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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

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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.

Keywords

Anaplasma; *Babesia*; *Borrelia*; *Ixodes scapularis*; Powassan virus; time-to-transmission

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., 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, 2,800 for anaplasmosis, 1,760 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 7–8.

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 injection of a larger amount of saliva with its array of compounds to minimize pain and irritation and modulate the host immune response.

A few similar studies have examined the probability of spirochete transmission in relation to duration of attachment of infected nymphs of two closely related tick species: *I. pacificus* and *I. ricinus* (Peavey et al., 1995; Kahl et al., 1998; Crippa et al., 2002). All of these studies exposed experimental hosts to simultaneous feeding by multiple potentially infected nymphs and none of them present data that clearly distinguishes experimental hosts fed upon by single versus multiple infected nymphs. No infection was recorded from 8 experimental hosts following 24 h of attachment by *I. pacificus* nymphs infected with *Bo. burgdorferi* (CA4) but the probability of transmission increased to 11% by 48 h, 25% by 72 h, and 80% for a complete nymphal blood meal (Peavey et al., 1995). In one of two European studies, there was no evidence of infection in 10 experimental hosts following 24 h of attachment by *I. ricinus* nymphs infected with *Bo. burgdorferi* (ZS7 or NE1849), whereas 1 of 7 (14%) mice became infected following 24 h of attachment by the same number of nymphs infected with another Lyme disease spirochete, *Borrelia afzelii* (NE496 or NE2963) (Crippa et al., 2002). As expected, the probability of transmission increased with duration of tick attachment for both Lyme disease spirochetes, reaching 50 and 100% for *Bo. burgdorferi* and *Bo. afzelii*, respectively, by 72 h.

Another European study on *I. ricinus* nymphs infected with uncharacterized *Bo. burgdorferi* sensu lato spirochetes originating from field-collected ticks produced dramatically different results (Kahl et al., 1998), with 4 of 6 (67%) experimental hosts infected following only 17 h of tick attachment (QU group) and 3 of 6 (50%) experimental hosts infected after 29 h of tick attachment. All experimental hosts became infected when ticks were allowed to remain attached for ≥47 h. One final study deserving mention, although the primary purpose was not to examine probability of transmission in relation to duration of tick attachment, indicated probable transmission of uncharacterized *Bo. burgdorferi* sensu lato spirochetes from field-collected infected females of *I. persulcatus* to 4 of 11 (36%) experimental hosts (based solely on host serological reactivity) 20–22 h after attachment (Alekseev et al. 1996). However, in contrast to the other studies mentioned in Table 1 and the text above, none of the two studies indicative of transmission within the first 24 h of tick attachment included well characterized *Bo. burgdorferi* sensu lato spirochetes known to belong to a human pathogenic member of the species complex.

4. Other *I. scapularis*-borne pathogens

The very sparse literature for probability of transmission of *Bo. miyamotoi, A. phagocytophilum, Ba. microti*, and Powassan virus (deer tick virus) in relation to duration to attachment by *I. scapularis* is summarized in Tables 3–6.

Bacterial disease agents (Borrelia miyamotoi disease and anaplasmosis)

In contrast to Lyme disease spirochetes, experimental studies have shown that both *Bo. miyamotoi* (wild strain) and *A. phagocytophilum* (wild strain) can be transmitted by single infected *I. scapularis* nymphs by 24 h of attachment (Des Vignes et al., 2001; Breuner et al., 2017). The minimum tick attachment duration allowing for transmission within the first 24 h has not yet been determined for either pathogen. Even though transmission can occur by 24 h, data from experimental studies indicate that, similar to Lyme disease spirochetes, the

probability of transmission of *Bo. miyamotoi* and *A. phagocytophilum* increases with the duration of time an infected tick is allowed to remain attached (Tables 3–4). For example, the probability of transmission of *Bo. miyamotoi* by a single attached nymph increased from 10% by 24 h to 31% by 48 h, 63% by 72 h, and 73% for a complete nymphal feed (Breuner et al., 2017; Table 3). Similarly, data from studies on transmission of *A. phagocytophilum* (NCH-1 and NTN-1) by multiple infected nymphs feeding together indicate that the probability of transmission increases 10-fold from 24 to 48–50 h (Hodzic et al., 1998; Katavolos et al., 1998; Table 4).

