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# Responses of juvenile blacklegged ticks (Acari: Ixodidae) to hosts of varying quality

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Blacklegged ticks (*Ixodes scapularis*) are the most medically and economically important vectors in North America. Each of their 3 life stages requires a blood meal from one of many potential host species, during which they can acquire or transmit pathogens. Host species, however, vary tremendously in their quality for ticks, as measured by differences in feeding and molting success. There should be clear fitness benefits for ticks that preferentially feed upon high-quality hosts (e.g., white-footed mice, *Peromyscus leucopus*), or at least avoid feeding on very low-quality hosts (e.g., Virginia opossums, *Didelphis virginiana*). Indeed, laboratory experiments have found some evidence of host preferences in *I. scapularis*; but these involve presenting ticks with hosts simultaneously and measuring movement towards hosts on a horizontal plane. In nature, however, host-seeking ticks encounter hosts sequentially and their movements are principally in a vertical plane. Here, we present the results of a study in which we measured the vertical movements of host-seeking juvenile blacklegged ticks before and after a host (*P. leucopus*, *Tamias striatus*, *Sciurus carolinensis*, or *D. virginiana*) was present, and whether the strength of their responses varies with host quality. We found ticks did not measurably alter the speed of their vertical movement in the presence of any hosts, regardless of host quality. Both larvae and nymphs quested slightly higher in the presence of hosts, but this did not vary by host species. These results call into question the existence of active host preferences, at least in this stage of the host-seeking process.

*Key words:* vector ecology, ecology, behavior

## Introduction

The blacklegged tick (*Ixodes scapularis*) is the most important arthropod vector of zoonotic pathogens in the United States, responsible for ≥75% of all locally acquired vector-borne disease cases (Eisen and Eisen 2018, Rosenberg et al. 2018). They, and other *Ixodes* ticks, vector the agents of Lyme disease, anaplasmosis, and babesiosis, as well as emerging pathogens such as Powassan virus (Fleshman et al. 2022, Foster et al. 2023). They can acquire, and later transmit, pathogens during their bloodmeals—one in each developmental stage—though the probability they become infected varies tremendously with the host species upon which they feed. For instance, larval ticks that feed on white-footed mice (*Peromyscus leucopus*) are more than 6 times as likely to become infected with *Borrelia burgdorferi* than if they feed on gray squirrels (*Sciurus carolinensis*) and >30 times that of larvae feeding on Virginia opossums (*Didelphis virginiana*; LoGiudice et al. 2003).

The identity of their bloodmeal host also has tremendous consequences for the tick's fitness (Keesing et al. 2009). While blacklegged ticks are host generalists, having been recorded attempting to feed on >125 mammalian, avian, and reptilian species (Lane et al. 1991, Keirans et al. 1996), they are successful on relatively few. Among the most permissive hosts (i.e., supportive of tick survival) are white-footed mice; a larval tick on a mouse has a 29% chance of successfully feeding, molting, and surviving over winter to emerge as a questing nymph (LoGiudice et al. 2003, Keesing et al. 2009, Brunner et al. 2011). In contrast, their chance of surviving this same set of transitions drops to 9% on gray squirrels and <1% on Virginia opossums (LoGiudice et al. 2003, Keesing et al. 2009, Brunner et al. 2011). Given these dramatic fitness consequences of feeding on high- or low-quality hosts, it is reasonable to expect that blacklegged ticks would have evolved "preferences," by which we mean behavioral or other mechanisms that increase the chances they feed upon the most permissive hosts available.

Observed distributions of ticks on host species are consistent with ticks having "preferences". The majority of blacklegged ticks appear to acquire their larval blood meal from a highly permissive host (e.g., mice in much of the Northeastern United States), as estimated from observed tick burdens on each species multiplied by the densities of those species (Spielman et al. 1985, LoGiudice et al. 2003, Brown et al. 2023). However, body burdens are likely a complex function of host densities (Ostfeld et al. 1996, Brunner and Ostfeld 2008), space use (Calabrese et al. 2011), immune responses (Brossard and Wikel 2004), and grooming behaviors (Shaw et al. 2003, Keesing et al. 2009), as well as the density and distribution of ticks in the environment (Calabrese et al. 2011), making it challenging to infer tick "preferences" for particular host species from these patterns. It is difficult to determine whether ticks actually discriminate among potential hosts or are simply more likely to be passively "swept up" by abundant hosts.

Laboratory experiments have shown that ticks, including *Ixodes* species, can respond to host-derived cues, presumably for the purposes of finding and feeding upon a host (Sonenshine and Roe 2014), and potentially for differentiating between host and nonhost species. For instance, larval *I. hirsti* and adult female *I. scapularis* will move toward warmer areas in artificial settings, potentially representing an attraction to host body heat (Oorebeek et al. 2009, Otálora-Luna et al. 2022), though this could also represent a ticks preference for a warmer environment (Lees 1948). Additionally, *I. ricinus* nymphs wave their forelimbs in response to and even approach the source of, vibrations derived from host movements (i.e., observers approaching ticks, making physical contact with ticks, or making physical contact with the surface ticks are on; Lees 1948, Vassallo and Pérez-eid 2002). If ticks are responding to temperature and vibrations derived from hosts (rather than the environment), it is likely a response to hosts in general, as all host species provide these cues, although it is possible some species generate stronger cues. For instance, ticks respond to $CO_2$ gradients, moving more, though not necessarily in the direction of the source (Sonenshine 2004, van Duijvendijk et al. 2017). While all tick hosts produce some amount of $CO_2$, larger-bodied hosts produce more (Heusner 1991, West et al. 2002). Thus, if ticks are using $CO_2$ to detect and select hosts, this would seem to lead to a "preference" for larger-bodied hosts. However, larger hosts are generally of lower quality to *I. scapularis* (LoGiudice et al. 2003, Keesing et al. 2009, Brunner et al. 2011)—the opposite of our expectation.

