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Review Article

Pathogen transmission in relation to duration of attachment by *Ixodes scapularis* ticks

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ABSTRACT

The blacklegged tick, *Ixodes scapularis*, is the primary vector to humans in the eastern United States of the deer tick virus lineage of Powassan virus (Powassan virus disease); the protozoan parasite *Babesia microti* (babesiosis); and multiple bacterial disease agents including *Anaplasma phagocytophilum* (anaplasmosis), *Borrelia burgdorferi* and *Borrelia mayonii* (Lyme disease), *Borrelia miyamotoi* (relapsing fever-like illness, named *Borrelia miyamotoi* disease), and *Ehrlichia muris eauclairensis* (a minor causative agent of ehrlichiosis). With the notable exception of Powassan virus, which can be transmitted within minutes after attachment by an infected tick, there is no doubt that the risk of transmission of other *I. scapularis*-borne pathogens, including Lyme disease spirochetes, increases with the length of time (number of days) infected ticks are allowed to remain attached. This review summarizes data from experimental transmission studies to reinforce the important disease-prevention message that regular (at least daily) tick checks and prompt tick removal has strong potential to reduce the risk of transmission of *I. scapularis*-borne bacterial and parasitic pathogens from infected attached ticks. The most likely scenario for human exposure to an *I. scapularis*-borne pathogen is the bite by a single infected tick. However, recent reviews have failed to make a clear distinction between data based on transmission studies where experimental hosts were fed upon by a single versus multiple infected ticks. A summary of data from experimental studies on transmission of Lyme disease spirochetes (*Bo. burgdorferi* and *Bo. mayonii*) by *I. scapularis* nymphs indicates that the probability of transmission resulting in host infection, at time points from 24 to 72 h after nymphal attachment, is higher when multiple infected ticks feed together as compared to feeding by a single infected tick. In the specific context of risk for human infection, the most relevant experimental studies therefore are those where the probability of pathogen transmission at a given point in time after attachment was determined using a single infected tick. The minimum duration of attachment by single infected *I. scapularis* nymphs required for transmission to result in host infection is poorly defined for most pathogens, but experimental studies have shown that Powassan virus can be transmitted within 15 min of tick attachment and both *A. phagocytophilum* and *Bo. miyamotoi* within the first 24 h of attachment. There is no experimental evidence for transmission of Lyme disease spirochetes by single infected *I. scapularis* nymphs to result in host infection when ticks are attached for only 24 h (despite exposure of nearly 90 experimental rodent hosts across multiple studies) but the probability of transmission resulting in host infection appears to increase to approximately 10% by 48 h and reach 70% by 72 h for *Bo. burgdorferi*. Caveats to the results from experimental transmission studies, including specific circumstances (such as re-attachment of previously partially fed infected ticks) that may lead to more rapid transmission are discussed.

1. Background

The blacklegged tick, *Ixodes scapularis*, is the primary vector to humans in the eastern United States of a suite of seven pathogenic microorganisms: the deer tick virus lineage of Powassan virus (Powassan virus disease); the protozoan parasite *Babesia microti* (babesiosis); and multiple bacterial agents including *Anaplasma phagocytophilum* (anaplasmosis), *Borrelia burgdorferi* and *Borrelia mayonii* (Lyme disease), *Borrelia miyamotoi* (relapsing fever-like illness, named *Borrelia miyamotoi* disease), and *Ehrlichia muris eauclairensis* (a minor causative agent of ehrlichiosis) (Mead et al., 2015; Eisen et al., 2017; Eisen and Eisen, 2018). Blacklegged ticks are naturally infected with all seven pathogens (Nelder et al., 2016; Pritt et al., 2016) and have been experimentally demonstrated to transmit each of them while feeding (Spielman et al., 1985; Telford et al., 1996; Scoles et al., 2001; Eisen and Lane, 2002; Ebel, 2010; Saito and Walker, 2015; Dolan et al.,

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2016). The numbers of human infections reported to the United States Centers for Disease Control and Prevention (CDC) in 2014 were 33,461 for Lyme disease, 2800 for anaplasmosis, 1760 for babesiosis, and 8 for Powassan virus disease (Adams et al., 2016). The national burden of *Borrelia miyamotoi* disease and ehrlichiosis caused by *E. muris eauclairensis* remains unknown. In addition to the human pathogens listed above, *I. scapularis* also may play a minor role as a vector of the tularemia agent, *Francisella tularensis* (Hopla, 1962).

