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Using A Dynamic Model To Assess Maintenance Of Deer Tick Virus Via Horizontal Transmission In Ixodes Scapularis Populations

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Using a dynamic model to assess maintenance of Deer Tick Virus via horizontal transmission in *Ixodes scapularis* populations

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Abstract

Introduction

Deer Tick Virus—a lineage of Powassan Virus—is an emerging tick-borne flavivirus associated with high rates of morbidity and mortality. Although DTV infection is rare, there has been an observed increase in the number of human cases in recent decades, necessitating more public health attention. *Ixodes scapularis* ticks are known to be the primary vector of DTV. However, the enzootic cycle has yet to be fully characterized and there is evidence that horizontal transmission alone may be insufficient for DTV maintenance; it is hypothesized that vertical and co-feeding transmission are also necessary for sustained transmission.

Methods

A dynamic model was developed to analyze DTV maintenance in the absence of vertical and co-feeding transmission. Multiple parameters—including host population density, host-to-larva and nymph-to-host transmission rates, and duration of host viremia—were modified to assess their impact on DTV transmission dynamics.

Results/Conclusions

DTV infection rates within the *I. scapularis* population declined dramatically within the tick population during the first year of the model's run-time, and DTV prevalence dropped to zero early in the second year. The model output indicates that, in isolation, horizontal transmission is unlikely to be sufficient for sustaining DTV long-term. A

combination of increased duration of host viremia, host population density, and transmission rates resulted in DTV stability within the tick population over time. Therefore, in order for viremic transmission to act as the sole form of transmission in nature, a combination of parameters must be modified, including host density, host viremic period, and/or horizontal transmission rates.

Acknowledgments

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Introduction

Powassan virus (POWV) is an emerging tick-borne virus associated with high rates of morbidity and mortality. POWV was first isolated in Ontario, Canada in 1958 from the brain of a 5-year-old who died of encephalitis; since then, human cases have also been identified in the United States and Russia [1]. Although POWV infection is rare, there has been an observed increase in the number of human cases over the past few decades, necessitating more public health attention [2].

Unlike other tick-borne diseases—such as Lyme disease and babesiosis—ticks can transmit POWV rapidly, with attachment durations as low as fifteen minutes for successful transmission [3]. This has important implications for intervention; some prevention measures, such as tick checks, may intervene too late to prevent POWV transmission. Due to the severity of disease, the observed rise in incidence, and the challenges of prevention, this virus has increasing public health relevance.

POWV is a positive-sense, single-stranded RNA virus in the genus *Flavivirus,* which is primarily comprised of arboviruses transmitted by mosquitoes or ticks [4]. In North America, there are two genetically distinct lineages of POWV with separate enzootic cycles: POWV I and POWV II; the latter is also known as deer tick virus (DTV) [5-7]. POWV I is transmitted by *Ixodes cookei* and *I. marxi* ticks and it is thought that medium-sized rodents serve as reservoir hosts [8]. POWV II/DTV, on the other hand, is maintained in an enzootic transmission cycle between *I. scapularis* and

small rodent hosts. While the two lineages of POWV are serologically indistinguishable, DTV is primarily transmitted by a human-biting tick, whereas the vectors of POWV lineage I only bite humans occasionally; therefore, DTV may have more public health relevance and as such is the focus of this report [9].

Clinical Illness and Epidemiology

Early symptoms of POWV infection in humans include fever, lethargy, and headache, but as the disease progresses, it can result in encephalitis and severe neurological sequelae such as seizures, paralysis, and coma. These conditions can cause lost-lasting neurologic sequelae in survivors, and the case-fatality rate is estimated to be around 10% [8, 10-12].

Infection with either lineage of POWV is diagnosed by detecting viral RNA or POWV-specific IgM in cerebrospinal fluid, detecting a large increase in POWV neutralizing antibodies in serum samples, or detecting POWV-specific IgM and neutralizing antibodies in the same or later sample [13]. Genomic sequencing is necessary to differentiate POWV lineage I from DTV.

Between 2006 and 2016, 99 cases of POWV disease in the United States were reported to ArboNET, the CDC's electronic passive surveillance system for arboviruses. The highest burden of disease was in people ≥50 years of age, and males were disproportionately affected. Of the reported cases, 90% were hospitalized and 11% died; all deaths occurred among people above 50 years of age [14]. Human risk factors for

DTV infection may include exposure to tick bites, spending time in wooded areas, and close contact with cats or dogs that are exposed to ticks [15].

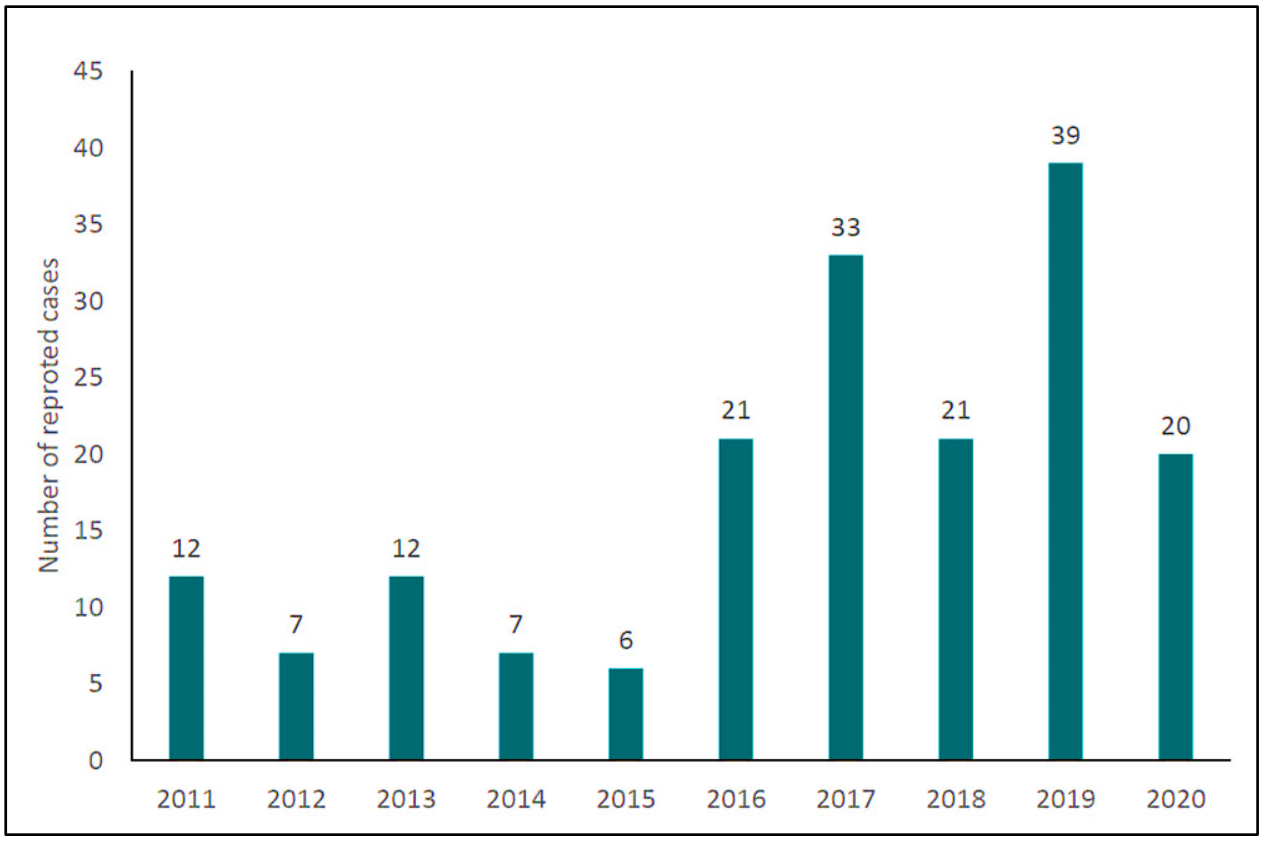

Figure 1. Annual number of POWV neuroinvasive human cases reported to ArboNET (2011-2020) [2]

The number of reported human cases was greater in more recent years, suggesting an increase in POWV incidence over time (Figure 1). The number of reported cases is likely an underestimate of the true disease burden, given that people may not seek care for less severe illness and some cases may not be tested for POWV. Disease onset occurred in all months except March, and cases were highest in late spring through early summer, representing a large window of POWV exposure risk [14].