$CO_2$ can also amplify tick responses to host odor, the unique profile of volatile chemicals a species produces, which might be used to differentiate species (Sonenshine 2004, van Duijvendijk et al. 2017, Long et al. 2023). In fact, in laboratory choice assays wherein ticks are presented with 2 or more hosts simultaneously, and can likely only sense host odor and $CO_2$, ticks appear to differentiate between host species (James and Oliver 1990, Shaw et al. 2003, Slowik and Lane 2009). For instance, Shaw et al. (2003) found that *I. scapularis* larvae were about 1.5 times as likely to orient towards and approach a white-footed mouse than the paired eastern chipmunk (*Tamias striatus*). Slowik and Lane (2009) found that *I. pacificus* larvae and nymphs, when presented with deer mice (*P. maniculatus*), western fence lizards (*Sceloperus occidentalis*), kangaroo rats (*Dipodomys californicus*) and California towhees (*Pipilo crissalis*) simultaneously, were more likely to approach and make contact with lizards—a high-quality host for this tick species (Slowik and Lane 2009). However, the effect sizes of these studies are moderate to small, and it is unclear how well these experiments translate to host findings in nature.

There are 3 issues that make it difficult to extrapolate existing lab research to natural settings. First, ticks are unlikely to have a simultaneous choice between potential hosts in nature, but rather hosts pass by host-seeking ticks such that ticks would have a series of choices about whether, or how, to respond to individual hosts in sequence. Second, experimental designs usually involve ticks orienting and moving horizontally towards hosts. However, *Ixodes* ticks, especially juvenile stages, are slow-moving—Slowik and Lane (2009) found that larval *I. pacificus* required ~10 min to contact a host 5–25 cm away—and tend to move vertically on vegetation (Lane et al. 2009). Third, it is difficult to know which aspects of tick behaviors—orientation, movement speed or distance (or arresting movement), or a particular position—are relevant host-seeking behaviors. Moreover, many definitions of questing, the common term used to encompass tick host-seeking behaviors, imply a tick's intent to contact a host (Sonenshine and Roe 2014), but the intent is impossible to measure. Thus, it is difficult to discern whether blacklegged ticks do, in fact, exhibit "preferences" for particular hosts, let alone whether the strength of these preferences corresponds to host quality.

Here we present the results of an experiment in which we measured characteristics of the vertical questing behaviors of larval and nymphal blacklegged ticks before and after a host was introduced. In particular, we were interested in if ticks' responses varied to hosts across the spectrum of host quality, from high-quality white-footed mice to moderately high-quality eastern chipmunks to lower-quality gray squirrels to very low-quality Virginia opossums. If ticks exhibit host preferences at this stage in the host selection process, we would expect any behavioral responses to the presence of a host to be stronger for higher quality hosts.

## Materials and Methods

### Tick Collection

Juvenile *I. scapularis* were collected via drag sampling at the Cary Institute of Ecosystem Studies (CIES) in the Hudson Valley of New York in June (nymphs) and August (larvae) of 2019 and housed in groups of 20 (nymphs) or 15 (larvae) in glass scintillation vials with moistened plaster of Paris held at room temperature when not in experimental trials. The ticks in a vial were treated as an experimental group. They were never mixed or combined with other groups. Experiments began with 5 groups of nymphs (100 total) and 5 groups of larvae (75 total), but we collected additional ticks from the CIES property in July (nymphs) and August–September (larvae) to replace ticks that had died. These additional ticks were held in separate vials and were treated as new groups in analyses.

### Host Collection

All hosts used in our experiments were wild-caught on the CIES property between June and September 2019. We targeted 4 host species that represent the range of host permissiveness for ticks, in descending order: white-footed mice (*P. leucopus*), eastern chipmunks (*T. striatus*), gray squirrels (*S. carolinensis*), and Virginia opossums (*D. virginiana*). Hosts were housed for the duration of the experiment in appropriately sized open-bottomed cages to allow previously attached ticks to fall off. Hosts were fed rodent block and apple slices (mice and chipmunks); black sunflower seeds, walnuts, and apple slices (gray squirrels); and puppy chow, hard-boiled eggs, and apple slices (opossums). All animals were supplied with fresh water. The use of animals for this experiment was approved by CIES IACUC (protocol number 2019-05).

### Apparatus Design

The testing apparatus (Fig. 1) consisted of a clear plastic food storage container (38 cm wide × 22 cm deep × 21 cm tall) with 5 cm thick slab of white Styrofoam glued to the bottom in which clear, acrylic rods (13 cm tall and 0.64 cm diameter for nymphs, 10 cm tall and

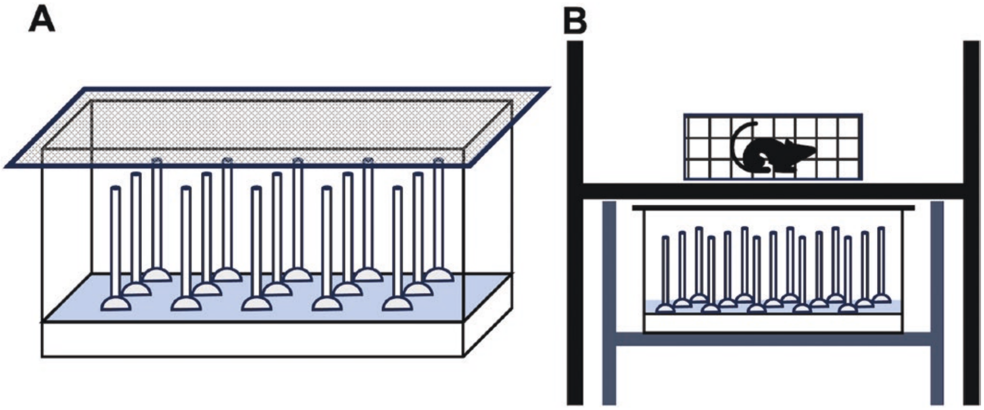

**Fig. 1.** Design of the experimental questing apparatus. A) The apparatus consisted of a plastic container with 15 (larva) or 20 (nymph) clear rods in white Styrofoam, sealed with white clay forming a base at the bottom of the rod. A layer of water, no more than halfway up the clay bases, prevented ticks from moving between rods. A fine mesh screen on top of the container prevented any solid materials from the hosts from entering the questing apparatus. B) Hosts were then placed in open bottom cages on a wire rack above the tick questing apparatus so that the ticks would not feel vibrations from hosts but would receive other cues.

