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Vertical transmission rates of *Borrelia miyamotoi* in *Ixodes scapularis* collected from white-tailed deer

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Abstract

Borrelia miyamotoi is a relapsing fever spirochete transmitted by ticks in the *Ixodes ricinus* complex. In the eastern United States, *B. miyamotoi* is transmitted by *I. scapularis*, which also vectors several other pathogens including *B. burgdorferi* sensu stricto. In contrast to Lyme borreliosis, *B. miyamotoi* can be transmitted vertically from infected female ticks to their progeny. Therefore, in addition to nymphs and adults, larvae can vector *B. miyamotoi* to wildlife and human hosts. Two widely varying filial infection prevalence (FIP) estimates - 6% and 73% - have been reported previously from two vertically infected larval clutches; to our knowledge, no other estimates of FIP or transovarial transmission (TOT) rates for *B. miyamotoi* have been described in the literature. Thus, we investigated TOT and FIP of larval clutches derived from engorged females collected from hunter-harvested white-tailed deer in 2015 (n = 664) and 2016 (n = 599) from Maine, New Hampshire, Tennessee, and Wisconsin. After engorged females oviposited in the lab, they (n = 492) were tested for *B. miyamotoi* infection by PCR. Subsequently, from each clutch

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Conflict of interest

The authors declare that they have no conflicts of interest.

produced by an infected female, larval pools, as well as 100 individual eggs or larvae, were tested. The TOT rate of the 11 infected females was 90.9% (95% CI; 57.1-99.5%) and the mean FIP of the resulting larval clutches was 84.4% (95% CI; 68.1-100%). Even though the overall observed vertical transmission rate (the product of TOT and FIP; 76.7%, 95% CI; 44.6-93.3%) was high, additional horizontal transmission may be required for enzootic maintenance of *B. miyamotoi* based on the results of a previously published deterministic model. Further investigation of TOT and FIP variability and the underlying mechanisms, both in nature and the laboratory, will be needed to resolve this question. Meanwhile, studies quantifying the acarological risk of *Borrelia miyamotoi* disease need to consider not only nymphs and adults, but larval *I. scapularis* as well.

Keywords

Borrelia miyamotoi; *Ixodes scapularis*; Transovarial transmission; Filial infection prevalence; White-tailed deer

Introduction

Borrelia miyamotoi is a relapsing fever group spirochete transmitted by ticks in the *Ixodes ricinus* complex (Scoles et al., 2001; van Duijvendijk et al., 2016; Breuner et al., 2017), which also transmit *B. burgdorferi* sensu lato spirochetes that may cause Lyme borreliosis, the most common vector-borne disease in Europe and North America (Mead, 2015).

Borrelia miyamotoi has been detected in Lyme borreliosis endemic areas in Eurasia and North America and is now known to cause human disease (Platonov et al., 2011; Gugliotta et al., 2013; Krause et al., 2013; Chowdri et al., 2013; Hovius et al., 2013; Krause et al., 2014; Sato et al., 2014; Molloy et al., 2015; Sudhinda et al., 2016; Jobe et al., 2016; Boden et al., 2016; Krause et al., 2016; Fiorito et al., 2017; Kadkhoda et al., 2017; Jiang et al., 2018; Sato et al., 2018) that has been referred to as *Borrelia miyamotoi* disease (BMD) and as hard tick-borne relapsing fever (HTBRF) (Krause and Barbour, 2015).

In the eastern United States, *B. miyamotoi* and *B. burgdorferi* sensu stricto (s.s.) are transmitted by the same vector species, *I. scapularis*, and so they coexist in the same geographical areas and interact with the same vertebrate host communities (Barbour et al., 2009). The two spirochetes, however, differ in how they are maintained enzootically. *Borrelia burgdorferi* s.s. spirochetes rely entirely on horizontal and transstadial survival for their maintenance in the vector population. In other words, *I. scapularis* larvae hatch uninfected from the eggs (Rollend et al., 2013) and only can acquire *B. burgdorferi* s.s. by feeding on an infected host or through non-systemic, co-feeding transmission (Gern and Rais, 1996; Piesman and Happ, 2001). An infected engorged larva will maintain *B. burgdorferi* transstadially and molt into an infected nymph, which subsequently can transmit the spirochetes to susceptible hosts, including humans. In contrast, in addition to horizontal transmission, *B. miyamotoi* can be transmitted vertically from infected females to their progeny (Scoles et al., 2001; Breuner et al., 2017). Consequently, larvae - as well as nymphs and adults - can vector *B. miyamotoi*. Transmission of *B. miyamotoi* from vertically infected larvae to laboratory mice has been demonstrated in *I. ricinus* (van Duijvendijk et al., 2016) and *I. scapularis* (Breuner et al., 2018). While the majority of Lyme borreliosis cases occur

in mid-summer coinciding with the peak nymphal *I. scapularis* activity period (Schwartz et al., 2017), recent epidemiological studies have revealed that the majority of BMD cases occur later (July to September) during the larval questing period (Molloy et al., 2015; Fiorito et al., 2017).

