factor $\xi < 1$ (unpublished data derived from reference 8).

Note that in all cases of transmission enhancement, the transmission probability is bounded above by 1. In coinfected mice or nymphs, *Bb* and *Bm* infections are transmitted independently with the same probabilities as in singly infected individuals, implying that the model is not neutral with respect to strain

interactions [9] and that coinfected individuals are intrinsically more infectious (see Supp Table S3 for details of the transmission probabilities that arise from these assumptions).

In addition to horizontal transmission, *Bm* may be transmitted vertically from an infected female to her offspring with probability v. Horizontal transmission is not affected by coinfection and evidence for vertical transmission of *Bb* in *P. leucopus* mice is lacking [10, 11, DMT unpublished data] and therefore not included in the model.

Mice recover from *Bb* infection, returning to a susceptible state, at constant rate (γ) during the summer interval. Recovery continues at the same rate throughout the winter interval. Thus, the probability of recovery over the entire winter interval (duration $\tau_m = 215$ days) is: $1 - \exp(-215\gamma)$. Overwinter recovery is accounted for at the beginning of each summer season by moving proportions $1 - \exp(-215\gamma)$ from M_{12}^T to M_2^T, and from M_1^T to M_0^T. In the model, coinfection does not affect the recovery rate and mice do not recover from *Bm* infection (i.e. infection is considered chronic). Although some evidence exists that recovery occurs at a low rate, life-long chronic infection is a reasonable approximation as *Bm* infection was shown to persist on average for 9 months [12] and the average life expectancy of wild *P. leucopus* mice is less than 6 months [13]. Our MSM model analysis of the field data showed that recovery from *Bb* was common, but recovery from *Bm* was rare. Ticks do not recover from *Bb* or *Bm* infections.

The complete system is described by discrete-time updates

$$\begin{bmatrix} M_0 \\ M_1 \\ M_2 \\ M_{12} \end{bmatrix}^{T+1} (t = 90) = \omega_M \begin{bmatrix} 1 & 1 - e^{-\gamma\tau_m} & 0 & 0 \\ 0 & e^{-\gamma\tau_m} & 0 & 0 \\ 0 & 0 & 1 & 1 - e^{-\gamma\tau_m} \\ 0 & 0 & 0 & e^{-\gamma\tau_m} \end{bmatrix} \begin{bmatrix} M_0 \\ M_1 \\ M_2 \\ M_{12} \end{bmatrix}^{T} (t = 240)$$

$$L_i^T(t = 90) = \omega_L {L_i}^{T-1}(t = 240),\ N_i^T(t = 90) = \omega_L {W_i}^{T-1}(t = 240),\ i = 0, 1, 2, 12$$

and the following differential equations. Subscript 1 corresponds to *Bb*, while subscript 2 corresponds to *Bm*:

$$\frac{dL^T}{dt} = \begin{cases} 0 & 90 \le t < \tau_E \\ L^{T-1}(240)\eta_E e^{-\eta_E(t-\tau_E)} - \lambda L^T\left(\overline{M}^T + D\right) & \tau_E \le t < \tau_L \\ L^{T-1}(240)\eta_E e^{-\eta_E(t-\tau_E)} + \Omega\eta_L e^{-\eta_L(t-\tau_L)} - \lambda L^T\left(\overline{M}^T + D\right) & \tau_L \le t \le 240 \end{cases}$$

$$\frac{dW_0^T}{dt} = \lambda L^T(M_0^T + (1-\beta_1^{ML})M_1^T + (1-\beta_2^{ML})M_2^T + (1-\xi\beta_1^{ML})(1-\sigma\beta_2^{ML})M_{12}^T + D)$$

$$\frac{dW_1^T}{dt} = \lambda L^T(\beta_1^{ML}M_1^T + \xi\beta_1^{ML}(1-\sigma\beta_2^{ML})M_{12}^T)$$