Parasitic disease agent (babesiosis)

Published data for transmission of *Ba. microti* in relation to tick attachment duration are restricted to a single study exposing experimental hosts to multiple *I. scapularis* nymphs infected with the Otis 4 and Lewis strains (Piesman and Spielman, 1980; Table 5). Infection was recorded from 1 of 11 (9%) experimental hosts following 36 h of tick attachment, with the probability of transmission rising to 50% by 54 h. Another notable study where individual *I. scapularis* nymphs dually infected with *Ba. microti* (GI strain) and *Bo. burgdorferi* (JD1) were allowed to stay attached to experimental hosts for 54 h resulted in 5 of 7 (71%) experimental hosts infected with *Ba. microti* (Piesman et al. 1987b; Table 5).

Viral disease agent (Powassan virus disease)

Published data for transmission of Powassan virus (deer tick virus, DTV-SPO) by *I. scapularis* nymphs are restricted to tick attachment durations from 15 to 180 min but indicate a very high probability of virus transmission to occur already by 15–30 minutes after attachment by a single infected tick (Ebel and Kramer, 2004; Table 6). This viral disease agent thus differs from other *I. scapularis*-borne pathogens in that it can be transmitted within minutes of tick attachment.

5. Further considerations

Based on the experimental data summarized in Tables 1–5, there is no doubt that the risk of transmission of bacterial and parasitic human pathogens, including Lyme disease spirochetes, increases with the length of time infected *I. scapularis* nymphs are allowed to remain attached. This finding agrees with reports that longer attachment durations (estimated using tick engorgement indices) for nymphal *I. scapularis* or *I. ricinus* ticks removed from humans are associated with elevated risk of infection with Lyme disease spirochetes (Sood et al., 1997; Nadelman et al., 2001; Wilhelmson et al., 2016; Hofhuis et al., 2017). Taken together, these observations reinforce the long-standing recommendation to conduct daily tick checks and promptly remove attached, potentially infected ticks.

Powassan virus can be transmitted within minutes of tick attachment (Ebel and Kramer, 2004) and both *A. phagocytophilum* and *Bo. miyamotoi* can be transmitted within the first 24 h of attachment by *I. scapularis* nymphs (Des Vignes et al., 2001; Breuner et al., 2017). However, the minimum time of attachment by *I. scapularis* or *I. pacificus* nymphs required for transmission of *Bo. burgdorferi* to occur has generated lively debate in the United States. This topic has been addressed in case studies and letters in response to case studies (e.g.,

Berger et al., 1995; Binnicker et al., 2012; Hynote et al., 2012; Piesman and Gray, 2012; Stricker et al., 2012) as well as in previous reviews (Kelly et al., 1999; Cook, 2015).

With regards to *Bo. burgdorferi* transmission by a single infected *I. scapularis* or *I. pacificus* nymph, there is no experimental evidence from rodent models for transmission within the first 24 h of attachment (Piesman et al., 1987a; Peavey et al., 1995; Piesman and Dolan, 2002; Des Vignes et al., 2001; Hojgaard et al., 2008). Additionally, there is no experimental evidence for *Bo. burgdorferi* transmission within the first 24 h of attachment by single *I. scapularis* nymphs co-infected with either *A. phagocytophilum* (Des Vignes et al., 2001; none of 3 dually infected nymphs removed 24 h after attachment transmitted *Bo. burgdorferi*; Levin and Fish, 2000: all co-infected nymphs were allowed to feed to completion) or *Ba. microti* (Piesman et al., 1987b: all co-infected nymphs were allowed to remain attached for 54 h). Nevertheless, the possibility that transmission of Lyme disease spirochetes could occur within 24 h of nymphal attachment under unusual circumstances should not be discounted.

Shih and Spielman (1993) demonstrated that infected *I. scapularis* nymphs that previously had been attached to an experimental host for 24–48 h and then removed and placed on a new experimental host effectively transmitted *Bo. burgdorferi* (JD1) within 24 h of their re-attachment. Partially fed ticks able to re-attach could result from detachment from dead animals (Piesman, 1991) or possibly by host grooming, although the latter scenario seems less likely to generate intact ticks capable of re-attaching if they had fed for long enough to already be firmly attached. How commonly humans may be bitten by nymphs that had previously taken a partial blood meal remains unknown. Occasional transmission by multiple and simultaneously feeding infected *I. scapularis* nymphs within the first 24 h of attachment were recorded both for *Bo. burgdorferi* (JD1) and *Bo. mayonii* (MN14–1420) (Piesman et al., 1987a; Dolan et al., 2016, 2017). However, simultaneous feeding by multiple infected ticks on a human most likely is an unusual event. Another possibility that cannot be discounted is that some *Bo. burgdorferi* strains may be transmitted more rapidly than those included in experimental transmission studies, but this remains speculative.