To understand better how *B. miyamotoi* is maintained in nature and to help predict disease risk from *B. miyamotoi*-infected larvae, we set out to obtain field estimates of the magnitude of vertical transmission. We measured both the transovarial transmission rate (TOT = the proportion of infected females that produce infected egg masses) and filial infection prevalence (FIP = the proportion of larvae in a clutch that are infected) (Burgdorfer and Varma, 1967). In the first description of *B. miyamotoi* in *I. scapularis*, Scoles et al. (2001) observed transovarial transmission (but did not quantify TOT) and reported FIP estimates of 6% and 73% for two vertically infected larval clutches. To our knowledge, no other previous data regarding TOT rates and FIP of *B. miyamotoi* from *I. scapularis* exist in the literature. We therefore investigated TOT and FIP from engorged female *I. scapularis* collected from hunter-harvested white-tailed deer (*Odocoileus virginianus*) in 2015 and 2016 from Maine, New Hampshire, Tennessee, and Wisconsin. White-tailed deer (hereafter referred to as deer) serve as an important host for adult *I. scapularis* (Main et al., 1981) and we previously detected amplification of *B. miyamotoi* in deer-fed adult female *I. scapularis* compared with questing adult ticks collected at the same time (Han et al. 2016).

Materials and Methods

Sample sites and collection

Ticks were collected at hunter-harvested deer check stations in Maine (ME), New Hampshire (NH), Tennessee (TN), and Wisconsin (WI) during the firearm season (November) in 2015 and 2016. Sampling sites spanned Lyme borreliosis endemic areas in the United States, where *B. miyamotoi* infected ticks (Barbour et al., 2009; Hamer et al., 2014) and BMD cases (Molloy et al., 2015; Jobe et al., 2016) were previously reported. TN was included as a representative area that is non-endemic for Lyme borreliosis, and where *B. miyamotoi* had previously been detected (Rosen et al., 2012). Check stations were located in the following counties: Kennebec and York (ME); Rockingham, Hillsborough, and Carroll (NH); Anderson (TN); and Monroe (WI).

Hunters brought freshly-killed deer carcasses to check stations, and each carcass was inspected for ticks for an average of 10 min, prioritizing the head, neck, and ventral regions. Feeding female ticks were collected using forceps and stored alive in ventilated polystyrene tubes plugged with pieces of mesh and caps modified to allow airflow. Collected ticks were labelled by deer ID and held at ambient temperature and 99% relative humidity (RH) during transport to the lab.

Oviposition conditions

Upon arrival at Michigan State University, the species, life stage, and sex of the collected ticks were confirmed morphologically using published keys (Keirans and Clifford, 1978; Sonenshine, 1979). In 2015, the body weights of all female ticks were measured (nearest 1

mg) to determine engorgement levels, and female *I. scapularis* > 10 mg were selected for the TOT experiment. In 2016, female ticks weighing > 20 mg were selected for monitoring, as the 2015 data showed that females with this weight had markedly higher oviposition success. Selected females were sterilized with 10% bleach for 30 seconds and then rinsed twice with water for 30 seconds to prevent fungal infection. Sterilized female ticks were placed individually in clear polystyrene tubes with ventilated caps and maintained at 22°C, 99% RH, and 16:8 L:D (hrs) photoperiod (Fig. 1). The date that oviposition commenced was recorded for each female. Female ticks were removed from tubes after oviposition ceased, typically 15 days after the first day of oviposition, and stored in 95% ethanol. Egg batches were monitored and allowed to hatch into larvae. In 2016, for logistical reasons, females were first stored for 42 - 87 days at 10°C, 99% RH in darkness to delay oviposition, before switching to the conditions described above to allow for oviposition (Fig. 1).

Screening for TOT and FIP

Total DNA from females was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) following the manufacturer's protocol for animal tissue, with minor modifications as previously described (Hamer et al., 2010). Each extraction batch included extraction negative controls consisting of extraction reagents without eggs or ticks. Presence of *B. miyamotoi* DNA was screened with a quantitative PCR (qPCR) that targeted 16S rRNA of *B. miyamotoi* as described previously (Tsao et al., 2004). We used sterile water as a negative PCR control and extracted DNA of *B. miyamotoi* MI029 strain as a positive PCR control (kindly provided by Dr. G. Margos; Margos et al., 2014). To determine the TOT rate of infected females, a pool of either 5 or 10 larvae from the larval clutch produced by each infected female was screened. If *B. miyamotoi* was not detected, at least 10 more pools of 5 larvae were screened for each female; if *B. miyamotoi* was detected, we then tested 100 individual eggs or larvae from the infected egg/larval batch to estimate its FIP.