While the rise in cases could be attributed to better surveillance or improved diagnosis, it may also reflect a true increase in prevalence. Human cases have been reported in 13 different states since a woman in New Jersey was diagnosed with POWV encephalitis in 1970, representing the first reported human case in the United States [2, 16]. There has been a shift in the epidemiology such that more cases are appearing in regions endemic for Lyme disease in the northeastern and northcentral U.S., suggesting that *I. scapularis* ticks—and therefore the DTV strain of POWV—may play an important role in the increase in cases [17]. This observation supports the hypothesis that the upward trend is in fact due to a true increase in prevalence.

Transmission

There are three modes of transmission that may be responsible for maintaining POWV in *I. scapularis* populations: horizontal transmission, vertical transmission, and co-feeding transmission. The conventional paradigm for understanding arbovirus transmission is that vectors become infected by feeding on a vertebrate host that is viremic with a titer sufficient to establish infection in the arthropod—i.e., via horizontal transmission (Figure 2a). Previous work in this laboratory has found that the POWV infection rate for *I. scapularis* nymphs was 27.8% (54/194) after feeding on viremic Balb/c mice as larvae; however, it is unclear if this is representative of the infection rates occurring in nature [18].

Balb/c mice are a laboratory strain of mice and may be more susceptible to DTV infection than wild rodent hosts, and thus develop viremia. However, for many rodent

species in nature, the viral concentration is only high enough in hosts to create a dose sufficient for transmission for a short period of time, if at all [19]. Therefore, other modes of transmission, such as vertical and co-feeding transmission, may be essential for sustaining the DTV enzootic cycle.

Tick-borne encephalitis virus (TBEV) is a tick-borne flavivirus found in Europe and Asia that is closely related to POWV [20]. Literature pertaining to TBEV transmission may therefore provide valuable insight into DTV transmission. Vertical transmission occurs when parents pass on infection to their offspring (Figure 2c). In the case of TBEV, transovarial transmission—where virus spreads to progeny via the ovaries—has been documented to occur at low rates and is believed to be a crucial component of TBEV maintenance [21, 22].

Co-feeding transmission, on the other hand, is a route of infection in which susceptible vectors become infected by feeding in close proximity to infected vectors; this can occur at very low titers or even in the absence of viremia (Figure 2b). The process is thought to be mediated by salivary proteins released by the feeding tick [23]. Co-feeding transmission provides benefits for both the vertebrate host and the tick as well as an evolutionary advantage for the virus. Without the need for high virulence, host mortality is reduced, which may allow the tick to complete its feeding period. Furthermore, horizontal transmission requires viremia to be above a certain threshold, the timing of which is fleeting and may occur after susceptible ticks have already

become fully engorged. Co-feeding transmission, however, is not limited by the timing or presence of viremia [24].

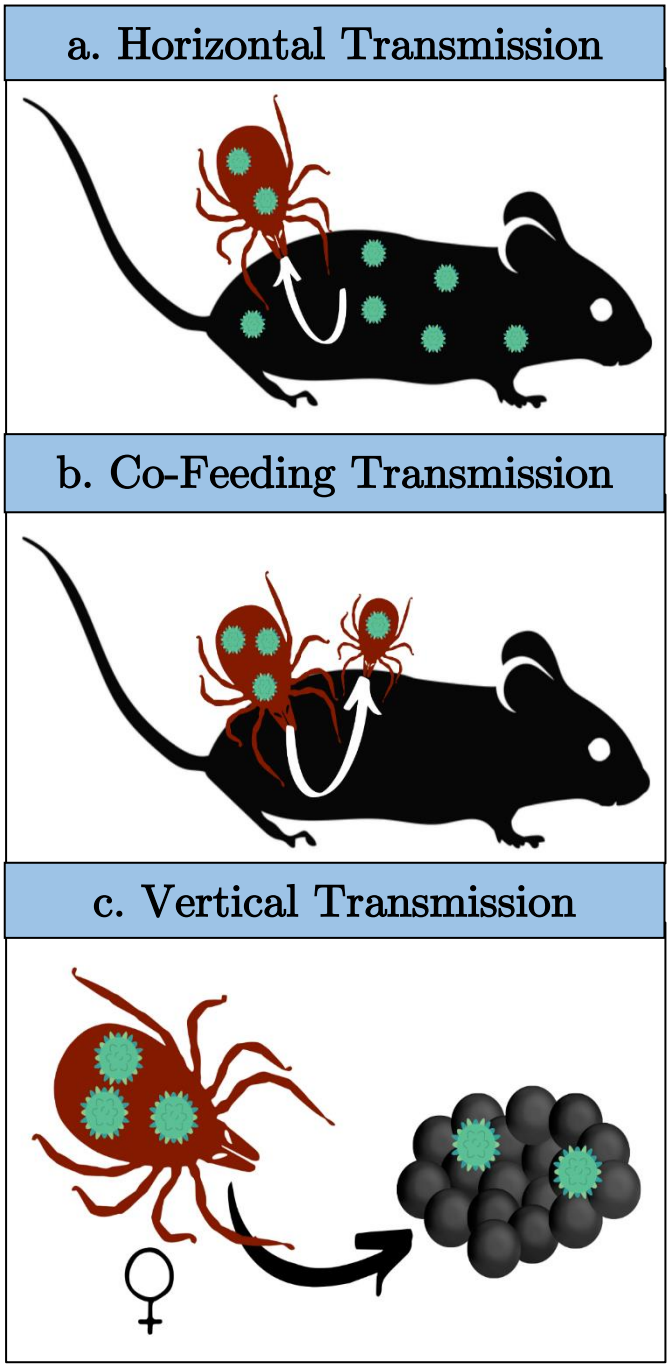

Figure 2. Diagram representing three modes of transmission: (a) horizontal—a tick ingests virus from host blood; (b) co-feeding—a larva becomes infected by feeding in proximity to infected nymph; and (c) vertical—the virus is transmitted from an infected female to her offspring

Efficient transmission between co-feeding ticks has been observed for TBEV [23]. This mode of transmission generally occurs between larvae and nymphs; therefore, seasonal activity of larvae and nymphs must overlap for co-feeding to occur, and may contribute to the geographic distribution of tick-borne viruses that rely on co-feeding for maintenance within the population [19]. Another consideration for the success of co-feeding transmission is the vertebrate host on which the feeding occurs, given that host species demonstrate variable efficiency of nonviremic transmission. For example, a greater proportion of co-feeding ticks became infected with TBEV on *Apodemus* mice compared to bank voles [24].

As mentioned previously, DTV is transmitted by *I. scapularis,* which is also the primary vector for other human illnesses such as Lyme disease, babesiosis, and granulocytic anaplasmosis. Therefore, it has been assumed that DTV shares the same reservoir host as these diseases: white-footed mice (*Peromyscus leucopus*) [25]. In a laboratory setting, *P. leucopus* mice did develop viremia following intraperitoneal inoculation, although it was early and short-lived [26]. Despite prior assumptions, the enzootic cycle for DTV has not yet been fully characterized, and there is emerging evidence that other rodent species may be reservoir hosts.

The ability for horizontal transmission alone to maintain DTV in *I. scapularis* populations likely depends on the duration of infectious viremia in hosts as well as the host-to-larvae and nymph-to-host transmission rates. Therefore, the aim of this report is to create a transmission dynamic model of a population of *I. scapularis* ticks and rodent

hosts in which only horizontal transmission occurs (i.e., vertical and co-feeding transmission rates are zero). Based on known parameters, the model assesses the ability of horizontal (viremic) transmission to maintain DTV within an *I. scapularis* population over time.