0.32 cm diameter for larvae) were inserted vertically and sealed at the base with modeling clay. Three interior walls were painted white to maximize visual contrast between the ticks and their background. A fine mesh screen lid was placed on top to prevent materials from host animals dropping into the apparatus. To prevent ticks from traveling between rods, we added water to a depth of halfway up the clay mounds prior to each trial.

The entire questing apparatus was held on a shelf of a wire rack underneath a separate, larger wire rack with a shelf placed <10 cm above the tops of each rod on which host cages were placed (Fig. 1), which prevented vibrations from host movements being felt by the ticks. Placing the host above the ticks, rather than to the side, also ensured that all ticks in the apparatus experienced the same gradient of host cues. Tick questing activity was recorded with an iPhone 6s (Apple, Cupertino, California) on a tripod in front of the apparatus, using a time-lapse photography application (OSnap!Pro, developer: Justin Cegnar) set to take a photograph every 5 s, though in later image analyses we only used every other image (i.e., 10 s intervals) for larval ticks. Earlier trials with more frequent photographs indicated ticks did not backtrack in these intervals.

Each trial began with a single tick placed on the clay base of each rod and no host above the apparatus. Ticks were then recorded for 10 min to measure baseline questing behaviors in the absence of hosts. A host in an open-bottomed cage was then placed above the apparatus and the recording continued for an additional 30 min. When not actively adding or removing a host, the experimenter was outside of the building, which itself had no other human or animal activity. After each trial, the apparatus was cleaned with 95% ethanol or isopropanol followed by distilled water and allowed to air dry, and the experimental room aired out with the door open for ≥30 min between trials. Each individual host was used in 3 trials, each with a randomly selected (without replacement) tick group. Dead or unresponsive ticks were replaced with ticks from replacement tick groups.

## Tracking Tick Movements

We tracked the pixel coordinates of the most visible ticks per trial, the number of which varied for each trial (i.e., excluding ticks in regions of the rod not visible to the observer for long stretches of the observation period due to shadows, reflective glare, or staying on the far side the clay base) using the Manual Tracking plugin in FIJI (a distribution of ImageJ2 Version 1.53; Schindelin et al. 2012). Tracking began 4.25 min after the experimenter placed the ticks in the apparatus and again 4.25 min after the experimenter added the hosts to exclude possible responses to these activities. These delays were established after tracking tick questing locations through time in trials with empty animal cages. During these control trials, we observed a noticeable change in tick questing heights and distance traveled between images after they were placed in the apparatus (i.e., ticks ascending and exploring the rods) and a slight change in height and distance traveled between images after the experimenter placed the host cage above the ticks. These responses faded within 4 min of the experimental disturbance. Additionally, our analyses of control trials with empty animal cages demonstrated that 4 min of recorded activity was sufficient to capture movement patterns of nymphs, and 6 min for the slower-moving larvae. These time periods captured any pattern of changes in tick questing height or distance traveled, and extending the time period of data collection did not add any additional information. Thus, we tracked nymph questing behaviors for 4 min and larva questing behaviors for 6 min with and without a host present, after an initial 4.25 min acclimation period. Because ticks often moved in and out of regions of the rods not visible to the observer, the majority of ticks had gaps in their pixel coordinates during the tracking periods. We excluded ticks that had gaps in their tracking larger than 25% of the total observation period (i.e., 1 min for nymphs and 1.5 min for larvae).

## Statistical Analyses

We compared the *speed* a tick traveled, measured in cm/min, and their average *height* from the water level during the tracking period when hosts were present or absent. We chose to use both these measurements, as ticks may increase or decrease their activity, but not alter their average vertical height on the rod. Because our goal was to estimate effect sizes for ticks confronting different species of hosts, or no hosts at all, we used a Bayesian statistical approach. This approach also had the advantage of providing more stable numeric solutions, especially with unbalanced designs, and avoiding the assumptions required by frequentist tests. We used Bayesian hierarchical generalized linear models in order to account for the potential influences of tick groups and host identity while estimating the effect of host presence and host species identity on tick behavior. All models were created in Stan language, a programming language for Bayesian statistics, (Stan Development Team 2019) and run in R using cmdstanr as an intermediary (Gabry et al. 2023). Nymphs and larvae were analyzed separately.

We modeled the average speed of an individual tick in group $g$ in trial $t$ in the absence and presence of a host of species $s$ as a gamma function parameterized with a mean ($\mu$ in the absence of a host, $v$ in the presence of a host) and variance ($\sigma$):

$$\textit{Activity host absent}_{tg} \sim \text{Gamma}\left(\mu_{tg},\ \sigma\right),$$

$$\textit{Activity host present}_{tg,s} \sim \text{Gamma}\left(\nu_{tg,s},\ \sigma\right).$$

We chose a gamma distribution because it is a continuous, positive distribution that allows for peaks above 0, and very long tails. We allowed the expected speed to vary by tick group and trial, together (trial-group; $tg$), for 2 reasons: first, because we had no expectation that tick groups would exhibit consistent differences in activity among trials and second, because time in since emergence and time in captivity, and thus perhaps tick activity, varied among tick groups, preventing a simple inclusion of a time covariate. Thus, we allowed intercepts to vary by trial group in all of our models, with these intercepts, represented by the vector $\alpha[tg]$, drawn from a shared distribution with hyperpriors $\alpha_\mu$ and $\alpha_\sigma$:

$$\log\left(\mu_{tg}\right) = \alpha\left[tg\right]$$

$$\alpha\left[tg\right] \sim \text{Normal}\left(\alpha_\mu,\ \alpha_\sigma\right).$$

We used a log link to constrain $\mu$ and $v$ to be positive (i.e., ticks cannot have negative speed). The expected speed after a host was introduced was modeled similarly, only allowing for the added effect of a host of species $s$:

$$\log\left(\nu_{tg,s}\right) = \alpha\left[tg\right] + \beta\left[s\right]$$

$$\beta\left[s\right] \sim \text{Normal}\left(0,\ 0.5\right).$$

We used a normal distribution for our species prior to allowing for ticks to increase or decrease their speed in the presence of the host. We constrained all priors, via prior predictive simulation, so that values less than 15 cm/min were most likely, with most of the probability mass laying below 5 cm/min, but still allowing for long tails (see Supplemental data for model code and complete priors).