In response to a nymphal *I. scapularis* tick attaching and starting to feed, *Bo. burgdorferi* spirochetes multiply in the gut, where they most commonly reside in unfed nymphs, escape into the hemocoel and then invade and multiply in the salivary glands (Ribeiro et al., 1987; Zung et al., 1989; De Silva and Fikrig, 1995; Piesman, 1995; Piesman et al., 2001). Before escaping the midgut to reach the salivary glands, spirochetes switch from expressing outer surface protein (Osp) A to Osp C and other surface proteins that facilitate establishment in the vertebrate host (Schwan et al., 1995; De Silva et al., 1996; De Silva and Fikrig, 1997; Schwan and Piesman, 2000, 2002; Ohnishi et al., 2001; Piesman et al. 2003; Tilly et al., 2008; Radolf et al., 2012). Although several studies have documented Lyme disease spirochetes from the salivary glands of unfed *I. scapularis* nymphs (Moskvitina et al., 1995; Piesman, 1995; Piesman et al., 2001; Dolan et al. 2017), this should not be taken as evidence that they can be transmitted shortly after tick attachment in sufficient numbers and expressing phenotypes leading to host infection. For example, spirochetes already present in the salivary glands of unfed nymphs prior to attachment (generalized infection following transstadial spirochete passage) may fail to express the appropriate surface proteins to

establish in the host. In this scenario, transmission of sufficient numbers of phenotypically infectious spirochetes to result in host infection may be delayed until either (i) a surface protein expression switch occurs in the spirochetes in the salivary glands following tick attachment or (ii) spirochetes already expressing the appropriate surface proteins for host invasion reach the salivary glands after switching from expressing OspA to OspC in the midgut in response to blood meal-related cues (for example temperature and pH) and then escaping into the hemocoel (Ohnishi et al., 2001). This is consistent with a recent finding where mice exposed to *I. scapularis* nymphs with *Bo. mayonii*-infected salivary glands became infected only when nymphs were allowed to remain attached for >24 h (Dolan et al., 2017).

Other important considerations relating to case studies of human infection with Lyme disease spirochetes is that bites by *I. scapularis* nymphs often go entirely undetected and that people underestimate the amount of time an *Ixodes* nymph has been attached prior to being detected. A summary of published data from the United States indicated that less than half of Lyme disease patients were aware of a tick bite in most studies, and that even fewer (typically 20–25%) could recall a tick bite at an erythema migrans rash site (Eisen and Eisen, 2016). Moreover, comparisons of self-assessed time of attachment by *I. scapularis* or *I. ricinus* nymphs and attachment time based on tick engorgement indices indicate that people consistently underestimate the actual time the tick was attached prior to being discovered (Sood et al., 1997; Logar et al., 2002; Wilhelmsson et al., 2013). A tick-bite victim's impression of how long a nymphal *Ixodes* tick was attached before it was detected and removed therefore should be regarded as an underestimate of the true attachment duration. Consequently, a self-assessed tick attachment duration of less versus more than 24 h is of questionable value for a medical treatment decision in a suspected Lyme disease patient.

Estimates for tick attachment duration based on scutal or coxal indices (Piesman and Spielman, 1980; Yeh et al., 1995; Falco et al., 1996; Gray et al., 2005; Meiners et al., 2006) of removed ticks can provide more reliable data on the approximate length of time a tick was attached. These indices have been used in numerous studies to estimate the duration of attachment in *Ixodes* ticks removed from humans (e.g., Falco et al., 1996; Sood et al., 1997; Nadelman et al., 2001; Logar et al., 2002; Huegli et al., 2009, 2011; Hynote et al., 2012; Wilhelmson et al., 2013, 2016). Notably, Gray et al. (2005) compared scutal and coxal indices of *I. ricinus* nymphs fed on experimental hosts for known durations of time and concluded that the coxal index provides a more accurate estimate for attachment times <36 h compared with the scutal index, which was found to substantially underestimate the attachment time at the 12 and 24 h attachment time points. Moreover, to be considered most accurate the index for a tick removed from a human should be compared with data series for indices from ticks of the same species and life stage fed for known durations of time on experimental hosts. The physical condition of the removed tick (including damage resulting from the removal process and variable storage conditions prior to examination) and the technical expertise of the person measuring its dimensions also are potential pitfalls to achieve accurate estimates for attachment duration. As noted by Gray et al. (2005), tick engorgement indices can be of great value as research tools but are less suitable for use in clinical settings.