$$\frac{dW_2^T}{dt} = \lambda L^T(\beta_2^{ML}M_2^T + (1-\xi\beta_1^{ML})\sigma\beta_2^{ML}M_{12}^T)$$

$$\frac{dW_{12}^T}{dt} = \lambda L^T(\xi\beta_1^{ML}\sigma\beta_2^{ML}M_{12}^T)$$

$$\frac{dN_i^T}{dt} = \begin{cases} 0 & 90 \le t < \tau_N \\ W_i^{T-1}(240)\eta_N e^{-\eta_N(t-\tau_N)} - \lambda N_i^T\left(\overline{M}^T + D\right) & \tau_N \le t \le 240 \end{cases} \quad \text{for } i = 0, 1, 2, 12$$

$$\frac{dM_0^T}{dt} = r\left(M_0^T + M_1^T + (1-v)(M_2^T + M_{12}^T)\right)\left(1 - \frac{\overline{M}^T}{K}\right) + \gamma M_1^T - \lambda M_0^T(\beta_1^{NM}N_1^T + \beta_2^{NM}N_2^T +$$

$$(\beta_1^{NM} + \beta_2^{NM} - \beta_1^{NM}\beta_2^{NM})N_{12}^T) - \mu M_0^T$$

$$\frac{dM_1^T}{dt} = \lambda M_0^T(\beta_1^{NM}N_1^T + \beta_1^{NM}(1-\beta_2^{NM})N_{12}^T) - \lambda M_1^T\beta_2^{NM}(N_2^T + N_{12}^T) - (\gamma+\mu)M_1^T$$

$$\frac{dM_2^T}{dt} = rv(M_2^T + M_{12}^T)\left(1 - \frac{\overline{M}^T}{K}\right) + \lambda M_0^T(\beta_2^{NM}N_2^T + (1-\beta_1^{NM})\beta_2^{NM}N_{12}^T) + \gamma M_{12}^T - \lambda M_2^T\alpha\beta_1^{NM}(N_1^T$$

$$+ N_{12}^T) - \mu M_2^T$$

$$\frac{dM_{12}^T}{dt} = \lambda\left(M_0^T\beta_1^{NM}\beta_2^{NM}N_{12}^T + M_1^T\beta_2^{NM}(N_2^T + N_{12}^T) + M_2^T\alpha\beta_1^{NM}(N_1^T + N_{12}^T)\right) - (\gamma+\mu)M_{12}^T$$

Note that $\beta_1 + \beta_2 - \beta_1\beta_2 = 1 - (1-\beta_1)(1-\beta_2)$ is the probability that at least one pathogen is transmitted from a coinfected nymph.

The model was solved numerically for t = 50 years with initial conditions $M_0^1(90)$ = 15.48, $M_1^1(90)$ = 0.00004, $M_2^1(90)$ = 5.57, $M_{12}^1(90)$ = 0 and all other variables 0 for the BI field sites and $M_0^1(90)$ = 9.372, $M_1^1(90)$ = 0.0008, $M_2^1(90)$ = 0.0002, $M_{12}^1(90)$ = 0 and all other variables 0 for the CT field sites.

With the default parameter values these initial conditions quickly produce trajectories that are in reasonable agreement with the field data. By t = 50 years the model state changes very slowly, and we assume it is close to steady state.