One of the larval clutches from Maine was sent to Yale University as part of a separate study by the Bockenstedt lab that aimed to obtain field isolates of *B. miyamotoi* for studies in mice. Briefly, a total of 90-100 larval ticks was divided between two immunodeficient C57BL/6 *rag*^{-/-} *Mus musculus* and allowed to feed to repletion. Presence of spirochetes in mouse blood was assessed by darkfield analysis after the completion of larval blood feeding. Total DNA was extracted from mouse blood using the Phusion Blood Direct PCR Kit (Thermo Fisher Scientific, Inc., Rockford, IL) and from 10 engorged larvae using the Macherey-Nagel Nucleospin Tissue DNA isolation kit (Takara Bio Inc., Shiga, Japan) by following the manufacturer protocols. *Borrelia miyamotoi* infection in mice and ticks was screened by PCR targeting a fragment of the flagellin gene (*Fla*), which was amplified with forward and reverse primers Fla-F 5'-GCATCATTAGCTGGAACACAA-GC (bp 366-389) and Fla-R 5'-AACTGGAGCGGCTGCTGGAGC (bp 547-567), respectively, with 66 °C annealing temperature and 35 PCR cycles. Transovarial transmission was inferred by assaying blood of challenged mice and engorged larvae by PCR. Sustained infection to molted nymphs was inferred by positive darkfield exams of blood smears from 2 *rag*^{-/-} mice that served as bloodmeal hosts for 10 nymphs (5 nymphs/mouse) that had molted from larvae. Because the entire ME clutch was used for challenging mice, however, FIP could not be measured.

To confirm the identity of *B. miyamotoi* DNA detected in these field-derived females (n = 10), larvae (n = 10) and eggs (n = 1), we sequenced a fragment of the 16S - 23S intergenic spacer region of *Borrelia* species (Bunikis et al., 2004).

Statistical analyses

We report prevalence estimates with binomial 95% confidence intervals (CI), and we use Fisher's exact test to evaluate differences among proportions (i.e., infection prevalence, oviposition success, hatching rate, TOT rate and FIP). To estimate the overall expected prevalence of infection in the next generation of larvae due to vertical transmission, we calculated the product of female infection prevalence, TOT rate and FIP.

Results

***Borrelia miyamotoi* infection prevalence and oviposition rates of deer-blood engorged females collected from hunter-harvested deer**

In 2015, we collected 664 female *I. scapularis* feeding on hunter-harvested white-tailed deer, of which 431 females (11–370 mg) were selected for further monitoring (Table 1). Of these, 230 (53.4%; CI 48.5–58.1%) oviposited, and the mean weight was 116 mg (Standard Error (SE) 6.0 mg) with the smallest weighing 20 mg. In 2016, we collected 1030 females, and monitored 599, of which ranged from 20–423 mg (Table 1). Of these, 262 (43.7%; CI 39.71–47.8%) oviposited, and the mean weight was 140 mg (SE 5.7 mg) with the smallest weighing 23 mg. The oviposition rate was significantly lower in 2016 than in 2015 ($p = 0.0024$).

The overall infection prevalence of *B. miyamotoi* in deer-blood engorged *I. scapularis* females was 2.6% (n = 1263; CI 1.8–3.7%). In 2015 we tested all collected female *I. scapularis* (n = 664) and detected a total 16 infected females (2.4%), comprising 2 ME (2.9%), 1 TN (0.8%) and 13 WI (3.0%) females. In 2016 we tested only females weighing > 20 mg (n = 599) and detected 17 infected females (2.8%), comprising 1 ME (8.0%), 2 NH (2.5%), and 14 WI (2.8%) females. There was no significant difference in engorged female infection prevalence between 2015 and 2016 at any site (all $p > 0.4$), and no significant difference in prevalence among the four sites was apparent (2015 and 2016 data pooled; Chi-sq = 2.84, 3 df, $p = 0.42$).