We modeled the average questing height of an individual tick in group $g$ in trial $t$, scaled between 0 (bottom) and 1 (top), using a beta distribution, parameterized with a mean ($\mu$ in the absence of a host, $v$ in the presence of a host), and variance ($\sigma$):

$$\textit{Height absent}_{tg} \sim \text{Beta}\left(\mu_{tg},\ \sigma\right)$$

$$\textit{Height present}_{tg} \sim \text{Beta}\left(\nu_{tg,s},\ \sigma\right)$$

We chose a beta distribution because it is a bounded continuous distribution—ticks could have an average questing height anywhere between the top and bottom of the rod. The beta distribution also allows for bimodal peaks, which would reflect a scenario where ticks were either at the top or bottom of the rod and rarely in the middle. We used a logit link to constrain $\mu$ to be between 0 and 1:

$$\text{logit}(\mu_{tg}) = \alpha\left[tg\right]$$

$$\text{logit}\left(\nu_{tg,s}\right) = \alpha\left[tg\right] + \beta\left[s\right]$$

and used normal distributions centered around 0 for our priors for the effect of trial-group and host species. These priors were broad enough to allow for peaks in the center of the rod (average height in the middle), peaks at the bottom of the rod (ticks rarely ascended the rod), or bimodal peaks (ticks were either at the bottom or the top of the rod).

We also fit 2 reduced versions of the speed and height models, 1 with a general host effect (i.e., the effect of a host does not vary by species) and 1 with no host effect (i.e., the expectations with and without a host are identical). We then compared the expected out-of-sample predictive capacity of each version of our height and speed models using the widely applicable information criteria (WAIC) to determine whether information about host species identity, or even the presence of a host, improved out-of-sample prediction.

## Results

We exposed nymphs to a total of 24 individual hosts (9 mice, 6 chipmunks, 6 squirrels, and 3 opossums) and larvae to a total of 19 individual hosts (9 mice, 6 chipmunks, 4 opossums—no squirrels were captured when larvae were available). After filtering out ticks with large gaps in tracking, we were left with 312 unique host + nymph observations and 294 unique host + larvae combinations in our analyses.

### Effect of Hosts on Tick Questing Speed

Neither nymphs nor larvae noticeably changed their speed in the presence of a host, regardless of species (Figs. 2 and 3; Tables 1 and 2). Estimates from our model including a species-specific effect showed the mean speed for nymphs was largely unchanged from observations before hosts were introduced to those after mice, squirrels, chipmunks, or opossums were added, well within the margin of error of these estimates (Fig. 2A, Table 1). Moreover, these minor differences in mean speeds are trivial compared with the large amount of variation between individual ticks, from no movement to over 10 cm/min. (Fig. 2Band Fig. S1).

Similarly, the mean movement rate of larvae prior to the host introduction was similar to that after chipmunks were introduced, and slightly less after mice and opossums were added, with little differentiation in posterior estimates (Fig. 3A, Table 2). And, again, small differences in mean movement rates belie the large variation between individual ticks within treatments; larvae were frequently observed not moving at all (Fig. 3B, Fig. S2). Notice that while some nymphs move twice as fast as larvae because a greater fraction of nymphs did not move (Figs. 2 and 3), the average speed of nymphs (without a host) was 63% of the average speed of larvae.

### Effect of Hosts on Tick Questing Height

Nymphs changed their average questing height slightly in the presence of hosts. Estimates from our model including a species-specific host effect showed nymphs average questing height increased by approximately 0.85 cm after chipmunks were introduced and slightly less with mice and squirrels. They did not change when opossums were introduced (Fig. 4A, Table 3). However, despite these differences in mean estimates, the posterior distributions still overlapped considerably (Fig. 4A, Table 3) and, again, these differences in averages are minor compared with the variability in the questing heights between individual nymphs (Figs. 4B, Fig. S3). Most nymphs were observed in the lower 2 cm of the rod, though many stayed near the top of the rod, with the remaining few scattered evenly along the middle (Fig. 4B).

The mean questing height of larvae prior also increased by less than 0.2 cm in the presence of hosts compared to without a host, and the credible intervals of these mean estimates overlapped considerably (Fig. 5A, Table 4). As with tick speed, the differences in mean heights with and without hosts were small compared to the large differences in questing heights of individual larvae (Fig. 5B, Fig. S4). Many larvae stayed within a centimeter of the bottom of the rod, while the rest were rather evenly distributed along its height (Fig. 5B).