Because *I. scapularis*-borne pathogens, with the notable exception of Powassan virus (Ebel and Kramer, 2004), are not thought to be commonly transmitted within the first few hours after tick attachment, disease-prevention messages underscore the importance of regular tick checks and prompt tick removal as a means to find and remove infected ticks before they have a chance to transmit disease agents (Hayes and Piesman, 2003; Stafford, 2007; Piesman and Eisen, 2008; CDC, 2017). This important message is based on experimental data from rodent models showing that risk of transmission resulting in host infection with *Ba. microti*, *A. phagocytophilum*, *Bo. burgdorferi*, *Bo. mayonii*, and *Bo. miyamotoi* increases with the length of time an infected tick is allowed to remain attached (Piesman and Spielman, 1980; Piesman et al., 1987a; Hodzic et al., 1998; Katavolos et al., 1998; Des Vignes et al., 2001; Breuner et al., 2017; Dolan et al., 2017). As these bacterial and parasitic pathogens are adapted to the extended attachment period of a hard tick, 3–4 d for an *I. scapularis* nymph, it is not surprising that the probability of transmission increases over the period of time a tick is feeding.

The most likely scenario for human exposure to an *I. scapularis*-borne pathogen is the bite by a single infected tick. Consequently, the most relevant experimental studies are those where the probability of pathogen transmission at a given point in time after attachment is determined using a single infected tick. However, some recent reviews (Cook, 2015; Richards et al., 2017) failed to make a clear distinction between data based on transmission studies where experimental hosts were fed upon by a single versus multiple infected ticks. This review summarizes data from experimental studies across the full range of *I. scapularis*-borne pathogens in order to clarify (i) how the probability of transmission of a given pathogen by a single infected tick to result in host infection changes with the length of time the tick is allowed to remain attached and (ii) what is known about the minimum time-to-transmission. Findings from studies based on transmission by single infected ticks are contrasted with data from studies where multiple infected ticks were allowed to feed simultaneously on an experimental host, a scenario most relevant to enzootic transmission cycles. Finally, caveats to experimental transmission studies, including circumstances that potentially could lead to more rapid transmission, are discussed. As used in this paper, the term transmission should be interpreted as transmission of a pathogen resulting in infection in a susceptible host.

2. Knowledge base

The published literature for experimental studies on pathogen transmission by *I. scapularis* ticks in relation to their known duration of attachment is very limited. As *I. scapularis* nymphs are considered the primary vectors to humans of pathogens transmitted by this tick species, nearly all studies focus on this life stage. Studies on the duration of tick attachment required for pathogen transmission and host infection where at least some experimental hosts were exposed to the feeding by a single infected tick include Powassan virus (Ebel and Kramer, 2004), *Ba. microti* (Piesman et al., 1987b), *A. phagocytophilum* (Des Vignes et al., 2001), *Bo. burgdorferi* (Piesman et al., 1987a; Des Vignes et al., 2001; Piesman and Dolan, 2002; Hojgaard et al., 2008), *Bo. mayonii* (Dolan et al., 2017), and *Bo. miyamotoi* (Breuner et al., 2017). Additional studies where experimental hosts were exposed for different periods of time to the feeding by two or more infected ticks, or where it cannot be clearly discerned whether or not individual infected hosts were exposed to a single or multiple infected ticks, include *Ba. microti* (Piesman and Spielman, 1980), *A. phagocytophilum* (Hodzic et al., 1998; Katavolos et al., 1998), *Bo. burgdorferi* (Piesman et al., 1987a; Piesman, 1993; Shih and Spielman, 1993; Ohnishi et al., 2001), and *Bo. mayonii* (Dolan et al., 2016, 2017). No published data are available for the duration of attachment required for transmission of *E. muris eauclairensis* or *F. tularensis* by *I. scapularis* ticks.