Overall, eleven females (4 in 2015 and 7 in 2016) infected with *B. miyamotoi* produced egg batches (Table 2). Oviposition rates of infected females were 40.0% (n = 10; CI 13.7–72.6%) in 2015 and 41.2% (n = 17; CI 19.4–66.6%) in 2016, whereas oviposition rates of uninfected females were 53.7% (n = 421; CI 48.8–58.5%) in 2015 and 43.8% (n = 582; CI 39.8–48.0%) in 2016. There was no statistical difference in the mean weight of oviposited females between infected (174.6 mg, SE 35.9 mg) and uninfected (127.7 mg, SE 4.2 mg; $t = 1.6662$, 491 df, $p = 0.0963$). There was no significant difference between the oviposition rates of infected and uninfected females in either year ($p = 0.53$ in 2015 and $p = 1.00$ in 2016). Combining data from both years, larvae hatched from 10/11 egg batches (90.9%, CI 57.1–99.5%) produced by infected females, although one batch produced only 4 larvae (i.e., most eggs did not hatch). Considering only egg batches in which the majority of eggs

hatched, larvae hatched from 9/11 infected females (81.8%, CI 47.8-96.8%). Overall 384/481 (79.8%; CI 75.9-83.3%) egg batches produced by uninfected females hatched into larvae. There was no significant difference between the hatching rates of infected and uninfected egg batches ($p = 0.827$).

TOT and FIP rates

TOT of *B. miyamotoi* was observed in 10 of 11 clutches (90.9%, CI 57.1-99.5%; Table 2) produced by *B. miyamotoi* infected females collected from white-tailed deer. Considering only vertically infected clutches from which larvae hatched ($n = 7$), average FIP was estimated as 84.4% (CI 68.1-100%; Table 2).

The following section provides details on the data obtained from each of the 11 *B. miyamotoi*-infected females, given the different approaches used. For the one infected clutch from TN, *B. miyamotoi* was not detected from a pool of 10 larvae nor from 13 pools of 5 larvae. One clutch from ME was small, comprising 28 larvae; these larvae were screened individually by qPCR for *B. miyamotoi* infection, demonstrating transovarial transmission and resulting in an estimate of 92.9% FIP. Transovarial transmission of *B. miyamotoi* in the other ME clutch was inferred by at Yale University by feeding its larvae on immunodeficient mice. Challenged mice showed spirochetemia after larval blood feeding and *B. miyamotoi* DNA was detected in their blood by PCR as well as in 9 of 10 (90%) engorged larvae, demonstrating TOT. Spirochetemia was also observed in mice challenged with nymphs that had molted from larvae and *B. miyamotoi* DNA was detected in those mice. Transovarial transmission was detected in pools of 10 larvae from 2/2 NH clutches, and FIP was subsequently estimated as 36.0% and 92.0%, respectively. Transovarial transmission was detected in pools of 5-10 larvae from 4/4 WI larval clutches from which all larvae hatched, and FIP ranged from 83.0% to 100.0%. From one WI egg batch that did not produce any larvae; *B. miyamotoi* was detected in a pool of 30 eggs indicating transovarial transmission, with FIP estimated as 73.0% from 100 individual eggs. From the remaining WI egg batch, 60 eggs and 4 hatched larvae were screened individually, demonstrating transovarial transmission with FIP estimated as 54.7%. The FIP values of these latter 2 egg batches were not included in the calculation of overall FIP due to potential differences in DNA extraction and qPCR detection efficiencies between larvae and eggs.

Prediction of larval infection prevalence

Larval infection prevalence of the next generation was predicted by multiplying the observed engorged female infection prevalence of each state (3.7% ME, 1.7% NH, 2.9% WI, 0.8% TN), and average TOT rate (90.9%) and FIP (84.4%). Predicted larval infection prevalence in the next generation ranged from 0.6% (TN) to 2.8% (ME), with 2.0% as the mean expected infection prevalence (Fig. 2).

All DNA extraction and PCR negative controls produced negative results and our positive controls produced positive results (for all assays, including those for ticks of all life stages). All 16S - 23S rRNA intergenic spacer region sequences (460 or 478 bp length; $n = 21$) showed 100% identity with published sequences for *B. miyamotoi* in GenBank. Sequences

of females and representative larval clutches have been deposited in GenBank (accession nos. [MF444832](#) - [MF444845](#), [MF460836](#) - [MF460842](#)).

Discussion

Although *B. miyamotoi* was first detected in the US nearly twenty years ago (Scoles et al., 2001), its enzootic cycle remains poorly understood. While the role of horizontal transmission still needs to be clarified, here we begin to evaluate the role of vertical transmission. As a first step, we sought to estimate transmission rates in field-infected and fed females. We investigated engorged female *I. scapularis* collected from hunter-harvested white-tailed deer, one of the most important hosts for adult *I. scapularis* (Main et al., 1981; Bosler et al., 1984; Telford et al., 1988), from four states in the eastern U.S., including three (Maine, New Hampshire, and Wisconsin) that are designated as high risk for Lyme borreliosis and one (Tennessee) that is currently considered to be of very low risk (Schwartz et al., 2017).