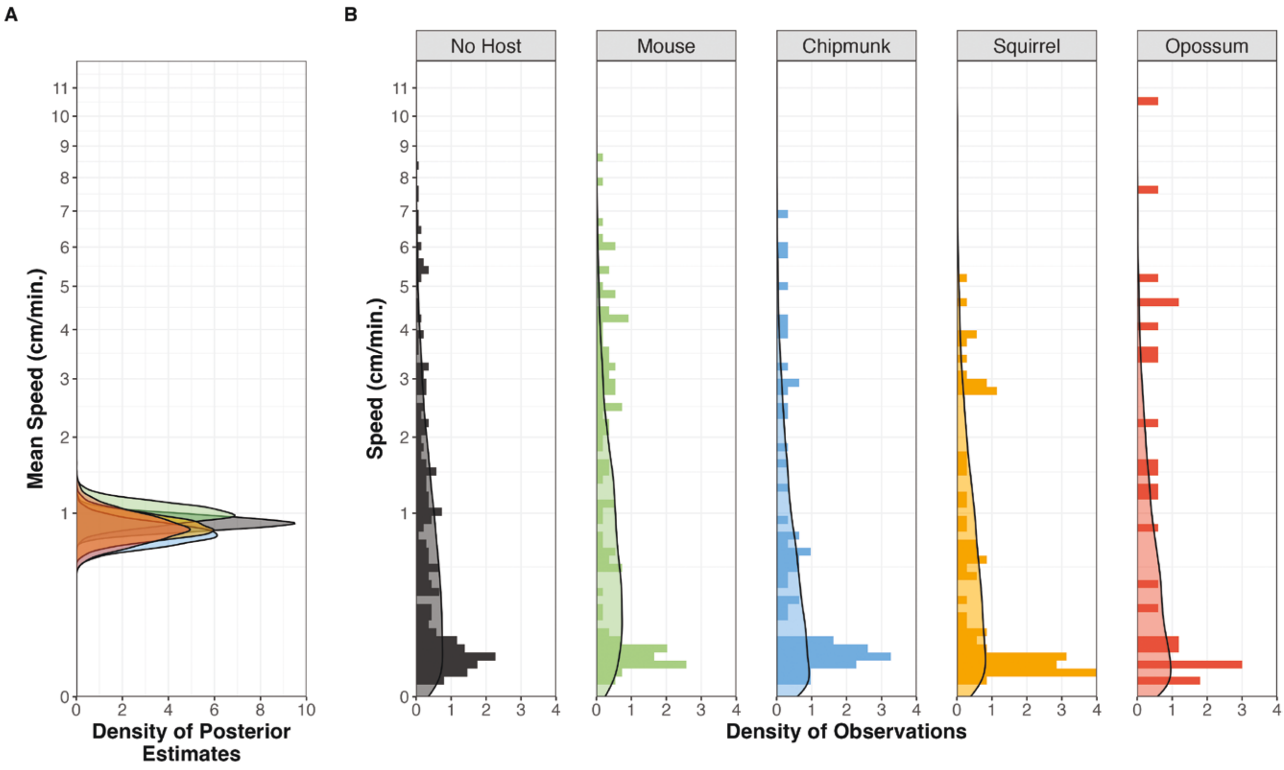

**Fig. 2.** Posterior estimates of mean nymphal tick speed without a host and with each host species present (A) from the speed model with a species-specific host effect. The distribution of nymphal tick speed (cm/min) without a host and with each host species present (B). Colored bars are density histograms of observed tick speeds and shaded curves are the predicted density of values based on posterior estimates from the speed model with a species-specific host effect. The *y*-axis is square-root scaled to capture the long tails of the distribution.

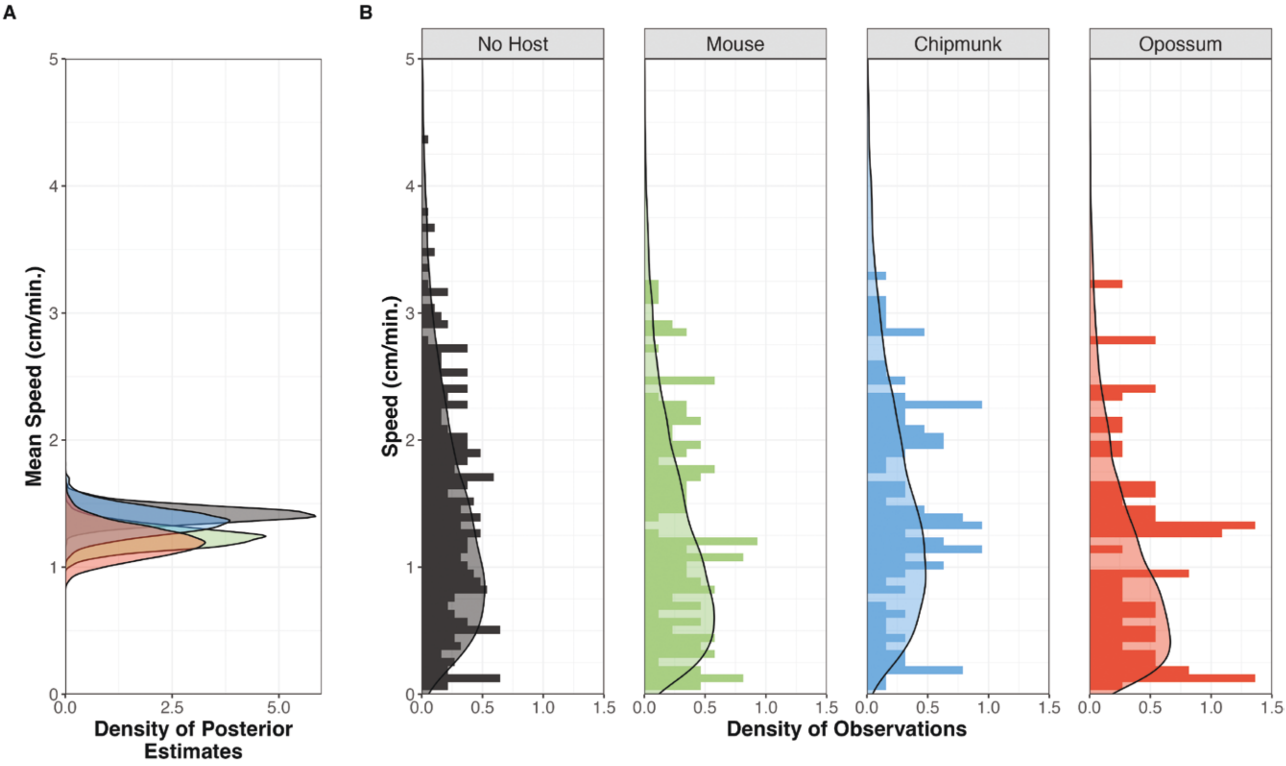

**Fig. 3.** Posterior estimates of mean larval tick speed without a host and with each host species present (A) from the speed model with a species-specific host effect. The distribution of larval tick speed (cm/min) without a host and with each host species present (B). Colored bars are density histograms of observed tick speeds and shaded curves are the predicted density of values based on posterior estimates from the speed model with a species-specific host effect.

## Model Comparison

For each response, movement speed and questing height, and each tick stage models with species-specific host effect performed equivalently or worse than 1 or both simpler models (Tables 5–8). The models of nymph speed with no host effect or a generic host effect were essentially equivalent (Δ WAIC = 0.9, 95% CI −0.59 to 2.39; Table 5), and all models of larva speed were equivalent (within 1 unit and 95% CIs that overlap 0; Table 6). The model of questing height with a generic host effect performed best at out-of-sample prediction for nymphs (Table 7). For the questing height of nymphs, the model with a species-specific effect performed better than a model with no host effect, on average, but the confidence intervals on these differences overlap a great deal (Table 7). The model of questing height with no host effect performed best at out-of-sample prediction for larva (Table 8).