Methods

Deer Tick Virus Transmission Dynamics

A transmission dynamic model was used to describe horizontal transmission of DTV in the absence of vertical or co-feeding transmission (Figure 3). The *I. scapularis* tick population is stratified as eggs (E), larvae (L), nymphs (N), and adults (A). Larvae, nymphs, and adults are further divided into questing (subscript q), feeding (subscript f), and engorged (subscript e) compartments. Feeding and engorged larvae and all stages of nymphs are stratified as susceptible (subscript s) or infectious (subscript i). All eggs and questing larvae are assumed to be susceptible due to the absence of vertical transmission

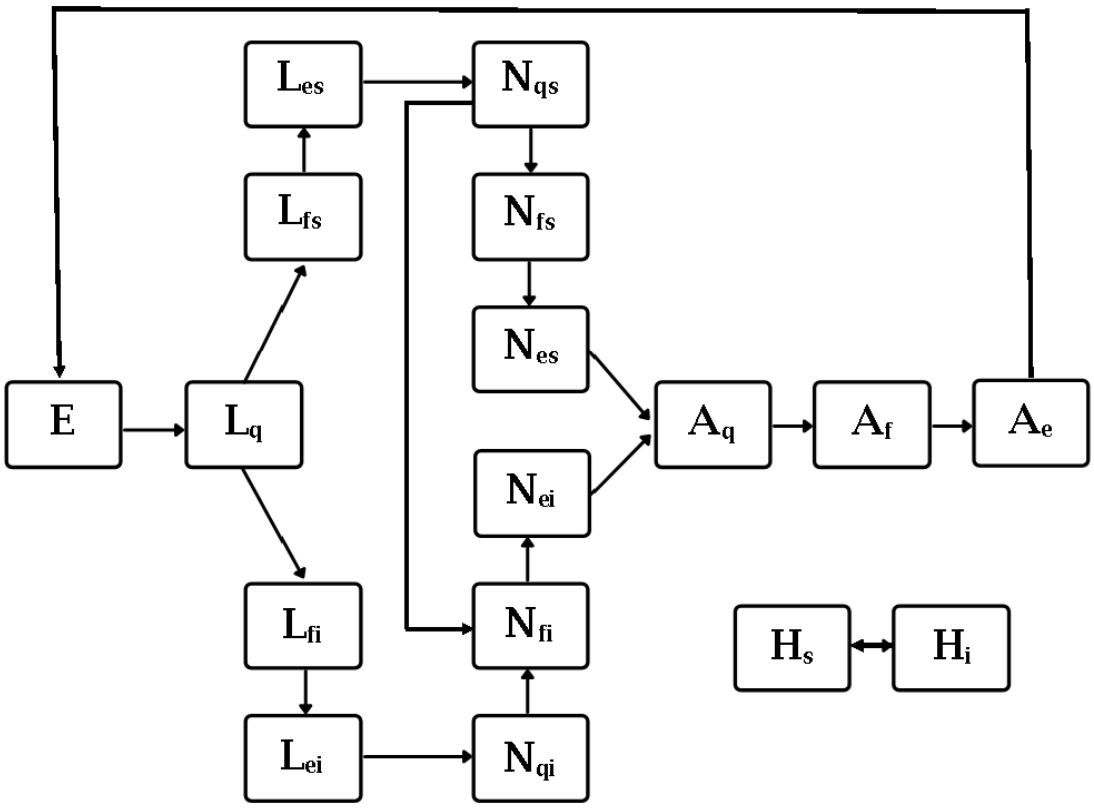

Figure 3. The transmission dynamic model of DTV transmission in tick and host populations. For simplicity, mortality rates are not depicted in the diagram above but are incorporated into the model for each compartment.

in this model. Infection status of adults is not taken into account under the assumption that adult ticks feed on a separate host (D), whereas immature ticks feed on a host (H) that plays a role in the POWV enzootic cycle. The immature host was likewise separated into susceptible (H_s) and infected (H_i) compartments.

The model assumes that horizontal transmission can occur when infected questing nymphs (N_{qi}) feed on a susceptible host (H_s) or when susceptible questing larvae or nymphs (L_{qs} or N_{qs}) feed on an infected host (H_i). Transmission dynamics in tick and host populations are influenced by horizontal transmission rates from nymphs to hosts (β_{nh}), hosts to larvae (β_{hl}), and host to nymphs (β_{hn}), the host recovery rate (γ), and parameters affecting tick/host population growth, including mortality rates, birth rates, host-attaching rates, feeding rates, and development rates (Figure 4).

Temperature-Dependent Parameters

Multiple variables in the model (e.g., developmental rates, tick oviposition rate) are dependent on temperature, which in turn is dependent on time. Historical daily temperature data were acquired from the National Centers for Environmental Information online database. Data were collected by the New Haven Tweed Airport Station (#USW00014758) in Connecticut in 2010; temperatures were reported as daily averages [27]. A model was fit to these data using harmonic regression (adjusted R^2: 0.9973) to inform temperature-dependent model parameters (Figure 5).

Model Equations

$$N_{ticks} = E + L_q + L_{fs} + L_{fi} + L_{es} + L_{ei} + N_{qs} + N_{qi} + N_{fs} + N_{fi} + N_{es} + N_{ei} + A_q + A_f + A_e$$

$$S = L_{fs} + L_{es} + N_{qs} + N_{fs} + N_{es}$$

$$I = L_{fi} + L_{ei} + N_{qi} + N_{fi} + N_{ei}$$

$$\frac{dE}{dt} = b(t)A_e - d_e(t)E - \mu_e E$$

$$\frac{dL_q}{dt} = d_e(t)E - \theta_l(t)\alpha_l(t)L_q - \mu_{ql}L_q$$

$$\frac{dL_{fs}}{dt} = \theta_l(t)\alpha_l(t)\left(\frac{H_s}{H} + (1-\beta_{hl})\frac{H_i}{H}\right)L_q - \rho_l L_{fs} - \mu_{fl}L_{fs}$$

$$\frac{dL_{fi}}{dt} = \theta_l(t)\alpha_l(t)\frac{Hi}{H}\,\beta_{hl}\,L_q - \rho_l L_{fi} - \,\mu_{fl}L_{fi}$$

$$\frac{dL_{es}}{dt} = \rho_l L_{fs} - d_l(t)L_{es} - \mu_{el}L_{es}$$

$$\frac{dL_{ei}}{dt} = \rho_l L_{fi} - d_l(t)L_{ei} - \mu_{el}L_{ei}$$

$$\frac{dN_{qs}}{dt} = d_l(t)L_{es} - \theta_n(t)\alpha_n(t)N_{qs} - \mu_{qn}N_{qs}$$

$$\frac{dN_{qi}}{dt} = d_l(t)L_{ei} - \theta_n(t)\alpha_n(t)N_{qi} - \mu_{qn}N_{qi}$$

$$\frac{dN_{fs}}{dt} = \theta_{n(t)}\alpha_n(t)\left(\frac{H_s}{H} + (1-\beta_{hn})\frac{H_i}{H}\right)N_{qs} - \rho_n N_{fs} - \mu_{fn}N_{fs}$$

$$\frac{dN_{fi}}{dt} = \theta_n(t)\alpha_n(t)\beta_{hn}\frac{H_i}{H}N_{qs} + \theta_n(t)\alpha_n(t)N_{qi} - \rho_n N_{fi} - \mu_{fn}N_{fi}$$

$$\frac{dN_{es}}{dt} = \rho_n N_{fs} - d_n(t)N_{es} - \mu_{en}N_{es}$$

$$\frac{dN_{ei}}{dt} = \rho_n N_{fi} - d_n(t)N_{ei} - \mu_{en}N_{ei}$$

$$\frac{dA_q}{dt} = d_n(t)(N_{es} + N_{ei}) - \theta_a(t)\alpha_a(t)A_q - \mu_{qa}A_q$$

$$\frac{dA_f}{dt} = \theta_a(t)\alpha_a(t)A_q - \rho_a A_f - \mu_{fa}\left(\frac{A_f}{D}\right)A_f$$

$$\frac{dA_e}{dt} = \rho_a A_f - \mu_{ea}A_e$$

$$H = Hs + Hi$$

$$\frac{dHs}{dt} = \mu_h H - \beta_{nh}\theta_n(t)\alpha_n(t)\left(\frac{N_{qi}}{N_q}\right)H_s + \gamma Hi - \mu_h Hs$$

$$\frac{dHi}{dt} = \beta_{nh}\theta_n(t)\alpha_n(t)\left(\frac{N_{qi}}{N_q}\right)H_s - \gamma Hi - \mu_h Hi$$

Figure 4. Differential equations that describe the change in population size over time for each compartment in the model

Temperature-dependent equations for development rate of eggs ($d_e(t)$), engorged larvae ($d_l(t)$), and engorged nymphs ($d_n(t)$) were derived from a model created by Wallace et al. and were informed by data collected in a 2004 study by Ogden et al. [28, 29]. Heaviside functions were incorporated into the maturation equations to simulate diapause at temperatures below 15°C, at which point development rates are assumed to be zero [28].

