Infected Ixodes scapularis nymphs maintained in prolonged questing under optimal environmental conditions for one year can transmit Borrelia burgdorferi (Borreliella genus novum) to uninfected hosts.

Kamalika Samanta, Jose Azevedo, Nisha Nair, Suman Kundu, and Maria Gomes-Solecki

Corresponding Author(s): Maria Gomes-Solecki, University of Tennessee Health Science Center

Review Timeline:

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| Submission Date    | April 18, 2022 |
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Editor: Catherine Brissette

Reviewer(s): Disclosure of reviewer identity is with reference to reviewer comments included in decision letter(s). The following individuals involved in review of your submission have agreed to reveal their identity: Fukai Bao (Reviewer #1)

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

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June 9, 2022

Dr. Maria Gomes-Solecki  
University of Tennessee Health Science Center  
Microbiology, Immunology and Biochemistry  
858 Madison Ave  
Memphis, TN 38163

Re: Spectrum01377-22 (Questing I. scapularis ticks maintained for one year under optimal environmental conditions transmit B. burgdorferi to uninfected mice)

Dear Dr. Maria Gomes-Solecki:

Reviewers were mainly positive and feel this should add to the field, but please address their concerns.

Thank you for submitting your manuscript to Microbiology Spectrum. When submitting the revised version of your paper, please provide (1) point-by-point responses to the issues raised by the reviewers as file type “Response to Reviewers,” not in your cover letter, and (2) a PDF file that indicates the changes from the original submission (by highlighting or underlining the changes) as file type “Marked Up Manuscript - For Review Only”. Please use this link to submit your revised manuscript - we strongly recommend that you submit your paper within the next 60 days or reach out to me. Detailed instructions on submitting your revised paper are below.

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Below you will find instructions from the Microbiology Spectrum editorial office and comments generated during the review.

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Sincerely,

Catherine Brissette

Editor, Microbiology Spectrum

Journals Department
American Society for Microbiology
1752 N St., NW
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Reviewer comments:

Reviewer #1 (Comments for the Author):

In this manuscript, authors produced nymphal ticks in the laboratory under controlled temperature, humidity and natural light/dark cycle conditions to allow them to remain in host-seeking (questing) phase for 1 year; then analyzed differences in B. burgdorferi infection prevalence of questing and diapause nymphs at 6 weeks post-molting (prime questing) as well as differences in infection prevalence of questing nymphs maintained under prolonged environmental induced questing over 12 months (prolonged questing); Lastly, analyzed the fitness of nymphal ticks subjected to environmental induced questing in transmission of B. burgdorferi to naïve mice over the course of the year.
This work is some interesting, but some questions exist still.
The authors hypothesize that infected I. scapularis nymphs that retain host-seeking behavior under optimal environmental conditions are fit to fulfill their role in transmission in the B. burgdorferi enzootic cycle. To test this hypothesis, they produced nymphal ticks in the laboratory under controlled temperature (22-25°C), humidity (80-90%) and natural daylight cycle conditions to allow them to retain host-seeking/questing behavior for ~1 year. They analyzed differences in B. burgdorferi infection prevalence in questing and diapause nymphs at 6 weeks post-molting (prime questing) as well as differences in infection prevalence of questing nymphs maintained under prolonged environmentally induced questing over 12 months (prolonged questing). They also analyzed the fitness of nymphal ticks subjected to prolonged questing in transmission of B. burgdorferi to naïve mice over the course of the year. They found that infected unfed I. scapularis nymphal ticks maintained under optimal environmental conditions in the laboratory survived for a year in a developmental state of prolonged questing (host-seeking), they retained an infection prevalence sufficient to effectively transmit transmission of B. burgdorferi to uninfected mice after tick challenge. The authors believe their study is important for understanding and possibly modeling Lyme disease expansion into former non-endemic regions due to global warming. (From the Abstract).