**Table 1.** Nymph speed

| Treatment group | Mean (95% CI) |
|---|---|
| No host | 0.89 (0.76–1.05) |
| White-footed mouse | 1.00 (0.79–1.25) |
| Eastern chipmunk | 0.79 (0.58–1.03) |
| Gray squirrel | 0.85 (0.64–1.10) |
| Virginia opossum | 0.85 (0.59–1.20) |

Estimates of mean nymph questing speed (cm/min) and 95% credible intervals in the absence of a host and in the presence of different host species from our model including a species-specific host effect term.

**Table 2.** Larval speed

| Treatment group | Mean (95% CI) |
|---|---|
| No host | 1.41 (1.28–1.55) |
| White-footed mouse | 1.23 (1.07–1.41) |
| Eastern chipmunk | 1.37 (1.17–1.58) |
| Virginia opossum | 1.20 (0.98–1.45) |

Estimates of mean larval questing speed (cm/min) and 95% credible intervals in the absence of a host and in the presence of different host species from our model including a species-specific host effect term.

## Discussion

Feeding upon a host is an essential step in a tick's life cycle; the host bloodmeal fuels the developmental transitions (e.g., molting) and provides the energy for the next questing period. Thus, finding and attaching to a bloodmeal host is key to blacklegged tick fitness. Additionally, host species vary tremendously in quality, profoundly affecting the tick's probability of surviving to the next life stage (Keirans et al. 1996, LoGiudice et al. 2003, Keesing et al. 2009, Brunner et al. 2011). We thus expect blacklegged ticks to not only respond to the presence of hosts, but to respond differently to hosts of high versus low quality. However, we found that neither nymphal nor larval ticks strongly altered their questing height or movement in the presence of nearby hosts, regardless of the species identity (i.e., quality) of the host.

We expected ticks to move more (i.e., increase their speed) in the presence of hosts because sensing host cues would "activate" the tick to seek out the host or move to a suitable position for host-finding.

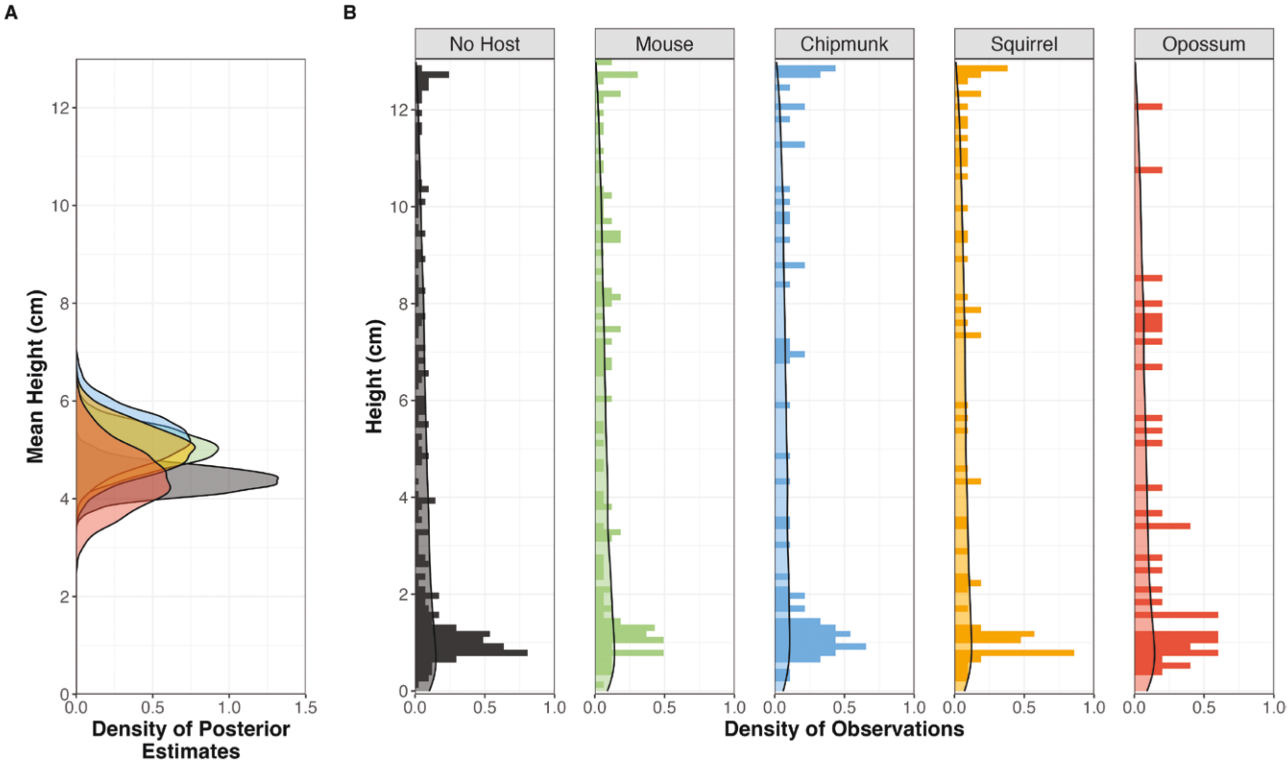

**Fig. 4.** Posterior estimates of mean nymph height on the rod (cm) without a host and with each host species present (A) from the height model with a species-specific host effect. The distribution of nymphal tick heights (cm) without a host and with each host species present (B). Colored bars are density histograms of observed tick heights and shaded curves are the predicted density of values based on posterior estimates from the height model with a species-specific host effect.

Alternatively, ticks might have had an arresting response after sensing the host and would thus move less—as occurs in adult *I. scapularis* (Carroll et al. 1996). We found no clear evidence of either pattern—instead, ticks appeared to maintain very similar levels of activity regardless of the presence or absence of hosts. Indeed, a model without a host effect was expected to make better predictions of nymph speed than a model accounting for the presence of hosts and models of larval speed were essentially equivalent. We did find that, on average, nymphs move less than larvae, but this was due to the large number of nymphs that barely moved—the maximum nymphal speed observed was twice that of larvae.