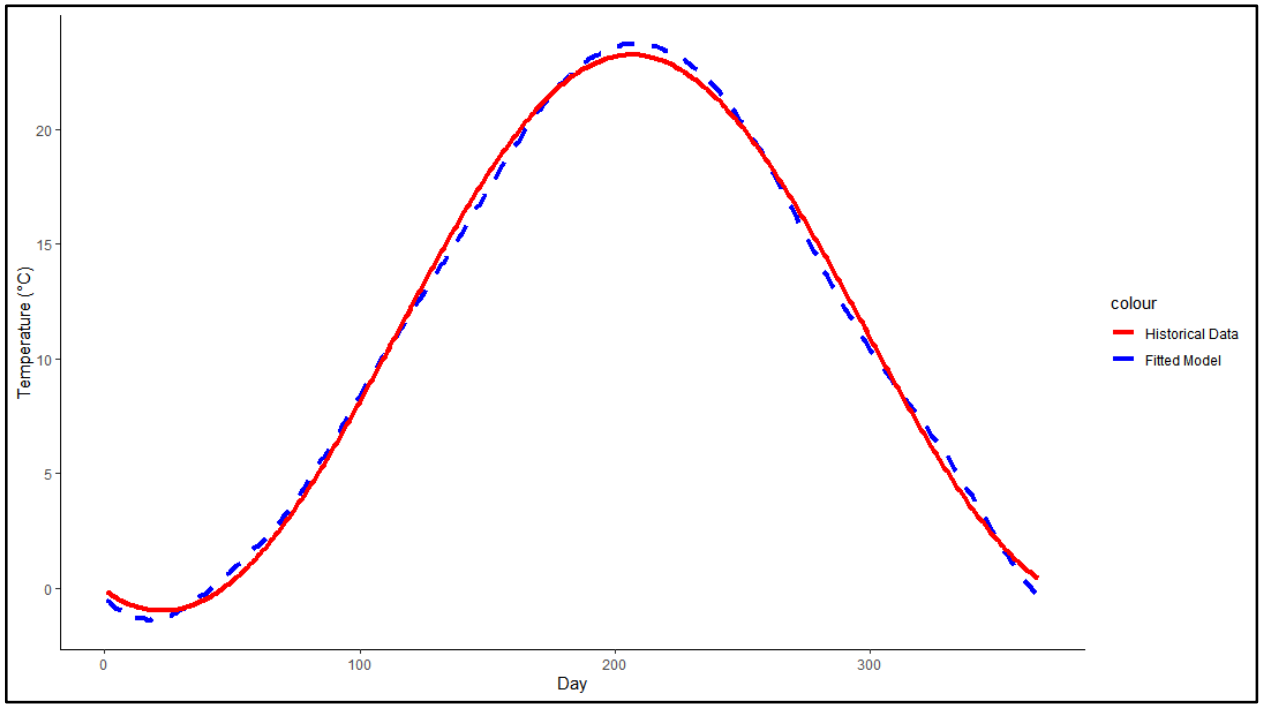

Figure 5. The seasonal temperature in New Haven, CT by day of the year. The red line represents historical data collected in 2010, and the blue dashed line was fit to this data using harmonic regression and used as the equation for daily temperature in the model.

Fecundity was also assumed to be temperature-dependent and oviposition rate was modeled using a quadratic equation developed by Mount et. al. This equation is applied when daily temperature is between 5°C and 29°C; otherwise, fecundity is assumed to be zero (Appendix Table 1) [30]. The model assumes that approximately one

half of engorged adults are female and that all females are capable of oviposition when temperature conditions are appropriate.

Mortality and Feeding Rates

Daily, per-capita mortality rates for tick eggs (μ_e), questing larvae (μ_{ql}), engorged larvae (μ_{el}), questing nymphs (μ_{qn}), engorged nymphs (μ_{en}), questing adults (μ_{qa},), and engorged adults (μ_{ea}) are held constant and were determined by field studies conducted by Ogden et al. [31]. Per-capita mortality rates for feeding larvae (μ_{fl}), feeding nymphs (μ_{fn}), and feeding adults (μ_{fa}) are assumed to be density-dependent as a result of host grooming behavior and host acquired resistance [31]. The daily mortality rate for engorged adults is greatest following oviposition ($\mu_{ea} = 0.5$), and lowest while in diapause ($\mu_{ea} = 0.002$) [29].

Once a tick attaches to a host, the larval feeding rate ($\rho_l = 0.33$), nymphal feeding rate ($\rho_n = 0.2$), and adult feeding rate ($\rho_a = 0.1$) are based on average feeding periods of 3 days, 5 days, and 10 days, respectively [31, 32].

Additional Parameters

The horizontal transmission rate from a viremic host to a susceptible larva ($\beta_{hl} = 0.278$) was determined in a laboratory setting [18]. The horizontal transmission rate from a viremic host to a susceptible nymph (β_{hn}) was set to 45%. Although this rate has not been established empirically, nymphs ingest more blood during feeding and consequently more virus, so the infection rate is expected to be higher. In any case, the

value selected should not influence the model outcomes; infected adults are assumed to feed on a separate host and are not a factor in DTV maintenance given the absence of vertical transmission in this model.

Questing activity is assumed to follow a seasonal pattern for each tick life stage and is based on observed seasonal activity of *I. scapularis* in the northeastern United States. Adult ticks undergo questing behavior twice a year; the first questing period spans from mid-February to May, and the second extends from the beginning of September to the end of the year [33]. The proportion of adults questing (θ_a) is modeled using a parabolic function to represent each questing period, with a peak in late March for the first period and a peak at the end of October for the second. Larvae and nymphs only have one questing period per year. Seasonal activity of nymphs spans from May to September, and the parabolic function representing the proportion of nymphs questing (θ_n) peaks in early summer [33, 34]. For larvae, seasonal questing activity spans from July to October, with a peak proportion of larvae questing (θ_l) in mid-August [33]. Outside of the questing period for each life stage, the proportion of ticks questing is 0.

While questing, daily host-attaching rates for larvae and nymphs ($\alpha_l(t)$ and $\alpha_n(t)$) are dependent on the density of immature hosts (H). Similarly, daily host-attaching rates for adults ($\alpha_a(t)$) are dependent on the density of mature hosts (D) at each time point [31].

Initial Conditions

The run-time for each model iteration is five years (1,825 days). The initial tick population size is 100,000 and the initial host population size is 200. Initial proportions of larvae, nymphs, and adults are based on findings from a field study and these values are listed in Appendix Table 2 [35]. For the first model iteration, the host recovery rate (γ) will be 0.5, representing a mean viremic period of two days, but γ will be decreased to represent longer viremic periods in subsequent model iterations. The transmission rate from an infected nymph to a susceptible host (β_{nh}) will start at 0.278, which is the measured value of β_{hl} in a laboratory setting [18]. Likewise, β_{nh} may be adjusted in subsequent iterations. For the first model, the initial host population size (H) is 200, with 1% of hosts infected at baseline. Later runs will increase host population size to simulate the impact of a higher host density on POWV transmission and tick population dynamics.