This is an interesting, focused and hypothesis-based work demonstrating that infected I. scapularis nymphs retain extended (over a year) host-seeking behavior under optimal environmental conditions and are fit to fulfill their transmission role in the enzootic cycle of B. burgdorferi. The demonstration of the ability of B. burgdorferi to survive long periods of time (over a year) in unfed ticks and conserve its infectiousness is also a very relevant finding to understanding the epidemiology of Lyme disease. It also triggers questions regarding the mechanism(s) underlying survival of the bacteria in apparently hostile conditions. The relevance of these important experimental results is that they may be useful to understanding the evolving epidemiology of Lyme disease and the diffusion of the disease from endemic to non-endemic areas under a background of climate change.

The use by the authors of infectious B. burgdorferi senso stricto mixed cultures recovered from wild footed mice organs is an important plus of these experiments as they recapitulate more closely the natural situation where the pathogen infects animals with a mixed population. The experiments to infect mice and I. scapularis tick larva were carried out using standard procedures; adequate controls for infection were included. The authors took special care in the generation of prime and prolonged questing nymphs and confirmation and quantification of their infection with B. burgdorferi by qPCR of the flaB gene and controls throughout the duration of the experiments. . Generation and assessment of infection of these nymphs with B. burgdorferi is a crucial step in these studies and the care with which the authors carried them out gives confidence on their results and their analysis. The molecular and immunological methods used to measure the efficiency of transmission and infection of the mice were adequate and provide further trust on the results obtained. Figure 7 clearly represents the protocol of the experiments, while the other the figures provide an excellent illustration of the results obtained.

The authors clearly demonstrate that host-seeking ticks had B. burgdorferi at higher frequency and concentration than non-host-seeking ticks and that the difference in frequency was significant and remained higher for over a year. More importantly, the nymphs so infected were proficient in their ability to transmit B. burgdorferi to naive mice as determined by qPCR, tissue culture of infected mice and antibody response. Thus, as the authors state, these results "raise important questions regarding prolonged survival of B. burgdorferi-infected host-seeking nymphal I. scapularis that could result in increased Lyme disease incidence if conditions of temperature and especially humidity in regions currently classified as non-endemic become amenable due to global warming."

This being said, I believe the authors should also articulate and describe a scenario of how these questing tick nymphs highly infected with B. burgdorferi will help to disseminate Lyme disease to terrains virgins of the disease. Will they migrate carried by rodent or birds? Or will the warmer weather facilitate their presence and numbers in some geographical areas where they already exist but were not previously a problem? In other words, how do the authors envision these ticks spreading Lyme disease to untouched areas. The authors should also expand their discussion as to how they see the tick metabolism interacting with B. burgdorferi metabolism to ensure persistence of viable B. burgdorferi for over a year and be efficiently transmitted to mice hosts (lines 191 and 192). In this context, what is their explanation for the NIP differences between diapause and questing ticks?

In the title Ixodes and Borreliella should be spelled fully, and a description of the statistical methods used to analyze the data should be added to the Material and Methods section; their description in the figure legends is not sufficient. What is the meaning of the abbreviation NS in Figure 2? Abbreviations used less than 3 times should not be employed and should be written out.
Preparing Revision Guidelines
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- Upload a compare copy of the manuscript (without figures) as a "Marked-Up Manuscript" file.
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Response to Reviewers

Re: Spectrum01377-22 (Questing I. scapularis ticks maintained for one year under optimal environmental conditions transmit B. burgdorferi to uninfected mice)

Reviewer comments:

Reviewer #1
This work is some interesting, but some questions exist still.
1. Abstract has a bad format which has so many introductions, but not contained logical methods, results, and conclusion.
   The abstract was reformatted to address these concerns.
2. Authors should provide a biosafety statement and permission.
   This was added to the first sentence on the Methods section.

Reviewer #2 (Comments for the Author):

This is an interesting, focused and hypothesis-based work demonstrating that infected I. scapularis nymphs retain extended (over a year) host-seeking behavior under optimal environmental conditions and are fit to fulfill their transmission role in the enzootic cycle of B. burgdorferi. The demonstration of the ability of B. burgdorferi to survive long periods of time (over a year) in unfed ticks and conserve its infectiousness is also a very relevant finding to understanding the epidemiology of Lyme disease. It also triggers questions regarding the mechanism(s) underlying survival of the bacteria in apparently hostile conditions. The relevance of these important experimental results is that they may be useful to understanding the evolving epidemiology of Lyme disease and the diffusion of the disease from endemic to non-endemic areas under a background of climate change.
I added the underlined concept to the discussion.