We also expected ticks to spend more time higher on the questing rod, closer to the host, when hosts were present. Indeed, the classic definition of tick questing is that ticks ascend emergent vegetation in order to contact hosts (Sonenshine and Roe 2014). Nymphs quested roughly 5 mm higher in the presence of a host, and models with a generic host effect performed better in explaining questing height than did models with no host. However, because there was considerable overlap in model estimates with and without hosts, and because we observed no differentiation between host species, the ecological relevance of these differences is not apparent. The differences with and without hosts were even smaller with questing larvae, although the model with a generic host effect again performed better than that with no host. Moreover, there was substantial variability in the questing height of individual nymphs and larvae. Most ticks stayed very low on the rod while a small subset remained near the top, regardless of whether a host was present.

**Table 3.** Nymph height

| Treatment group | Mean (95% CI) |
|---|---|
| No host | 4.38 (3.84–4.96) |
| White-footed mouse | 5.04 (4.23–5.86) |
| Eastern chipmunk | 5.25 (4.32–6.23) |
| Gray squirrel | 5.02 (4.06–6.00) |
| Virginia opossum | 4.42 (3.26–5.74) |

Estimates of mean nymph questing height (cm) and 95% credible intervals in the absence of a host and in the presence of different host species from our model including a species-specific host effect term.

Though our findings are not consistent with previous studies that found evidence of host preferences by measuring ticks' propensity to moved towards particular host species, given an array of simultaneously presented options (James and Oliver 1990, Shaw et al. 2003, Slowik and Lane 2009) our study design introduced elements of biological realism that these previous laboratory studies on tick host preferences lacked. We measured the natural behaviors of ticks (i.e., vertical movements) and recreated how ticks naturally encounter host species (i.e., one at a time, rather than simultaneously). However, there are still important limitations to our study design.

First, hosts were suspended above ticks so that ticks could sense the odor and $CO_2$ of a nearby host, but not make contact. While this arrangement is unrealistic, it allowed all the ticks in the apparatus to experience the same gradient of host odors and $CO_2$. Second, we did not account for other factors that influence a tick's vertical movements, such as desiccation risk—ticks are less likely to be found in higher vegetation layers under desiccating conditions (Vail and Smith 1998, Randolph and Storey 1999)—or the need to avoid pathogenic fungi or predators in the soil (Fischhoff et al.

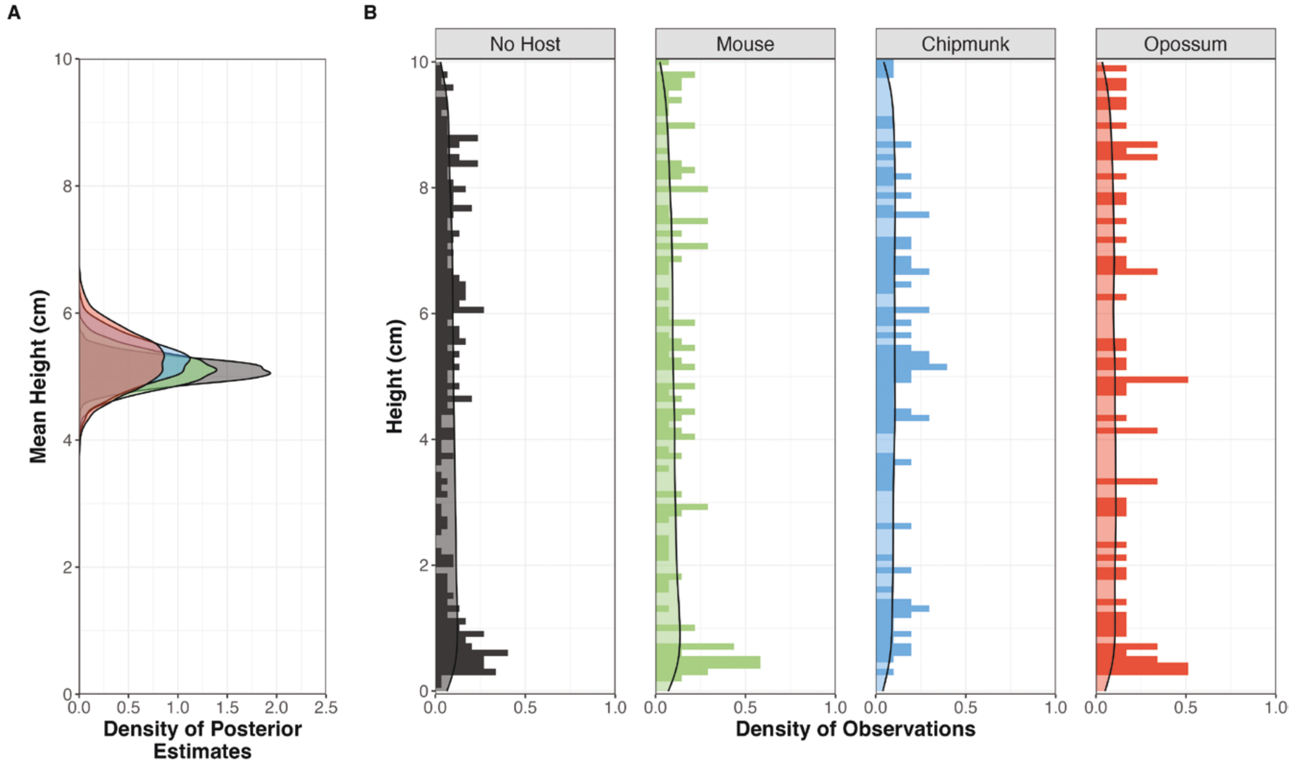

**Fig. 5.** Posterior estimates of mean larvae height on the rod (cm) without a host and with each host species present (A) from the height model with a species-specific host effect. The distribution of larval tick heights (cm) without a host and with each host species present (B). Colored bars are density histograms of observed tick heights and shaded curves are the predicted density of values based on posterior estimates from the height model with a species-specific host effect. Means and credible intervals from models with species-specific host effect.