Model Outcomes

The primary outcome of interest for this model is the growth rate of the infected compartment (I) over time. The first iteration of the model will use parameters as specified as above, which are thought to most closely reflect true values. Model output with a negative growth rate for the infected compartment would indicate that POWV cannot be maintained in the tick population under these conditions. In this case, multiple variables will be adjusted in order to identify the circumstances under which horizontal transmission alone would be sufficient to sustain POWV. Adjusted variables

will include the horizontal transmission rate from nymph to host (β_{nh}) and host to larva (β_{hl}), the host recovery rate (γ), and the initial immature host population size (H) to assess their impact on the outcome of interest.

Results

Initial Tick Population and POWV Dynamics

The model output demonstrates annual seasonality of tick life stages during the 5-year run-time as expected (Figure 6a). On day 1, the initial proportion of infected hosts and nymphs was 0.01 (Appendix Table 2). The host recovery period for this iteration was two days, representing fleeting viremia for immature hosts. Host-to-larva and nymph-to-host transmission rates (β_{hl} and β_{nh}) were 0.278 and the host-to-nymph transmission rate (β_{hn}) was 0.45. Under these conditions, POWV declined dramatically within the tick population during the first year and disappeared completely within the first quarter of the second year (Figure 6b).

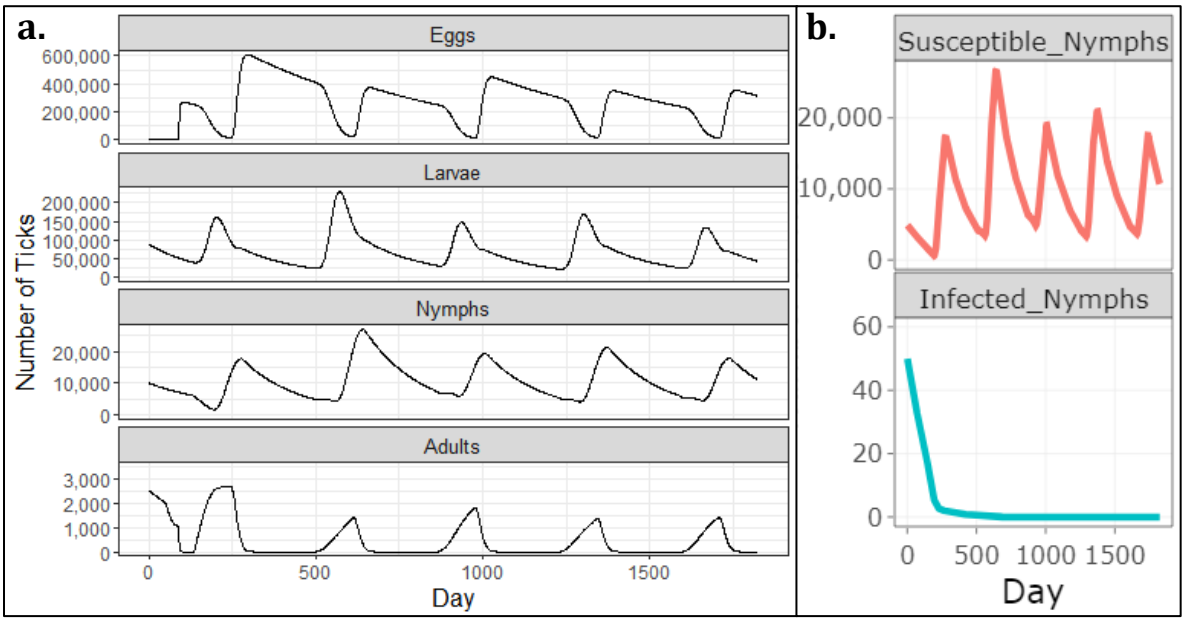

Figure 6. **(a)** Population dynamics of each tick life stage over a 5-year period; **(b)** Number of susceptible vs. infected questing nymphs during this time frame

Based on these findings, it is unlikely that horizontal transmission alone is responsible for maintaining DTV in *I. scapularis* populations in the northeastern United

States. However, many of the model parameters do not have known literature values and thus are merely estimates. Therefore, future model iterations adjust the hypothesized parameter values to determine the conditions necessary for DTV stability in this model.

Impact of host recovery rate (γ)

In the next set of model iterations, the host recovery rate (γ) is modified to analyze the impact of varying durations of host viremia. Current evidence points to a very short or absent viremic period for hosts of DTV, but the enzootic cycle has yet to be fully characterized and further research is needed to ascertain the true levels and duration of viremia in rodents.

Linear regression (calculated for the period where I>0) was used to visualize trends in DTV infection over time. In the first model iteration, γ is 0.5, resulting in a very steep decline in infected larvae and questing nymphs ($slope = -0.1287\ \frac{ticks}{day}$, Figure 7a). When the value of γ is changed to 0.0357, representing a viremic period of 28 days or approximately one month, the slope of the trend line remains negative ($slope = -0.0726\ \frac{ticks}{day}$), but the decline in DTV is more gradual and seasonality of DTV infection is observed (Figure 7b). When γ is modified to be 0.00274 and 0.00137, representing a one-year and two-year viremic period, respectively, the overall trend in DTV infection remains negative, ($slope = -0.0286, -0.0269\ \frac{ticks}{day}$, Figure 7c,d). For a one-year viremic period, POWV disappears from the tick population in the first quarter

of the fourth year. Although there are still two infected ticks at the end of the run for the two-year viremic period, infection rates would quickly reach zero if the run-times were extended. An additional observation is that the maximum number of POWV cases during the 5-year time period increases and the decline in infected ticks slows as γ decreases.