The overall *B. miyamotoi* infection prevalence of engorged females collected in 2015-2016 ($n = 1263$) was 2.6% (95% CI 1.8-3.7%). There was no apparent geographic difference in infection prevalence, but small sample sizes at some sites resulted in low power to discern differences at such low prevalence. There also was no significant difference in overall infection prevalence between years. Considering the overall infection prevalence of engorged females as well as just those data from Wisconsin from either/both years, the infection prevalence values observed in this study are significantly lower than previously observed at the Wisconsin site in 2010 (Han et al., 2016, 7.1%, Fisher's exact test, $p = 0.0057$). Published studies exist regarding the infection prevalence of other tick-borne pathogens in deer-fed *I. scapularis* ticks, but few have looked explicitly at *B. miyamotoi* infection. A recent examination of male *I. scapularis* collected in 2002 from hunter-harvested deer check stations located throughout New Jersey found 2.7% (95% CI 1.6-4.3%) to be infected (Egizi et al., 2018). The female *I. scapularis* attached to these deer were not assayed for pathogens, but based on Han et al. (2016), *B. miyamotoi* infection prevalence of engorging females on white-tailed deer tends to be higher than that of attached males. Thus if we take 2.7% infection as a conservative estimate for infection prevalence of engorged females, the level is similar to or greater than that estimated in our current study. We can also try to glean comparable information from laboratory studies. Rollend et al. (2013) reviewed laboratory records for 1214 female *I. scapularis* collected from three sites in Connecticut and fed on laboratory rabbits or sheep and reported the infection prevalence of resultant egg batches for *B. miyamotoi* as 1.6% (95% CI 0.9-2.4%). This result is similar to the estimates for infection prevalence of engorged females reported above (and note, females fed on different host species), but because vertical transmission may have been $< 100\%$, this value should be considered a minimum infection prevalence of engorged females.

We observed high TOT of *B. miyamotoi* from the infected *I. scapularis* engorged with deer blood; infected progeny were detected from 10 of 11 females (90.9%, CI 57.1-99.5%; Table 2). FIP, however, varied from 36.0% to 100.0% for 7 larval clutches; this wide variation is consistent with previously reported estimates of 6% and 73% (Scoles et al., 2001).

In an attempt to view our results in a larger context, here we cautiously compare our data with historical studies published prior to the detection of *B. miyamotoi* in *I. scapularis* (Scoles et al., 2001), on the apparent vertical transmission of “*B. burgdorferi*” that were later refuted by laboratory transmission experiments (Patrican, 1997). Because direct observation of spirochetes and the use of cross-reactive immunoassays would not have differentiated between the two *Borrelia* species, these researchers most likely detected *B. miyamotoi* (or another yet-to-be discovered relapsing fever *Borrelia*) rather than *B. burgdorferi* in infected larvae (Rollend et al., 2013).

Magnarelli et al. (1987) tested 34 moribund female *I. scapularis* collected from hunter-harvested deer or domestic dogs in Connecticut after their oviposition and found 7 clutches of larvae produced by *Borrelia* infected females to be infected with spirochetes. If these spirochetes were *B. miyamotoi*, this infection prevalence of engorged females (20.6%; 95% CI 8.7-37.9%) is much higher than the values observed in our current study, and is trending higher than the 7.1% (95% CI 3.6-12.3%) we previously observed in deer-fed females collected from hunter-harvested deer in 2010 in Wisconsin (Han et al., 2016). The researchers reported 3.3% to 27.3% FIP, but these values may be underestimates, as it is unclear whether the denominator included larvae of *B. burgdorferi* positive/*B. miyamotoi*-negative females.

Investigations of vertical transmission of *B. burgdorferi* by *I. pacificus* have also been published. Lane and Burgdorfer (1987) fed field-collected *I. pacificus* females (n = 100) on laboratory rabbits and assayed the females and their clutches for *Borrelia* infection. Because the immunoassay they used did not differentiate between *B. burgdorferi* and *B. miyamotoi* (Rollend et al. 2013) (or other uncharacterized *Borrelia* spp.), the minimum *B. miyamotoi* infection prevalence of the field-collected females fed on rabbits may have ranged from 1% - 3%, which is similar with values seen in our current study and that of Rollend et al. (2013). To quantify TOT and FIP, the researchers assayed 50 eggs and up to 100 larvae from each of the 3 *Borrelia*-infected *I. pacificus* females. Only one of the infected females transmitted spirochetes to offspring; depending on whether females actually were infected with *B. burgdorferi*, *B. miyamotoi*, or both, the TOT estimate would be 33% - 100%. From the one female that transmitted spirochetes to offspring, FIP was 100% and transstadial survivorship of *Borrelia* spirochetes was also 100% for subsequent nymphs and adults. In tissue smears from this infected female, a disseminated heavy infection was observed, with spirochetes visible in multiple tissues including the central ganglion, Malpighian tubules, midgut, ovaries, and salivary glands. In contrast, spirochetal infection was not as generally disseminated in the two females that did not transmit spirochetes to offspring, despite moderate to heavy infection of spirochetes in their ovaries. These data are suggestive of a positive relationship between spirochete load and the probability of transovarial transmission, but are highly speculative because we do not definitively know spirochete identity (i.e., *B. miyamotoi* v. *B. burgdorferi*) of the infected ticks.