The use by the authors of infectious B. burgdorferi senso stricto mixed cultures recovered from wild footed mice organs is an important plus of these experiments as they recapitulate more closely the natural situation where the pathogen infects animals with a mixed population. The experiments to infect mice and I. scapularis tick larva were carried out using standard procedures; adequate controls for infection were included. The authors took special care in the generation of prime and prolonged questing nymphs and confirmation and quantification of their infection with B. burgdorferi by qPCR of the flaB gene and controls throughout the duration of the experiments. Generation and assessment of infection of these nymphs with B. burgdorferi is a crucial step in these studies and the care with which the authors carried them out gives confidence on their results and their analysis. The molecular and immunological methods used to measure the efficiency of transmission and infection of the mice were adequate and provide further trust on the results obtained. Figure 7 clearly represents the protocol of the experiments, while the other the figures provide an excellent illustration of the results obtained.
I highlighted the fact that we used multiple strains of B. burgdorferi in the study in the
The authors clearly demonstrate that host-seeking ticks had B. burgdorferi at higher frequency and concentration than non-host-seeking ticks and that the difference in frequency was significant and remained higher for over a year. More importantly, the nymphs so infected were proficient in their ability to transmit B. burgdorferi to naïve mice as determined by qPCR, tissue culture of infected mice and antibody response. Thus, as the authors state, these results "raise important questions regarding prolonged survival of B. burgdorferi-infected host-seeking nymphal I. scapularis that could result in increased Lyme disease incidence if conditions of temperature and especially humidity in regions currently classified as non-endemic become amenable due to global warming."

This being said, I believe the authors should also articulate and describe a scenario of how these questing tick nymphs highly infected with B. burgdorferi will help to disseminate Lyme disease to terrains virgins of the disease. Will they migrate carried by rodent or birds? Or will the warmer weather facilitate their presence and numbers in some geographical areas where they already exist but were not previously a problem? In other words, how do the authors envision these ticks spreading Lyme disease to untouched areas. The authors should also expand their discussion as to how they see the tick metabolism interacting with B. burgdorferi metabolism to ensure persistence of viable B. burgdorferi for over a year and be efficiently transmitted to mice hosts (lines 191 and 192). In this context, what is their explanation for the NIP differences between diapause and questing ticks?

I added a little more in the discussion lines 192-196 and lines 209-211 to include these excellent comments:

192-196: This is corroborated by findings in *Ixodes ricinus* that suggest that vector-borne pathogens can alter phenotypic traits in the vector so that microorganism transmission is enhanced (Herrmann, 2015). They found that the level of energy reserves, hydration status and *Borrelia* infection influence *I. ricinus’* walk over a humidity gradient (Herrmann, 2012) and that *Borrelia*-infected nymphal ticks have higher fat content than uninfected ticks (Herrmann, 2013).

209-211: We speculate that warmer weather may facilitate survival of increased numbers of questing infectious nymphal ticks in some geographical areas where they already exist but were not previously a problem.

In the title *Ixodes* and *Borreliella* should be spelled fully, and a description of the statistical methods used to analyze the data should be added to the Material and Methods section; *Ixodes* and *Borreliella* were included in title and a description of statistical methods were added in the methods section.

Their description in the figure legends in not sufficient. What is the meaning of the abbreviation NS in Figure 2? Abbreviations used less than 3 times should not be employed and should be written out.

These issues were addressed in the revised manuscript.
June 26, 2022

Dr. Maria Gomes-Solecki  
University of Tennessee Health Science Center  
Microbiology, Immunology and Biochemistry  
858 Madison Ave  
Memphis, TN 38163

Re: Spectrum01377-22R1 (Infected Ixodes scapularis nymths maintained in prolonged questing under optimal environmental conditions for one year can transmit Borrelia burgdorferi (Borrelia genus novum) to uninfected hosts.)

Dear Dr. Maria Gomes-Solecki:

This work raises important questions regarding tick borne pathogens and climate change. The very minor concerns of the reviewers were addressed.

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Sincerely,

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Editor, Microbiology Spectrum  

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