Parameter estimation

We used Approximate Bayesian Computation (ABC) to estimate parameter values for the mechanistic model consistent with the data from each of the two field locations (BI and CT). Because the mouse and tick population dynamics do not depend on the epidemiological dynamics, we simplified the parameter space with a two-stage estimation process. In stage 1, we considered a reduced model limited to the mouse and tick population dynamics. We estimated mouse and tick demographic parameters consistent with field observations of mouse density and tick burdens using ABC with uniform priors for all parameters. In stage 2, we considered the full eco-epidemiological model. We estimated, or re-estimated, all model parameters consistent with field observations for mouse density, tick burdens, and infection prevalence. For demographic parameters where stage 1 produced a clearly non-uniform posterior, we approximated this distribution with a log-normal function and used it as the prior for stage 2. For transmission parameters where we have point estimates based on experimental observations we used a log-normal prior with mean equal to the experimental value. For all other parameters we used uniform priors (stage 1 output and full prior specifications are in Supp Table S2). A total of 5×10^5 trials (simulation runs with unique parameter sets drawn from the prior distributions) were computed for each field site using the easyabc package in R [14]. Trials for which the parameter combination resulted in *Bb*, *Bm*, or both being entirely absent were removed, leaving 3.61×10^5 trials for BI and 2.64×10^5 trials for CT. Rejection sampling was applied to these remaining trials with an acceptance tolerance of 0.005 using the abc package in R [15]. This algorithm uses Euclidian distance to calculate the similarity between the empirical and model timeseries. A total of 1804 trials were accepted for BI and 1321 for CT. We calculated the 10%, 50%, and 90% quantiles of the accepted parameter distributions for all the parameters used in the mechanistic model and we compared the demographic and epidemiological trajectories from the model with the field data from BI and CT.

Sensitivity analysis

The sensitivity of the model to the parameterization was assessed using the Fourier Amplitude Sensitivity Test (FAST) method [16] implemented in R using the fast package [17]. The model has a total of 24 parameters for which the fast algorithm generates 3427 parameter sets. Parameter values were chosen from between the 10% and 90% quantiles of the BI and CT posterior distributions given in Supp Table S5. For each parameter set the model was solved to a presumed steady state as described above. The fast algorithm was then used to determine the sensitivity of each summary state variable (mouse density, larval burden, nymph burden, mouse *Bb* prevalence, mouse *Bm* prevalence, nymph *Bb* prevalence, and nymph *Bm* prevalence) to each parameter at 31 time points between day 90 and 240.

Endemic threshold

We examined how the presence or absence of *Bb* and *Bm* in this system depends on key ecological and epidemiological factors. A standard approach is to evaluate the basic reproduction numbers R_0 which determine the viability thresholds at $R_0 = 1$. However, the complex seasonality of the system makes it challenging to construct R_0. Instead, we implicitly determined how the thresholds $R_0 = 1$ depend on certain parameters by examining whether numerical solutions of the system tend to an asymptotic state where the infection is present or absent. Because the population dynamics of *Bb* and *Bm* are driven by many common processes, we focused on the parameters that differed between the pathogens and can thus result in divergent viability thresholds. Mice do not recover from *Bm* infection, but they do recover from *Bb* infection at rate γ. *Bm* has a lower mouse-to-tick transmission rate (β_i^{ML}) than *Bb*. *Bm* is transmitted vertically in mice at rate v, but *Bb* is not. Therefore, we examined how the viability of *Bb* and *Bm* depends on (1) the density of tick eggs at the beginning of each season (Ω) which effectively scales the size of all tick life-stages, (2) the probability that a mouse survives the winter (ω_M) which facilitates *Bm* persistence due to chronic infection, and (3) the probability of vertical transmission of *Bm* (v) which potentially interacts with the probability that a mouse survives the winter (ω_M). We fixed all other parameters at the median values estimated for each field site, as in Supp Table S2. For the given ω_M and