In our current study, there is additional, albeit sparse, support for a positive relationship between spirochete load and the probability of transovarial transmission. Only one of 11 infected females in our study did not transmit *B. miyamotoi* to her progeny. The quantitative PCR threshold cycle (C_t) value of the female that did not transmit spirochetes to offspring

(C_t 39.7) was above the upper 95% confidence limit of the mean C_t value for the infected females that did transmit spirochetes to offspring (mean C_t = 21.5, 95% CI 20.6-22.4). This suggests that the relative spirochete burden of the 'non-TOT' female was significantly lower than that of TOT females. To confirm *B. miyamotoi* infection of the 'non-TOT' female, we sequenced DNA of the female and observed 100% identity with published *B. miyamotoi* sequences in GenBank.

Overall *B. miyamotoi* spirochete load in engorged females and/or heavy disseminated infections as seen by Lane and Burgdorferi (1987) may be indicative of ovarian tissue that has been successfully colonized by *B. miyamotoi*. It is unknown whether the high spirochete load may result from an initially high spirochete number in flat females and/or efficient spirochete amplification during feeding or through horizontal transmission from the adult tick's host (Han et al. 2016). Thus, future experiments that study spirochete dynamics within the engorged female, and mechanisms (e.g., timing and route) by which eggs are colonized by *B. miyamotoi*, may also reveal the relative importance of the route by which the female tick originally acquired the spirochetes, either prior to the adult blood meal (i.e., vertically with subsequent transstadial survivorship or horizontally with subsequent transstadial survivorship) or during the adult bloodmeal. Ecologically, given that the infection prevalence of engorged females is an important parameter influencing the overall contribution of vertical transmission, future research should document how infection prevalence of engorged females varies temporally, geographically, and among potential reservoir hosts used by blacklegged ticks (as well as for other *I. ricinus* complex ticks to understand how *B. miyamotoi* is maintained in those enzootic cycles).

How important is vertical transmission for *B. miyamotoi* maintenance?

Overall, it appears that the prevalence of infection of engorged infected *I. scapularis* females is usually low (< 3%), that TOT is high (e.g. > 90%), and that FIP also can be high, but appears to be quite variable. By multiplying the observed engorged female infection prevalence, TOT rate and FIP together, we estimate that larval *I. scapularis* infection prevalence of the next generation would be ~2.0% (Fig. 2). This value is not significantly different from what Piesman et al. (1986) estimated for the transovarial transmission of *B. burgdorferi* in questing larval *I. scapularis*, which given their method of detection, probably was *B. miyamotoi*. They collected larval *I. scapularis* from vegetation in Massachusetts (northeastern U.S.), fed them in the laboratory on naive hamsters. They found 2 of 274 subsequently molted nymphs to be infected, which represents a minimum of 0.7% (95% CI 0.1-2.9%) of transovarially infected *B. miyamotoi* prevalence in questing larval *I. scapularis*. Our predicted value of 2.0% in questing larvae based on observed TOT and FIP in this study also is consistent with reported prevalence of questing nymphal and adult *I. scapularis* (Barbour et al., 2009; Hamer et al., 2014).

This consistent prevalence of infection among larvae, nymphs and adults may seem to support the importance and adequacy of transovarial transmission for the maintenance of *B. miyamotoi* in the enzootic cycle. Our observed values of TOT and FIP, however, may not be high enough to allow *B. miyamotoi* infection to persist in the host population without input via horizontal transmission (Fine, 1975). Fine (1975) developed a deterministic

mathematical model to investigate the sufficiency of vertical transmission for the maintenance of a vector-borne pathogen. According to that model, the maintenance of infection by vertical transmission alone can occur when the symbiote fitness criterion is met. This criterion is expressed as $\alpha\beta(r + v) > 1$, where r and v are defined respectively as the maternal and paternal vertical transmission rates, and α and β respectively are the relative fertility and survival rates of infected to uninfected hosts (in this case, the tick) (Fine, 1975). Fine interpreted the product of $\alpha\beta$ as a quantitative definition of symbiotic relationships between host (in this case, the tick) and agent as mutualistic, commensal, or parasitic if the product $\alpha\beta$ were > 1 , $= 1$ and < 1 , respectively. Hosts receive selective advantage, no selective effect and selective disadvantage from the infection with 'mutual', 'commensal' and 'parasite' agent, respectively, when compared with uninfected individuals (Fine, 1975).