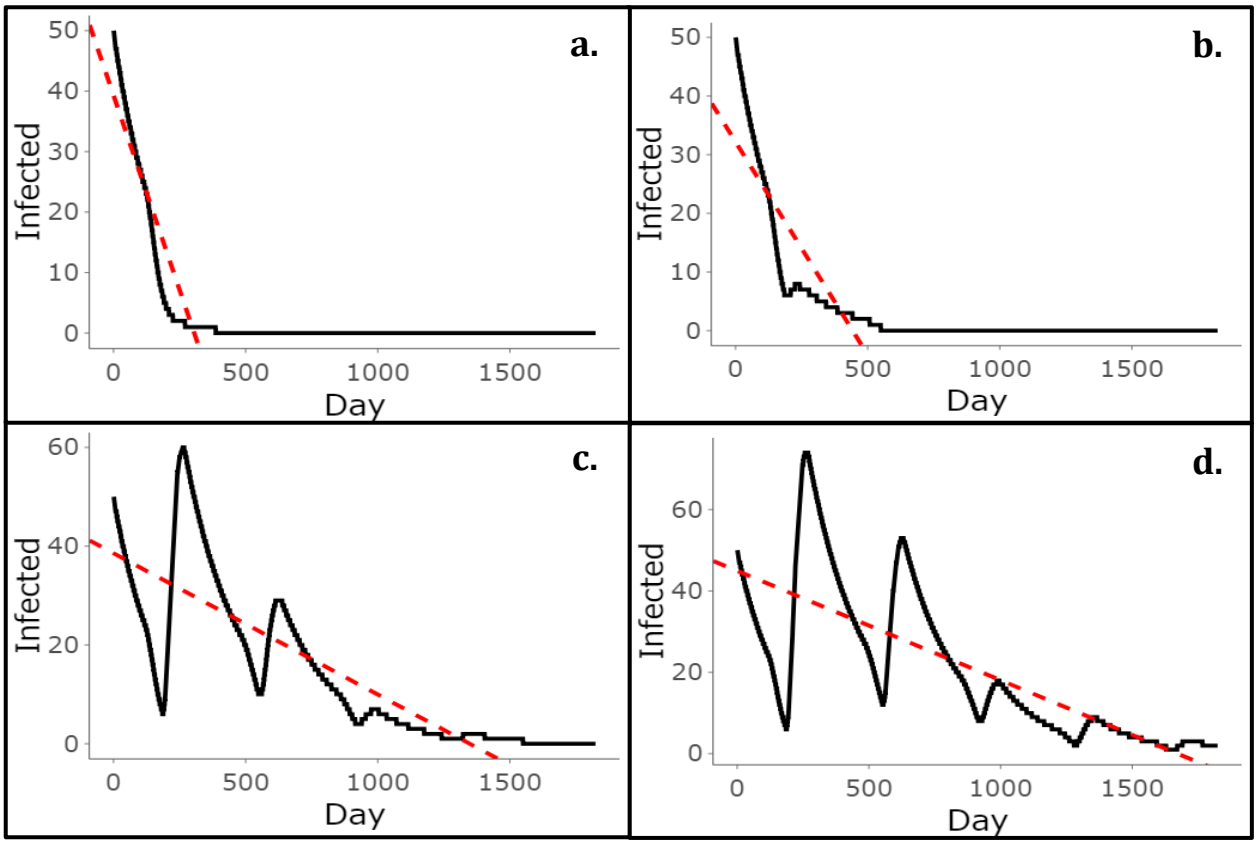

Figure 7. Change in the number of infected larvae and infected questing over a 5-year period where γ is (a) 0.5, (b) 0.0357, (c) 0.00274, and (d) 0.00137. The red dotted line is the trend line associated with the rate of DTV decline.

Given that the average lifespan of the immature host in this model is two years, a lower value of γ is unlikely to result in further changes to DTV transmission dynamics. Therefore, it appears that modifications to this parameter alone are insufficient to reverse the decline in DTV infection over time. Even if future research identifies a reservoir host that develops sustained viremia, horizontal transmission would likely remain inadequate as an isolated means of viral maintenance.

Impact of transmission rates (β_{nh} and β_{hl})

POWV was on track to completely disappear from the *I. scapularis* population even at the theoretical maximum viremic period. Therefore, the next step was to analyze the effect of greater transmission rates (β_{nh} and β_{hl}). Although β_{hl} was identified in a laboratory experiment, is is unknown if this value is representative of transmission in nautre, and thus this parameter will be modified to understand the conditions necessary for POWV maintenance via viremic transmission. For the following model iterations, viremia was assumed to be chronic (i.e., last for up to two years). This may not be realistic for POWV in nature, but chronic viremia enables better visualization of the effects of higher transmission rates.

When β_{nh} and β_{hl} both equal 0.278, the slope of the regression line for the infected compartment is -0.0269 ticks/day (Figure 8a). If either β_{hl} or β_{nh} is set equal to one—meaning that infection occurs with every contact between an infected host or tick and a susceptible host or tick—the slope of the regression line becomes steeper ($slope =$

-0.0443 or $-0.0278\ \frac{ticks}{day}$, respectively) with a noted increase in the y-intercept (Figure 8b,c). While the infected tick population is declining more rapidly, the concomitant increase in the y-intercept extends the duration of DTV infection within the tick population, though it is still trending towards zero. However, if both β_{hl} and β_{nh} are set equal to one, the trend is reversed; the infected compartment increases over time with a slope of 4.95 ticks/day (Figure 8d). Under these conditions, it is feasible that horizontal transmission alone could maintain DTV within the *I. scapularis* population.

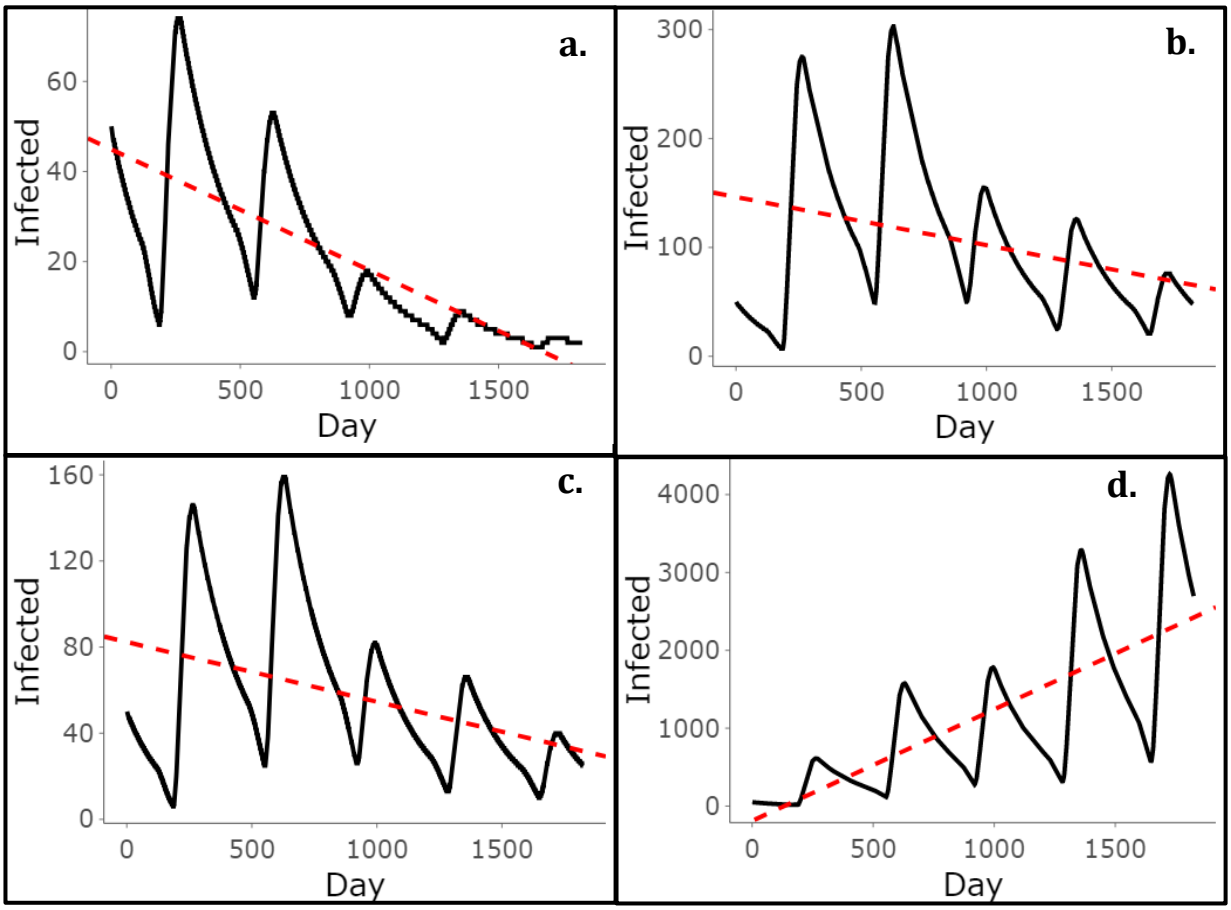

Figure 8. Change in the number of infected larvae and questing nymphs over a 5-year period where γ is 0.00137 and **(a)** $\beta_{nh} = 0.278, \beta_{hl} = 0.278$, **(b)** $\beta_{nh} = 0.278, \beta_{hl} = 1$, **(c)** $\beta_{nh} = 1, \beta_{hl} = 0.278$, and **(d)** $\beta_{nh} = 1, \beta_{hl} = 1$. The red dotted line is the trend line associated with the rate of DTV decline.

Impact of Host Density

The next four model iterations analyzed the influence of host density on POWV maintenance. The initial host population ranged from 100 to 400 (step=100) and the host recovery period was two years for each. β_{hl} and β_{nh} were set equal to each other, and the input value was selected by identifying the minimum value at which the slope of the regression line was positive (step=0.05). Although this is an imperfect measure and a positive slope does not necessarily indicate long-term maintanence of POWV outside of the model run-time, this criterium allows for relative comparison.