v parameters, we solved the system numerically to determine threshold values of Ω required for asymptotic states with *Bb* or *Bm* present. The numerical solutions used initial conditions $M_0^1(90) = 21.05$, $M_1^1(90) = 0.0001$, $M_2^1(90) = 0.0001$, $M_{12}^1(90) = 0$, $L^1(90) = 8993$, $N_0^1(90) = 19652$ and all other variables 0 for the BI field sites and $M_0^1(90) = 9.372$, $M_1^1(90) = 0.0001$, $M_2^1(90) = 0.0001$, $M_{12}^1(90) = 0$, $L^1(90) = 26520$, $N_0^1(90) = 20392$ and all other variables 0 for the CT field sites. These initial conditions place the system close to the demographic steady state but with a small number of mouse infections in states M_1 and M_2. The solution was computed for 50 years. For each year, the total number of mouse infections was calculated at the beginning of the continuous-time period of each year. For the final year, the maximum infection prevalence in mice was also determined. If the total number of mouse infections at the beginning of the year showed a year-on-year decrease for at least the last 10 simulation years and the maximum mouse infection prevalence was less than 10^{-5} then the asymptotic state was defined as absent, otherwise it was defined as present. The Ω thresholds for presence/absence were calculated using the R function 'bisection' with 20 iterations. The function we supplied to the bisection algorithm solves the differential equation model to near steady-state and returns -1 if the specified infection is absent or 1 if it is present. The bisection algorithm then approximately determines the values of Ω at which the return value of the function switches between -1 and 1.

Results

Estimation of transition intensities between mouse infection states

Infection status was assessed from a total of 879 observations (479 unique *P. leucopus* mice) from the BI sites and a total of 932 observations (535 unique mice) from the CT sites (Supp Table S4; Supp Fig 1). For the BI sites, a total of 395 observations were recaptures of the same mouse, the remaining observations were mice only singly captured. Single captures cannot provide any information about infection state transitions and were therefore not included in the analysis. There were 204 observation pairs in which a mouse remained in state 2 (*Bm* infected), 53 in which a mouse transitioned

from state 2 to state 12 (coinfected), 88 in which a mouse remained in state 12, and 36 in which a mouse transitioned from state 12 to state 2 (Supp Table S6). All other state transitions were observed rarely, or never. Using the Mark program, we produced maximum likelihood estimates (with 95% confidence intervals) for the survival probability between field sessions of S = 0.74 (0.69 – 0.77), the observation probability p = 0.53 (0.48 – 0.58), and state transition probabilities ψ_{ij} (Supp Table S7). Very similar estimates for ψ_{ij} were obtained using the R package msm [18] to estimate continuous-time transition rates and then using these to calculate the transition probabilities over discrete time intervals. The small number, or absence, of observations for state transitions involving the uninfected or *Bb* infected states resulted in very broad confidence intervals for these transition probabilities that limit meaningful interpretation. For *Bm* infected states we see that between any two field sessions (a period of 2 weeks) mice infected with *Bm* only (state 2) either remain in that state (probability 0.81) or become coinfected with *Bb* (state 12, probability 0.18). Coinfected mice either remain in that state (probability 0.74) or become infected with *Bm* only (probability 0.25).

For the CT sites, there were also a total of 395 observations involving the same mouse at different sample points. The remaining observations related to mice only observed once and so do not provide any information about state transitions. There were 145 observation pairs in which a mouse remained in state 2 (*Bm* infected), 73 in which a mouse transitioned from state 2 to state 12 (coinfected), 51 in which a mouse remained in state 12, and 48 in which a mouse transitioned from state 12 to state 2 (Supp Table S6). In contrast to the BI sites, there are also 18 observation pairs in which a mouse remained in state 0 (uninfected), 26 in which a mouse transition from state 0 to state 2, 11 in which a mouse transitioned from state 0 to state 12, and 12 in which a mouse transitioned from state 2 to state 0. All other state transitions were observed rarely. The Mark program produced maximum likelihood estimates (with 95% confidence intervals) for the survival probability between field sessions of S = 0.59 (0.55 – 0.62), observation probability of p = 0.77 (0.72 – 0.82), and state transition probabilities ψ_{ij} (Supp Table S7). Very similar estimates for ψ_{ij} were obtained by using the R package msm [18] to estimate continuous-time transition

rates and then using these to calculate the transition probabilities over discrete time intervals. The small number of observations for state transitions involving the *Bb*-only infected states resulted in wide confidence intervals for these transition probabilities that limit meaningful interpretation. For *Bm* infected states we see that between any two field sessions most uninfected mice remain in that state (probability 0.34), become infected with *Bm* (state 2, probability 0.46), or become coinfected (state 12, probability 0.18). Most mice infected with *Bm* only either remain in that state (probability 0.63) or become coinfected with *Bb* (state 12, probability 0.31). Most coinfected mice either remain in that state (probability 0.52) or become infected with *Bm* only (probability 0.46) (Supp Table S7).