Results from older studies involving detection of uncharacterized *Borrelia burgdorferi* sensu lato spirochetes from the salivary glands of unfed field-collected *Ixodes* ticks or experimental transmission of uncharacterized *Bo. burgdorferi* sensu lato spirochetes by *Ixodes* ticks should be interpreted with caution, especially when spirochetes were identified using microscopy or immunofluorescence assays. These studies may not have reliably distinguished *Bo. burgdorferi* sensu lato spirochetes from *Bo. miyamotoi*, which later was shown to (i) be present in *I. scapularis* as well as the closely related *Ixodes pacificus* in far western North America and *Ixodes ricinus* and *Ixodes persulcatus* in Eurasia (Wagemakers et al., 2015), (ii) be effectively transmitted from a female *I. scapularis* tick to her offspring as well as occur very commonly in the salivary glands of unfed nymphs (Scoles et al., 2001; Rollend et al., 2013; Breuner et al., 2017); and (iii) share some antigens and proteins with *Bo. burgdorferi*, raising the possibility of cross-reactivity in immunofluorescence assays previously thought to be specific to *Bo. burgdorferi* sensu lato spirochetes (Krause et al., 2014, 2015).

Data for probability of pathogen transmission to result in host infection in relation to duration of attachment by a single infected *I. scapularis* nymph or multiple and simultaneously feeding infected nymphs are summarized in Tables 1–6, and the minimum recorded time-to-transmission for a single infected tick or multiple and simultaneously feeding infected ticks are summarized in Tables 7 and 8.

3. *Bo. burgdorferi* and *Bo. mayonii* (Lyme disease spirochetes)

The Lyme disease spirochete, *Bo. burgdorferi*, is by far the most intensely studied pathogen with regards to the duration of tick attachment required for infection of experimental hosts to occur. Despite a large number ($n = 89$) of experimental rodent hosts having been exposed for 24 h to single *I. scapularis* nymphs infected with various *Bo. burgdorferi* strains (including JD1 and B31), there is no evidence of transmission by a single infected nymph within the first 24 h of attachment (Table 1). By 48 h after attachment of a single infected nymph, the probability of transmission to result in host infection appears to be approximately 10%, increasing to reach 50% by 63–67 h, 70% by 72 h, and > 90% for a complete feed. A similar pattern was documented for the recently recognized Lyme disease spirochete, *Bo. mayonii* (MN14-1420), with lack of evidence for transmission by single infected nymphs 24 and 48 h after attachment but successively increasing probability of transmission and host infection by 72 h (31%) and for a complete nymphal feed (53%) (Table 2). These data provide a strong justification for Lyme disease prevention messaging to encourage daily tick checks and prompt tick removal as a means to reduce the risk of transmission by attached infected ticks.

In contrast to the findings for single infected *I. scapularis* nymphs, simultaneous feeding by multiple infected nymphs resulted in occasional transmission of both *Bo. burgdorferi* and *Bo. mayonii* already by 24 h after attachment (Tables 1 and 2). By 48 h after attachment the probability of transmission of *Bo. burgdorferi* is 6-fold higher when multiple infected *I. scapularis* nymphs feed together as compared with single infected nymphs, and, albeit less pronounced, this general trend persists to the 72 h attachment time-point. Results for *Bo. mayonii* are similar, with numerically higher probability of transmission when multiple infected nymphs feed together, as compared with single infected nymphs, for all examined attachment duration time-points. The reason(s) for increased likelihood of infection in the host when multiple infected ticks feed together are not clear but may be related to passage of higher numbers of spirochetes or more effective suppression of the host immune response (facilitating spirochete establishment) through