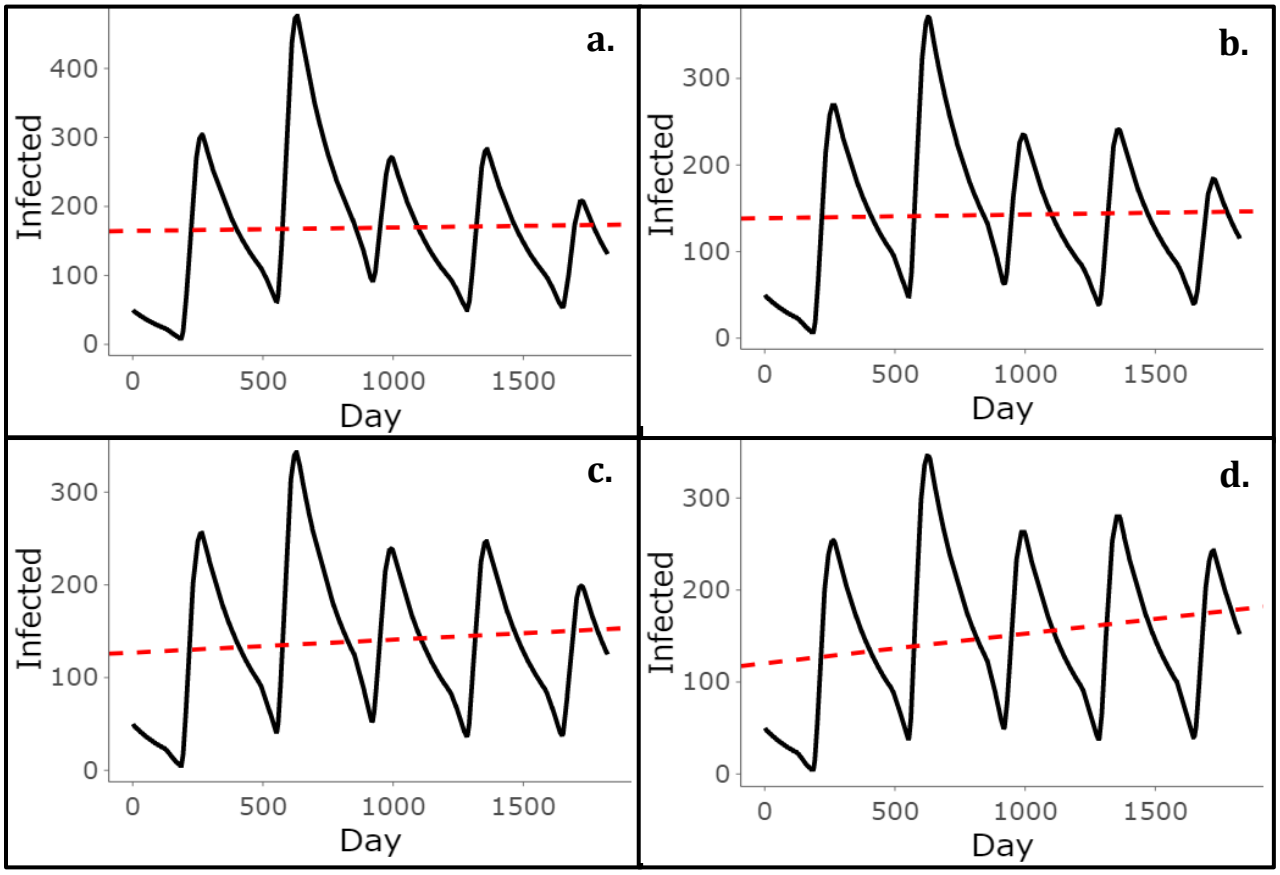

Figure 9. Change in the number of infected larvae and questing nymphs over a 5-year period where γ is 0.00137 and (a) H=100, $\beta_{nh} = 0.9$, $\beta_{hl} = 0.9$, (b) $H = 200$, $\beta_{nh} = 0.65$, $\beta_{hl} = 0.65$, (c) $H = 300$, $\beta_{nh} = 0.55$, $\beta_{hl} = 0.55$, and (d) $H = 400$, $\beta_{nh} = 0.5$, $\beta_{hl} = 0.5$. The red dotted line is the trend line associated with the rate of DTV decline.

With a host population size of 100—half of the input value for all prior models—β_{hl} and β_{nh} must be very high in order for the slope of the regression line to be positive—90% at minimum (Figure 9a). When host population size is doubled (H=200), the minimum transmission rates to maintain a positive slope are lower ($\beta_{hl} = \beta_{nh} = 0.65$, Figure 9b). This trend is also seen when host population size is increased to 300 and 400; the minimum transmission rates are 55% and 50%, respectively (Figure 9c,d). It appears that the decline in minimum transmission rate narrows as host density increases.

Discussion

Under the initial assumptions, POWV disappeared from the *I. scapularis* population in less than two years. Even when the duration of host viremia and the nymph-to-host transmission rate were extended to their theoretical maximums, POWV prevalence within the tick population declined over time. Notably, these two variables have not been identified in the literature as of yet; however, even at their greatest values, they are insufficient to sustain continued POWV infection. Therefore, it is improbable that horizontal transmission alone is responsible for POWV maintenance in the northeastern United States.

Based on known parameters, it seems that the only way for horizontal transmission to be the sole mode of POWV transmission in nature is if the nymph-to-host transmission rate must is very high, and/or the host-to-larva transmission rate exceeds what has been identified in the laboratory between viremic Balb/c mice and *I. scapularis* larvae. Furthermore, viremic transmission may be sufficient for POWV maintenance if a rodent host is identified with sufficiently high population density and a longer viremic period than those of the rodent hosts that have already been identified in the literature.

In a study conducted from 2018 to 2020, researchers collected host-seeking *I. scapularis* nymphs and identified the source of the infectious larval bloodmeal for DTV-infected ticks. They discovered that the majority had fed on shrews and none fed on a mouse [36]. In addition, recent studies have identified voles as a potential candidate

host. Researchers found high seroprevalence of TBEV in bank voles (*Myodes glareolus*) in nature and a laboratory study confirmed that TBEV infection results in prolonged viremia (~28 days) and the majority of voles do not develop clinical illness [37-39]. These traits implicate bank voles as a strong candidate for an amplification host of TBEV and thus voles should also be considered as a potential reservoir host for POWV as well.

In the United States, POWV antibodies have been found in wild birds, hares, squirrels, chipmunks, mice, rats, voles, weasels, groundhogs, skunks, opossums, foxes, antelopes, white-tailed deer, and raccoons [40-45]. In a recent study, three potential reservoir hosts—groundhogs, striped skunks, and fox squirrels—were inoculated with DTV. One of four groundhogs and one of four squirrels inoculated with DTV had detectable viremia with a low mean peak serum titer of $10^{2.4}$ PFU/mL. Viremia was not detectable in any of the four skunks inoculated with DTV [46]. Although detecting viremia was rare and titers were low, this study points to the ability of POWV to cause viremia in diverse mammalian hosts. More research is required to assess host competency among candidate hosts, and it is unclear what viremia titers are sufficient to infect ticks via horizontal transmission.

However, POWV is highly unlikely to cause chronic infection, unlike the persistent infections that have been observed in *P. leucopus* for *B. burgdorferi* [47]. A more realistic expectation for the duration of viremia falls between 2 and 28 days [39, 48]. Meeting all of the conditions required for POWV stability in the model is unlikely

to occur in nature given our current knowledge of tick and host population dynamics. Therefore, it is highly likely that co-feeding and/or vertical transmission play an essential role in the POWV transmission cycle. This finding supports the results of a modeling study conducted by Nonaka et al. in which the authors conclude that sustained prevalence of POWV may be attributed to a combination of low vertical, intermediate co-feeding, and high horizontal transmission rates [49].

Limitations

Given the seasonality of tick population dynamics, the model was limited to one geographic region (the northeastern United States). *I. scapularis* range is influenced by many environmental and geographic variables such as climate, habitat, elevation, density of host species, and anthropogenic changes [50]. Temperature may be the most important factor for seasonal activity of ticks, but other variables such as relative humidity, photoperiod, and rainfall patterns can also influence activity and were not explicitly included in the model [51].