Here we use Fine's model as an initial quantitative exploration of the relative importance of vertical transmission. When we substitute our vertical transmission value of 76.7% (product of 90.9% TOT and 84.4% FIP) (or 93.3%, the upper 95% confidence limit) for the sum ($r + v$) and set the symbiote fitness criterion > 1 , then the value of $\alpha\beta > 1.30$ (or 1.07), which suggests that *B. miyamotoi* infection provides a $> 30\%$ (or 7%) increase in the fertility or survival of infected versus uninfected *I. scapularis*. Currently there have been no studies to indicate either positive or negative effects of *B. miyamotoi* infection on the fertility or survival of *I. scapularis*.

Our study was not designed to compare fitness effects of *B. miyamotoi* infection, but our data allow us to compare the oviposition and hatching rates of infected and uninfected females. The overall oviposition rate was significantly lower in 2016 than in 2015, which could be related to a change in our laboratory practices between the years (in 2016, to accommodate workflow logistics, we delayed oviposition by keeping females at a lower temperature and darkness for up to 87 days). Within each year, however, there was no statistical difference between the oviposition rates of *B. miyamotoi* infected and uninfected females. In addition, we did not observe any significant difference in hatching rates between infected and uninfected egg batches. Therefore, our results did not show any measurable positive or negative effect of *B. miyamotoi* infection on oviposition rates of *I. scapularis* females or of hatching success of their egg batches. Future research should compare fitness parameters of infected v. uninfected ticks throughout the entire life cycle.

For now, if we assume no fitness effects of *B. miyamotoi* infection on *I. scapularis*, then the value of $\alpha\beta$ is set to 1.0 and the sum of the maternal and paternal vertical transmission rates ($r + v$) would need to be > 1.0 for vertical transmission to maintain *B. miyamotoi*. Thus, under this assumption, our observed vertical transmission rate of 76.7% appears to be insufficient (but closer to being sufficient if we take the upper 95% CI value of 93.3%) to support the maintenance of *B. miyamotoi* infection in the tick population and suggests that horizontal transmission involving reservoir hosts and/or co-feeding, non-systemic transmission likely is required for *B. miyamotoi* enzootic maintenance. If so, identifying reservoir hosts and quantifying horizontal transmission parameters would increase our understanding regarding the biological processes underlying *B. miyamotoi* maintenance. Additional mathematical modeling (e.g., Hartemink et al., 2008) that considers the vertebrate host population may be helpful to further evaluate the contributions of vertical and

horizontal transmission for *B. miyamotoi* maintenance and extend our knowledge about the disease ecology of *B. miyamotoi*.

Implications for public health

First recognized as a human pathogen in Russia in 2011 (Platonov et al., 2011), the number of human case reports of *B. miyamotoi* infection since then has been increasing in the United States and Eurasia (Gugliotta et al., 2013; Krause et al., 2013; Chowdri et al., 2013; Hovius et al., 2013; Krause et al., 2014; Sato et al., 2014; Molloy et al., 2015; Sudhindra et al., 2016; Jobe et al., 2016; Boden et al., 2016; Krause et al., 2016; Fiorito et al., 2017; Kadkhoda et al., 2017; Jiang et al., 2018; Sato et al., 2018). This increase undoubtedly is due in part to increased surveillance effort and improved diagnostics to detect BMD.

Nevertheless, the range of *I. scapularis* continues to expand (Dennis et al., 1998; Eisen et al., 2016), so the incidence of BMD is likely to increase over time, as has already been seen with Lyme borreliosis, human anaplasmosis, and human babesiosis (Mead et al., 2015; Schwartz et al., 2017).

One of the epidemiologically important biological differences between *B. miyamotoi* and several other *I. scapularis*-borne pathogens is that transovarial transmission occurs in *B. miyamotoi* (Scoles et al., 2001; Barbour et al., 2009), but is non-existent for most other *I. scapularis*-borne pathogens (Macleod and Gordon, 1933; Patrican, 1997; Dumler et al., 2001; Rollend et al., 2013) (Powassan/deer tick virus is an exception; Ebel and Kramer, 2004) (see van Duijvendijk et al., 2016, for differences in the *I. ricinus* system). Thus, while nymphs pose the greatest epidemiological risk for several *I. scapularis*-borne pathogens, larvae may pose an important, and possibly the greatest, risk for BMD due to their high abundance and tiny size, as is already suggested by the seasonality of BMD incidence seen in two studies from the Northeast (Molloy et al., 2015; Fiorito et al., 2017). Future studies should quantify the infectious dose of *B. miyamotoi* in infected larvae; as well as measure the acarological risk for *B. miyamotoi* (i.e., the density of infected ticks; Szekeres et al., 2017; Ruyts et al., 2017) as a function of all questing life stages.