**Table 4.** Larval height

| Treatment group | Mean (95% CI) |
|---|---|
| No host | 4.62 (4.26–4.97) |
| White-footed mouse | 4.63 (4.10–5.16) |
| Eastern chipmunk | 4.74 (4.14–5.33) |
| Virginia opossum | 4.79 (4.02–5.57) |

Estimates of mean larval questing height (cm) and 95% credible intervals in the absence of a host and in the presence of different host species from our model including a species-specific host effect term; model comparisons.

**Table 5.** Nymph speed model comparison

| Model | WAIC (SE) | Δ WAIC (95% CI) |
|---|---|---|
| No host effect | 839.26 (112.29) | 0.00 |
| Generic host effect | 840.16 (112.33) | 0.9 (–0.59–2.39) |
| Species-specific host effect | 842.78 (112.33) | 3.52 (–0.52–7.56) |

Comparison of nymph speed models with no host effect, a generic host effect, and a species-specific host effect using WAIC and differences from the minimum WAIC (Δ WAIC).

**Table 6.** Larval speed model comparison

| Model | WAIC (SE) | Δ WAIC (95% CI) |
|---|---|---|
| Generic host effect | 1,421.18 (37.84) | 0.00 |
| Species-specific host effect | 1,421.82 (37.69) | 0.64 (–3.48–4.76) |
| No host effect | 1,421.95 (37.41) | 0.76 (–5.47–6.99) |

Comparison of larval speed models with no host effect, a general host effect, and a species-specific host effect using WAIC and differences from the minimum WAIC (Δ WAIC).

**Table 7.** Nymph height model comparison

| Model | WAIC (SE) | Δ WAIC (95% CI) |
|---|---|---|
| Generic host effect | –284.32 (38.37) | 0.00 |
| Species-specific host effect | –282.32 (38.19) | 2.00 (–0.27–4.27) |
| No host effect | –278.62 (38.28) | 5.70 (–0.42–11.82) |

Comparison of nymph height models with no host effect, a general host effect, and a species-specific host effect using WAIC and differences from the minimum WAIC (Δ WAIC).

**Table 8.** Larval height model comparison

| Model | WAIC (SE) | Δ WAIC (95% CI) |
|---|---|---|
| No host effect | –8.90 (13.12) | 0.00 |
| Generic host effect | –8.41 (12.84) | 0.48 (–0.97–1.93) |
| Species-specific host effect | –4.83 (13.31) | 4.06 (2.49–5.63) |

Comparison of larval height models with no host effect, a general host effect, and a species-specific host effect using WAIC and differences from the minimum WAIC (Δ WAIC).

2018, Burtis et al. 2019), either of which might modify questing behaviors. In our experiment specifically, because we prevented ticks from moving between questing rods using a layer of water, we may have created a humidity gradient with higher humidity at the bottom of the rod, which may have had some influence on tick behaviors. However, this also likely represents a natural scenario, as humidity tends to decrease from the ground up. It also reduced the overall risk of desiccation during the trials. Lastly, our collection and use of ticks did not allow us to accurately account for the age of ticks in our analyses. As ticks age, they lose their energy reserves, which may have reduced tick responsiveness to host cues and propensity to move on the rods during our experiment (Randolph and Storey 1999, Herrmann and Gern 2012, McClure and Diuk-Wasser 2019). This could also decrease ticks' ability to behave in ways that suggest they have host preferences. However, our statistical framework accounted for trial, and thus qualitative differences in the time since emergence, and we still found no evidence that host species impacted tick questing behaviors. And in any case, our results represent responses (or the lack of responses) of wild ticks to wild hosts, implying a degree of ecological realism.

While our results strongly suggest that host "preferences," if they exist, are not active responses to nearby hosts, there may be fixed strategies that lead to higher contact rates with higher-quality hosts. Indeed, the low questing positions of most larvae and nymphs might increase their odds of contacting small-bodied, and generally higher quality hosts, that are found lower to the ground. Ticks that never ascend emergent vegetation and stay within the leaf litter may still have access to hosts that move through the different layers of the leaf litter. For instance, blood meal analyses of blacklegged ticks collected in New England found that 26% of nymphs had gotten their larval blood meal from shrews, which often move through and under forest leaf litter (Goethert et al. 2023). Indeed, many studies simply assume ticks quest at the right height to contact the hosts they desire (Mejlon and Jaenson 1997, Tietjen et al. 2020). It is also possible that ticks actively select or reject hosts later in the process, perhaps at the point of contact (when ticks could choose whether or not to climb onto the host) or after having climbed onto a host (when ticks could choose to either bite or drop off the host) (Keesing et al. 2009). Future work should carefully examine these later steps for evidence that ticks tend to select higher-quality hosts.

Finding a bloodmeal host is a necessary step in the blacklegged tick life cycle and the identity of that host has profound consequences for both tick survival and the probability a tick acquires a pathogen of human interest. While there is good reason to expect ticks to exert some control over the host they feed on, it is not yet clear how such "preferences" manifest, at least for blacklegged ticks. Our results suggest that juvenile ticks do not actively change their questing behavior—movements or heights—in response to hosts. If there are active preferences, they presumably manifest later in the host-finding and feeding process.

## Supplementary data

Supplementary data are available at *Journal of Medical Entomology* online.

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## Author contributions

Emily Burton (Conceptualization [Equal], Formal analysis [Equal], Investigation [Lead], Methodology [Equal], Writing—original draft [Lead], Writing—review & editing [Equal]), Richard Ostfeld

(Conceptualization [Equal], Formal analysis [Supporting], Funding acquisition [Equal], Methodology [Supporting], Writing—review & editing [Equal]), and Jesse Brunner (Conceptualization [Equal], Formal analysis [Equal], Funding acquisition [Equal], Methodology [Equal], Writing—review & editing [Equal])

## Data availability

Data from this study is available in Dryad: https://doi.org/10.5061/dryad.k6djh9wg9. Code from this study is available in Zenodo: https://doi.org/10.5281/zenodo.12775408.

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