In contrast to the 24 h time-point, it is clear from experimental studies that some single infected *I. scapularis* (approximately 1 in 10) do transmit *Bo. burgdorferi* by 48 h of attachment (Des Vignes et al., 2001; Piesman and Dolan, 2002). As data for probability of transmission by single infected nymphs are lacking between the 24 and 48 h attachment time-points (Table 1), it remains unknown whether recorded transmission occurred shortly after the 24 h time point or closer to 48 h. Studies to clarify the minimum duration of attachment required for transmission by single infected nymphs are of interest for Lyme disease spirochetes as well as other *I. scapularis*-borne pathogens, but represent major undertakings within ranges of tick attachment durations where transmission events and subsequent infection in experimental hosts are expected to occur only rarely.

6. Conclusions

- Powassan virus can be transmitted within minutes after attachment by an infected *I. scapularis* nymph but there is no doubt that the risk of transmission of bacterial and parasitic human pathogens, including Lyme disease spirochetes, increases with the length of time (number of days) infected *I. scapularis* nymphs are allowed to remain attached. This is the basis for 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 pathogens from infected attached ticks.
- As the most likely scenario for human exposure to an *I. scapularis*-borne pathogen is the bite by a single infected tick, 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.
- Both *A. phagocytophilum* and *Bo. miyamotoi* can be transmitted within the first 24 h of attachment by a single infected *I. scapularis* nymph but there is no experimental evidence for transmission of Lyme disease spirochetes by a single infected *I. scapularis* nymph within the first 24 h of attachment.
- Although the most likely outcome is for single infected *I. scapularis* nymphs to fail to transmit Lyme disease spirochetes within the first 24 h of attachment, such transmission could still occur under unusual circumstances such as when an infected tick previously having taken a partial blood meal (e.g., forced to detach from a dead animal before taking a complete blood meal) encounters and bites a human to complete its blood meal.
- Bites by *I. scapularis* nymphs often go entirely undetected and tick-bite victims typically underestimate how long a nymph was attached before it was detected and removed. A self-assessed tick attachment duration of less versus more than 24 h therefore is of questionable value for a medical treatment decision in a suspected Lyme disease patient

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Table 1

Transmission of *Bo. burgdorferi* resulting in infection in experimental rodent hosts in relation to duration of attachment by a single infected *I. scapularis* nymph versus multiple and simultaneously feeding infected nymphs.

Strain	% experimental hosts infected in relation to duration of attachment by infected ticks (no. experimental hosts infected / no. exposed to bites by infected ticks)										Reference
	16 h	**24 h**	**36–37 h**	**41–43 h**	**47–49 h**	**53–55 h**	**59–61 h**	**63–67 h**	**72 h**	**Complete feed**	
Transmission by a single infected nymph											
Wild		0 (0/18)			12 (2/16)				79 (15/19)	94 (15/16)	Des Vignes et al., 2001
Wild		0 (0/8)			50 (2/4)				75 (3/4)		Des Vignes et al., 2001
JD1		0 (0/2)			0 (0/3)					100 (2/2)	Piesman et al., 1987a
JD1		0 (0/16)			0 (0/17)				56 (9/16)		Des Vignes et al., 2001
B31		0 (0/16)			12 (2/17)				71 (12/17)		Des Vignes et al., 2001
B31		0 (0/27)			26 (7/27)	41 (11/27)	44 (12/27)	89 (24/27)			Piesman and Dolan, 2002
B31					0 (0/31)	10 (3/30)		24 (8/33)	79 (23/29)		Hojgaard et al., 2008
		0 (0/87)			**11 (13/115)**	**25 (14/57)**	**44 (12/27)**	**53 (32/60)**	**73 (62/85)**	**94 (17/18)**	
Transmission by multiple and simultaneously feeding infected nymphs											
JD1		8 (1/12)			45 (5/11)					91 (10/11)	Piesman et al., 1987a
JD1			7 (1/14)	25 (3/12)	75 (6/8)						Piesman, 1993
JD1	0 (0/8)	0 (0/9)	14 (1/7)		100 (10/10)				100 (6/6)		Shih and Spielman, 1993
B31			0 (0/1)	0 (0/2)	0 (0/2)	100 (2/2)	100 (2/2)	100 (3/3)	100 (1/1)		Ohnishi et al., 2001
	0 (0/8)	**5 (1/21)**	**9 (2/22)**	**21 (3/14)**	**68 (21/31)**	**100 (2/2)**	**100 (2/2)**	**100 (3/3)**	**100 (7/7)**	**91 (10/11)**	