This analysis of mouse infection states indicates that the prevalence of *Bm* infection was very high in these populations and uninfected mice were uncommon. Mice infected with *Bb* only were rare; however, around 28% of observations recorded mice coinfected with *Bb* and *Bm*. The small number of mice observed in the uninfected or *Bb*-only infected states limits insight into the relative transmission efficiencies of *Bm* and *Bb*, or the effect of an existing *Bm* infection on *Bb* susceptibility. The analysis showed that recovery from *Bb* was common, while recovery from *Bm* was rare (Fig. 2). It is curious, that 12 mice in the CT region appear to have recovered from single *Bm* infections, but no mice in either region appear to have recovered from *Bm* while in the coinfection state.

Mechanistic mathematical model

Parameter estimation

For both field locations (BI and CT) the posterior distributions for all parameters estimated by ABC are tightly focused for mouse carrying capacity (K), the day larvae emerge from eggs (τ_L), the day nymphs emerge from diapause (τ_N), daily tick-host encounter rate (λ), the density of tick eggs at the beginning of the season (Ω), the probability of tick overwinter survival (ω_L), and the density of non-competent hosts (D) (Supp Table S5; Supp Fig. S4). These distributions indicate that the field data constrains these parameters quite tightly. In contrast, the posterior distributions for the daily mouse intrinsic growth rate (r), daily mouse mortality rate (μ), the proportion of mice that survive the winter

(ω_M), the day unengorged larvae emerge from diapause (τ_E), the early unengorged larval emergence rate from diapause (η_E), the daily late larval emergence rate from eggs (η_L), the daily emergence rate of nymphs (η_N), the daily engorged tick detachment from host rate (δ), and the increased *Bm* transmission probability to *Bb* infected mice (α) are close to their uniform priors (Supp Table S5; Supp Fig. S2, S3). These distributions indicate that these parameters are not constrained by the field data; the relative sensitivity of the model to these parameters is insufficient to produce a signal that can be detected in the field data. Finally, the probability of *Bm* vertical transmission (v), the probability of host-tick and tick-host transmissions (β_1^{ML}, β_1^{NM}, β_2^{ML}, β_2^{NM}), the increased *Bm* transmission probability from coinfected mice (σ), and the decreased *Bb* transmission probability from coinfected mice (ξ) are close to their lognormal priors. These distributions indicate that these parameters are not further constrained by the field data; the relative sensitivity of the model to these parameters is insufficient to produce a signal that can be detected in the field data.

The posterior distributions are similar for both field locations for all parameters except for mouse reproduction carrying capacity (K), the density of tick eggs at the beginning of each season (Ω), the density of non-competent hosts (D), and the daily *Bb* recovery rate in mice (γ). The posterior distribution for mouse reproductive carrying capacity (K) is centered around higher values on BI than in CT which reflects the higher mouse densities on BI evident in the field data (Supp Fig. S1). The posterior distribution for the density of tick eggs at the beginning of each season (Ω) is centered around higher values on BI than in CT; this reflects the higher larval burden on BI evident in the field data (Fig. 1). The posterior distribution for the density of non-competent hosts (D) is centered around higher values and is more dispersed for BI than for CT. Finally, the posterior distribution for the daily rate of recovery in *Bb* infection in mice (γ) is centered around higher values for BI compared to CT. However, the variance is also quite high so the signal for the difference between sites is weak (Fig 1; Supp Fig. S4).