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<p>A. Field collections of <i>Ixodes scapularis</i></p> <p>1) Collect engorged females from hunter-harvested white-tailed deer.</p>
<p>B. Laboratory: Oviposition</p> <p>1) Sterilize and weigh females;</p> <p>2) Store females individually at 22°C, 99% RH with 16:8 L:D (hrs) photoperiod schedule.</p>
<p>C. Screen females for <i>Borrelia miyamotoi</i></p> <p>1) Remove females 15 days post onset of oviposition;</p> <p>2) Assay each female for infection.</p>
<p>D. Quantify TOT/FIP</p> <p>1) TOT - Screen pools of larvae from each infected female (minimum of 10 pools, if no infections found);</p> <p>2) FIP - Screen 100 individual larvae from each infected clutch.</p>

Fig. 1.
Workflow for estimating transovarial transmission (TOT) and filial infection prevalence (FIP) of *Borrelia miyamotoi*-infected deer-fed *Ixodes scapularis* females in 2015 (RH – Relative Humidity, L:D - Light : Dark lighting schedule). See text for modifications made in 2016 to improve workflow logistics.

Fig. 2. Predicted *Borrelia miyamotoi* prevalence (95% CI) in the next generation of larvae; calculations based on observed female infection prevalence, TOT rate, and FIP from ticks at each sampling site. ME = Maine, NH = New Hampshire, WI = Wisconsin, TN = Tennessee. The overall predicted infection prevalence value for these sites combined (= Total) is also shown.

Table 1.

Infection prevalence of *Borrelia miyamotoi* positive (pos) female *Ixodes scapularis* ticks collected from hunter-harvested white-tailed deer.

State	2015			2016		
	No. of females	No. of pos.	% Infection prevalence (95% CI [†])	No. of females	No. of pos.	% Infection prevalence (95% CI [†])
Maine	69	2	2.9 (0.5-11.0)	13	1	7.7 (0.4-37.9)
New Hampshire	32	0	0.0 (0.0-13.3)	85	2	2.4 (0.4-9.0)
Tennessee	133	1	0.8 (0.0-4.7)	--	--	--
Wisconsin	430	13	3.0 (1.7-5.2)	501	14	2.8 (1.6-4.8)
Total	664	16	2.4 (1.4-4.0)	599	17	2.8 (1.7-4.6)

[†] 95% confidence interval for a proportion including continuity correction

Table 2.

Transovarial transmission (TOT) and filial infection prevalence (FIP) of *Borrelia miyamotoi* from 11 infected female *Ixodes scapularis* ticks collected from hunter-harvested white-tailed deer in Tennessee, Maine, New Hampshire, and Wisconsin.

State	Egg Batch ID	Weight of female (g)	C _t ^φ Value of female DNA	TOT	FIP		
					No. of larvae tested	No. of positive larvae	% Infection prevalence (95% CI [¥])
Tennessee	F15-011	0.052	39.7	No	75	0	0 (0-6.1)
Maine	F15-042	0.046	18.7	Yes	28	26	92.9 (75.1-98.8)
	F15-044 [‡]	0.062	20.7	Yes	NA	NA	NA
New Hampshire	F16-573	0.221	21.6	Yes	100	36	36.0 (26.8-46.3)
	F16-596	0.354	23.4	Yes	100	92	92.0 (84.4-96.2)
Wisconsin	F15-279	0.191	21.8	Yes	100	100	100.0 (95.4-100.0)
	F16-099	0.262	21.6	Yes	100	98	98.0 (92.3-99.7)
	F16-276	0.306	22.3	Yes	200	178	89.0 (83.6-92.8)
	F16-311	0.293	22.5	Yes	100	83	83.0 (73.9-89.5)
	F16-108	0.043	20.8	Yes	64	35	54.7 (41.8-67.0) [‡]
	F16-288	0.091	21.6	Yes	100	73	73.0 (63.0-81.2) [‡]
Mean % (95% CI) [¥]				90.9% (57.1-99.5%)		84.4 (68.1-100%) [€]	

[‡] All larvae were infested on a RAG-deficient mouse

[‡] FIP was estimated from unhatched eggs (F16-288) and unhatched eggs and 4 hatched larvae (F16-108)

[¥] 95% confidence interval for a proportion including continuity correction or for mean

[€] The estimate of the average FIP of larval ticks did not include F16-108 and F16-288

^φ Threshold cycle of quantitative PCR