Table 2

Transmission of *Bo. mayonii* resulting in infection in experimental rodent hosts in relation to duration of attachment by a single infected *I. scapularis* nymph versus multiple and simultaneously feeding infected nymphs.

Strain	% experimental hosts infected in relation to duration of attachment by infected ticks (no. experimental hosts infected / no. exposed to bites by infected ticks)				Reference
	24 h	**48 h**	**72 h**	**Complete feed**	
Transmission by a single infected nymph					
MN14–1420				40 (2/5)	Dolan et al., 2016
MN14–1420	0 (0/24)	0 (0/17)	31 (5/16)	57 (8/14)	Dolan et al., 2017
	0 (0/24)	**0 (0/17)**	**31 (5/16)**	**53 (10/19)**	
Transmission by multiple and simultaneously feeding infected nymphs					
MN14–1420	33 (1/3)	0 (0/3)	67 (2/3)	88 (7/8)	Dolan et al., 2016
MN14–1420	0 (0/3)	25 (1/4)	71 (5/7)	83 (5/6)	Dolan et al., 2017
	17 (1/6)	**14 (1/7)**	**70 (7/10)**	**86 (12/14)**	

Table 3

Transmission of *Bo. miyamotoi* resulting in infection in experimental rodent hosts in relation to duration of attachment by a single infected *I. scapularis* nymph.

Strain	% experimental hosts infected in relation to duration of attachment by infected ticks (no. experimental hosts infected / no. exposed to bites by infected ticks)				Reference
	24 h	**48 h**	**72 h**	**Complete feed**	
Transmission by a single infected nymph					
Wild	10 (3/30)	31 (11/35)	63 (22/35)	73 (22/30)	Breuner et al., 2017

Table 4

Transmission of *A. phagocytophilum* resulting in infection in experimental rodent hosts in relation to duration of attachment by a single infected *I. scapularis* nymph versus multiple and simultaneously feeding infected nymphs.

Strain	% experimental hosts infected in relation to duration of attachment by infected ticks (no. experimental hosts infected / no. exposed to bites by infected ticks)								Reference
	12–16 h	**24 h**	**30 h**	**36 h**	**40 h**	**48–50 h**	**72 h**	**Complete feed**	
Transmission by a single infected nymph									
Wild		67 (2/3)				100 (1/1)	50 (2/4)		Des Vignes et al., 2001
Transmission by multiple and simultaneously feeding infected nymphs									
NCH-1	0 (0/1)	0 (0/1)			0 (0/5)	100 (5/5)	100 (1/1)	100 (1/1)	Hodzic et al., 1998
NTN-1	0 (0/12)	9 (1/11)	0 (0/5)	67 (8/12)		85 (11/13)			Katavolos et al., 1998
	0 (0/13)	**8 (1/12)**	**0 (0/5)**	**67 (8/12)**	**0 (0/5)**	**89 (16/18)**	**100 (1/1)**	**100 (1/1)**	

Table 5

Transmission of *Ba. microti* resulting in infection in experimental rodent hosts in relation to duration of attachment by multiple and simultaneously feeding infected *I. scapularis* nymphs.

Strain	% experimental hosts infected in relation to duration of attachment by infected ticks (no. experimental hosts infected / no. exposed to bites by infected ticks)			Reference
	36 h	48 h	54 h	
Transmission by a single infected nymph[a]				
GI			71 (5/7)	Piesman et al., 1987b
Transmission by multiple and simultaneously feeding infected nymphs				
Otis 4; Lewis	9 (1/11)	17 (2/12)	50 (6/12)	Piesman and Spielman, 1980

[a] Individual nymphs were dually infected with *Ba. microti* and *Bo. burgdorferi*.