Mechanistic analysis of the model

The mechanistic model trajectories have very low *Bb* prevalence at the beginning of the season but increases rapidly once the nymphs emerge around day 110 (Fig. 4A-D; Supp Table S5). A gradual, constant decline in prevalence from day 140 is observed because most nymphs have fed on a host by then and the model dynamics are driven by recovery and mortality. Most *Bb* infections in mice occur as coinfections with *Bm* (Supp Table S4), reflecting the high prevalence of *Bm* throughout the season, but single infections do occur (Fig. 4A-D). The seasonal pattern of *Bm* prevalence is more complex. At the beginning of the season a high prevalence of single *Bm* infections is observed, likely due to persistent infection in the overwintered population and ongoing vertical transmission. The prevalence of single infections arising from tick-borne horizontal transmission in the previous season which decreases quickly due to mortality, but the prevalence of single infections from vertical transmission increases due to a burst of rapid population growth (infected offspring join the population). This growth slows as the population approaches carrying capacity and, around the same time, the nymphs emerge and intense *Bb* transmission rapidly converts single *Bm* infections to coinfections. From day 140 most of the nymphs have fed and there is a gradual, constant decline in coinfections due to mortality and recovery from *Bb* infection, the latter of which leads to a corresponding increase in single *Bm* infections. Single *Bm* infections due to vertical transmission also gradually increase due to ongoing population turnover (Fig. 4A-D).

The probability that a mouse is in any given infection state over the course of the season was calculated from trajectories of the mechanistic model with default parameter values (Supp Fig. S5A-D). At the beginning of the season there is a very low probability that a mouse is infected with *Bb*, but a high probability that it is infected with *Bm*. As the overwintered nymphs emerge, the probability that a mouse is coinfected increases rapidly and then declines gradually because most of the nymphs have fed and mice recover from *Bb* infections.

Sensitivity analysis

The sensitivities of summary state variables for infection prevalence (Supp Fig. S6, S7) and sensitivities for demographic state variables (Supp. Fig. S8) were calculated. For mouse *Bb* prevalence,

the key parameter early in the season is γ (daily *Bb* recovery rate), which is related to persistence of the infection in the overwintered population or early season infection. Later in the season the key parameters are Ω (density of eggs at the beginning of each season), ω_L (proportion of ticks that survive winter) which are related to the size of the tick population, and D (density of non-competent hosts) which is related to the concentration of ticks that bite mice. Other influential parameters are ω_M (proportion of mice that survive winter) early in the season, μ (mouse mortality rate), and ξ (decrease in *Bb* transmission probability from coinfected mice). The parameter τ_N (day nymphs begin to emerge from diapause) is also important early in the season because it effectively determines when transmission begins. For mouse *Bm* prevalence, the key parameter early in the season is ω_M (proportion of mice that survive the winter), reflecting persistence of infection in the overwintered population. Later in the season the key parameters are Ω (density of eggs at the beginning of the season), ω_L (proportion of ticks that survive the winter), D (density of non-competent hosts), μ (mouse mortality rate) and, to a lesser extent, v (probability of *Bm* vertical transmission).

For nymph *Bb* and *Bm* prevalences, the key parameter early in the season is τ_N (day nymphs begin to emerge from diapause) which effectively determines when transmission starts. Later in the season the key parameter is D, which relates to the concentration of feeding on mice. For both *Bb* and *Bm*, other influential parameters are Ω (density of eggs at the beginning of each season), ω_L (proportion of ticks that survive the winter), and μ (mouse death rate). For *Bb*, β_1^{ML} (probability of *Bb* transmission from mice to larvae), ξ (decrease of *Bb* transmission probability from coinfected mice), and γ (daily rate of mouse recovery from *Bb* infection) are influential. For *Bm*, β_2^{ML} (probability of *Bm* transmission from mouse to larva) is an influential parameter.

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