In addition, this model simplified the enzootic cycle to a single immature and mature host, whereas in nature ticks feed on multiple animal species at different rates. While larvae tend to feed on small animals, nymphs feed on larger mammals on occasion. Hosts may have differential impact on POWV transmission in terms of horizontal or co-feeding transmission. Varying host densities and grooming behaviors may also influence tick population dynamics.

Furthermore, the host population in the model was only divided into infected and susceptible compartments. Host immunity was not taken into account due to an assumed high turnover rate of rodents in nature. However, even if host immunity were to affect transmission, it would further limit maintenance of POWV and would not affect the principle findings from the model.

Future Directions

Future research is needed to empirically measure co-feeding and vertical transmission rates for POWV in *I. scapularis* populations. In the meantime, the dynamic model in this report could be expanded to include these modes of transmission. Additional field studies are also needed to better understand the POWV enzootic cycle. Co-feeding transmission rates may vary between host species, so it is crucial to identify candidate hosts to inform laboratory transmission studies.

Furthermore, findings from the model encourage increased POWV surveillance, particularly in regions where human-biting tick species overlap. *I. scapularis* is currently the only known vector of the DTV lineage of POWV, but co-feeding may faciliate cross-species transmission. Prior work in this laboratory has already found that two additional human-biting tick species—*Ambloyomma americanum* and *Dermacentor variabilis*—can be infected with DTV and transmit it to Balb/c mice [18]. If DTV were to become established in either of these species, it could mean an increase in human POWV infections and would present a larger threat to public health. However, while horizontal transmission rates measured for these species are comparable to that of *I. scapularis,* it

is possible that vertical and co-feeding transmission are less efficient in these species, meaning that introduction of POWV to *A. americanum* or *D. variabilis* populations would be short-lived. Measuring rates of vertical and co-feeding transmission in these species is therefore necessary to understand the potential threat of cross-species spread and to inform surveillance endeavors.

Conclusion

In nature, DTV maintenance in *I. scapularis* is likely achieved via a combination of transmission routes rather than horizontal transmission in isolation. More research is needed to fully characterize the enzootic cycle and transmission dynamics of DTV. POWV is an emerging threat that requires more public health attention; human POWV cases are rare but severe and increasing in incidence over time. Climate change is driving shifts in the geographic ranges of ticks, which may present new opportunities for cross-species transmission of DTV. Therefore, it is vital that DTV dynamics are better understood to improve surveillance and prevention measures.

Appendix

Parameter	Description	Equation	Source
$T(t)$	Daily average temperature at time t	$T(t) = 10.97 - 11.04\cos\left(\frac{2\pi t}{366}\right) - 4.646\sin\left(\frac{2\pi t}{366}\right)$	[27]
$d_e(t)$	Egg maturation rate	$d_e(t) = 0.0552 * \exp\left(-\left(\frac{\mathrm{T(t)} - 25.83}{4.946}\right)^2\right) * \mathrm{HS}(T(t) - 15)$	[28, 29]
$d_l(t)$	Larval development rate	$d_l(t) = 0.04001 * \exp\left(-\left(\frac{T(t) - 26.68}{9.533}\right)^2\right) * \mathrm{HS}(T(t) - 15)$	[28, 29]
$d_n(t)$	Nymphal development rate	$d_n(t) = 0.03173 * \exp\left(-\left(\frac{T(t) - 25.83}{9.042}\right)^2\right) * \mathrm{HS}(T(t) - 15)$	[28, 29]
$\alpha_l(t), \alpha_n(t)$	Daily host-attaching rates for larvae, nymphs	$\alpha_l(t) = \alpha_n(t) = 0.001271\mathrm{H}^{0.515}$	[31]
$\alpha_a(t)$	Daily host-attaching rates for adults	$\alpha_a(t) = 0.008571\mathrm{D}^{0.515}$	[31]
$\theta_l(t),$	Larva proportion questing	$\theta_l(t) = \begin{cases} \frac{(t-230)^2}{-2000} + 1, & 185 < t < 275 \\ 0, & t \le 185, t \ge 275 \end{cases}$	[33]
$\theta_n(t)$	Nymph proportion questing	$\theta_n(t) = \begin{cases} \frac{(t-182.5)^2}{-4000} + 1, & 120 \le t \le 245 \\ 0, & t < 120, t > 245 \end{cases}$	[33, 34]
$\theta_a(t)$	Adult proportion questing	$\theta_a(t) = \begin{cases} \frac{(t-83)^2}{-1400} + 1, & 45 < t < 120 \\ \frac{(t-304.5)^2}{-3600} + 1, & 245 < t < 365 \\ 0, & t \le 45, 120 \le t \le 245 \end{cases}$	[33]
ρ_l	Larval feeding rate	$\rho_l = 0.33$	[31]
ρ_n	Nymphal feeding rate	$\rho_n = 0.2$	[32]
ρ_a	Adult feeding rate	$\rho_a = 0.1$	[31]
$b(t)$	Birth rate/fecundity	$b(t) = -24.6T(t)^2 + 836T(t) - 4106$	[30]
μ_e	Egg mortality rate (daily, per-capita)	$\mu_e = 0.002$	[31]
μ_{ql}	Daily, per-capita mortality rate for questing larvae	$\mu_{ql} = 0.006$	[31]
μ_{qn}	Daily, per-capita mortality rate for questing nymphs	$\mu_{qn} = 0.006$	[31]
μ_{qa}	Daily, per-capita mortality rate for questing adults	$\mu_{qa} = 0.006$	[31]
μ_{el}	Daily, per-capita mortality rate for engorged larvae	$\mu_{el} = 0.003$	[31]

μ_{en}	Daily, per-capita mortality rate for engorged nymphs	$\mu_{en} = 0.002$	[31]
μ_{ea}	Daily, per-capita mortality rate for engorged adults	$\mu_{ea} = 0.5$ when $b(t) > 0$ $\mu_{ea} = 0.002$ when $b(t) = 0$	[29, 31]
μ_{fl}	Daily, per-capita mortality rate for feeding larvae (density-dependent)	$\mu_{fl} = 0.65 + 0.049 \ln\left(\frac{1.01 + L_{fs} + L_{fi}}{H}\right)$	[31]
μ_{fn}	Daily, per-capita mortality rate for feeding nymphs (density-dependent)	$\mu_{fn} = 0.5 + 0.049 \ln\left(\frac{1.01 + N_{fs} + N_{fi}}{H}\right)$	[31]
μ_{fa}	Daily, per-capita mortality rate for feeding adults (density-dependent)	$\mu_{fa} = 1 - [0.01 + 0.04 \ln\left(\frac{1.01 + A_f}{D}\right)]$	[31]
β_{hl}	Horizontal transmission rate from host to larva	0.278	[18]
β_{hn}	Horizontal transmission rate from host to nymph	0.45	[18]
β_{nh}	Horizontal transmission rate from nymph to host	Varying input	NA
γ	Host recovery rate	Varying input	NA

Table 1. The equations for parameters related to DTV transmission and tick/host population growth. T represents daily temperature at time t.

Compartment	Description	Initial Value
E	Number of eggs	0
L_q	Number of questing larvae	87,600
L_{fs}	Number of feeding susceptible larvae	0
L_{fi}	Number of feeding infected larvae	0
L_{es}	Number of engorged susceptible larvae	0
L_{ei}	Number of engorged infected larvae	0
N_{qs}	Number of questing susceptible nymphs	4,900
N_{qi}	Number of questing infected nymphs	50
N_{fs}	Number of feeding susceptible nymphs	0
N_{fi}	Number of feeding infected nymphs	0
N_{es}	Number of engorged susceptible nymphs	4,900
N_{ei}	Number of engorged infected nymphs	50
A_q	Number of questing adults	1,250
A_f	Number of feeding adults	0
A_e	Number of engorged adults	1,250
Hi	Number of infected immature hosts	2
Hs	Number of susceptible immature hosts	198
D	Number of mature hosts	200

Table 2. Initial population sizes for tick, immature host, and mature host compartments

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