Table 6

Transmission of Powassan virus (deer tick virus) resulting in infection in experimental rodent hosts in relation to duration of attachment by *I. scapularis* nymphs.

Strain	% experimental hosts infected in relation to duration of attachment by infected ticks (no. experimental hosts infected / no. exposed to bites by infected ticks)				Reference
	15 min	**30 min**	**1 h**	**3 h**	
Transmission by single infected nymph or multiple and simultaneously feeding infected nymphs					
DTV-SPO	83 (5/6)[a]	100 (3/3)[b]	100 (9/9)[c]	100 (5/5)[d]	Ebel and Kramer, 2004

[a] Including at least 2 mice exposed to a single infected nymph based on the total number of infected nymphs (n=8) recorded across all mice in relation to the number of infected mice (n=5).

[b] Including 2 mice exposed to a single infected nymph based on the total number of infected nymphs (n=4) recorded across all mice in relation to the number of infected mice (n=3).

[c] Including at least 5 mice exposed to a single infected nymph based on the total number of infected nymphs (n=13) recorded across all mice in relation to the number of infected mice (n=9).

[d] Including 4 mice exposed to a single infected nymph based on the total number of infected nymphs (n=6) recorded across all mice in relation to the number of infected mice (n=5).

Table 7

Minimum recorded time of attachment for a single infected *I. scapularis* tick resulting in transmission that produced a detectable infection in an experimental host.

Pathogen[a]	Pathogen strain/ isolate	Tick life stage	Experimental host to confirm transmission	Minimum duration of tick attachment examined	Minimum recorded duration of attachment by single infected tick resulting in transmission	Reference
Powassan virus	DTV-SPO	Nymph	White mouse	15 min	15 min	Ebel and Kramer, 2004
Anaplasma phagocytophilum	Wild[b]	Nymph	White mouse	24 h	24 h	Des Vignes et al., 2001
Borrelia burgdorferi	B31; Wild[b]	Nymph	White mouse	24 h	48 h[d]	Des Vignes et al., 2001; Piesman and Dolan, 2002
Borrelia mayonii	MN14–1420	Nymph	White mouse	24 h	72 h	Dolan et al., 2017
Borrelia miyamotoi	Wild[c]	Nymph	White mouse	24 h	24 h	Breuner et al., 2017

[a] No data in the published literature for *Ba. microti, E. muris eauclairensis* or *F. tularensis*.

[b] Field-collected infected ticks were used in the transmission experiment.

[c] The infected ticks used in the transmission experiment originated from a field-collected female that passed spirochetes to her offspring.

[d] Shih and Spielman (1993) documented one instance of transmission of *Bo. burgdorferi* strain JD1 by 36 h after nymphal attachment but it cannot be deduced from the description of the experiment whether this involved one or more infected nymphs.

Table 8

Minimum recorded time of attachment for multiple and simultaneously feeding infected *I. scapularis* ticks resulting in transmission that produced a detectable infection in an experimental host.

Pathogen[a]	Pathogen strain/ isolate	Tick life stage	Experimental host to confirm transmission	Minimum duration of tick attachment examined	Minimum recorded duration of attachment by infected ticks resulting in transmission	Numbers of infected ticks attached	Reference
Powassan virus	DTV-SPO	Nymph	White mouse	15 min	15 min	Not clear	Ebel and Kramer, 2004
Babesia microti	Otis 4; Lewis	Nymph	Hamster	36 h	36 h	Not clear	Piesman and Spielman, 1980
Anaplasma phagocytophilum	NTN-1	Nymph	White mouse	12 h	24 h	Not clear	Katavolos et al., 1998
Borrelia burgdorferi	JD1	Nymph	Hamster	24 h	24 h	2	Piesman et al., 1987a
Borrelia burgdorferi	Wild[b]	Female	White rabbit	24 h	48 h	8–9	Piesman et al., 1991
Borrelia mayonii	MN14–1420	Nymph	White mouse	24 h	24 h	6	Dolan et al., 2016

[a] No data in the published literature for *Bo. miyamotoi, E. muris eauclairensis* or *F. tularensis*.

[b] Field-collected infected ticks were used in the transmission experiment.