Duration of Adult Female *Ixodes dammini* Attachment and Transmission of *Borrelia burgdorferi*, with Description of a Needle Aspiration Isolation Method

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The relationship between the attachment duration of adult female *Ixodes dammini* and the transmission of *Borrelia burgdorferi* was studied. Sixteen rabbits were exposed to spirochete-infected female ticks for specified intervals. All five rabbits exposed to ticks that fed to repletion (>120 h) became infected, as did two of three exposed for 48 h. In contrast, five rabbits exposed to a cumulative total of 53 infected female *I. dammini* for 36 h failed to become infected, as did three rabbits exposed for 24 h. A needle aspirate method facilitated the isolation of spirochetes from host skin.

Lyme disease is principally a summertime illness in the northeastern and midwestern United States, where *Ixodes dammini* is the primary tick vector of *Borrelia burgdorferi*. The peak in onset of human illness (June and July) [1] is directly preceded by the active questing period of nymphal *I. dammini* (May and June) [2]. Surprisingly few cases occur during the cooler months (October-April) when adult female *I. dammini* are active, even though the infection rate of adults (50%) is roughly twice that of nymphs (25%) [3].

A possible explanation for these seemingly paradoxical observations is that the relatively large size of adult female *I. dammini* [4] makes it likely that these ticks will be quickly detected by human hosts and removed before they can transmit spirochetes. This theory assumes that risk of transmission in both nymphal and adult female *I. dammini* is related to duration of attachment. Indeed, a study with rodents as hosts demonstrated that the duration of attachment of nymphal *I. dammini* is directly correlated with the risk of transmission of *B. burgdorferi* [5]. Similar data are lacking for adult female *I. dammini*. Accordingly, we studied the relationship between duration of attachment of female *I. dammini* and the efficacy of transmission of *B. burgdorferi* to laboratory rabbits.

Materials and Methods

Female New Zealand white rabbits (1.9-3.4 kg), a host known to be susceptible to infection with *B. burgdorferi* [6], were subjected to tick feedings for specific intervals. Naturally infected adult female *I. dammini* were collected from vegetation on the Calder Conservation and Ecology Study Center of Fordham University (Armonk, Westchester Co., NY). Lyme disease is highly endemic in this area, and ~50% of adult *I. dammini* collected from this study site are infected with *B. burgdorferi* (unpublished data). Female ticks were held at 4°C, 97% relative humidity, for 1-4 months before being allowed to feed on rabbits.

A total of 20-30 ticks were placed on each rabbit in standard ear bags. At the specified interval, the ear bags and all attached ticks were removed from hosts; ticks were grasped with fine forceps, and gentle but constant pressure was applied to remove them. Within 48 h after ticks were removed, the midgut contents from attached ticks were dissected in PBS and examined for Lyme disease spirochetes by darkfield and direct fluorescent antibody microscopy as previously described [7].

Rabbits were examined for spirochetes at 1 month after tick-feeding. Each rabbit was sacrificed via intramuscular inoculation of 150 mg of ketamine HCl and 15 mg of xylazine (Rompun); wedges of skin, 5-8 mm², from the external and interior surface of each ear were removed, and the surfaces were sterilized with 70% ethanol and placed into 4 ml of modified BSK II medium in snap-cap tubes [8].

Needle aspiration of skin also was done. Briefly, 0.5 ml of PBS or BSK II was injected from a tuberculin syringe, containing a 26-gauge needle, intradermally into the external or interior surface of each ear. A second needle was simultaneously inserted into the bleb forming at the site of the inoculation, and fluid was aspirated into a syringe. About 0.25-0.3 ml of fluid was generally obtained via needle aspiration. This aspirate was directly injected into BSK II medium.

Cultures were held at 34°C and examined for spirochetes by darkfield microscopy weekly for 4 weeks. Eight cultures were usually obtained from each rabbit: one external and one interior skin biopsy left ear; one external and one interior skin biopsy right ear; one PBS aspirate each interior left and right ear; and one BSK aspirate each exterior left and right ear. Only 1 of 128 cultures was contaminated. To confirm that spirochetes isolated from rabbits were *B. burgdorferi*, we did an indirect fluorescent antibody test on several isolates with monoclonal antibody H5332, which is believed to be species-specific for *B. burgdorferi* [9].

Results

Sixteen rabbits were exposed to female *I. dammini* infected with *B. burgdorferi* (table 1). All five rabbits upon which ticks
other rabbits were not tested.

Table 2. Efficacy of skin biopsy and needle aspiration for the isolation of Borrelia burgdorferi

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>Duration of tick attachment (h)</th>
<th>No. ticks infected/no feeding</th>
<th>Culture results</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>24</td>
<td>2/4</td>
<td>−</td>
</tr>
<tr>
<td>11</td>
<td>24</td>
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</tr>
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<td>12</td>
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</tr>
<tr>
<td>14</td>
<td>48</td>
<td>NT</td>
<td>1/1*</td>
</tr>
<tr>
<td>15</td>
<td>48</td>
<td>2/2</td>
<td>2/2</td>
</tr>
<tr>
<td>16</td>
<td>48</td>
<td>2/2</td>
<td>2/2</td>
</tr>
<tr>
<td>Total</td>
<td>21/28</td>
<td>9/12</td>
<td>10/13</td>
</tr>
</tbody>
</table>

NOTE. Data are no. cultures positive/no. examined. NT = not tested.
* Isolates reacted with monoclonal antibody H5332 at ≥1:8 dilution; isolates from other rabbits were not tested.

were allowed to feed to repletion (≥6 days of attachment) became infected. Moreover, two of three rabbits exposed to infected ticks for 48 h became infected. In contrast, five rabbits exposed to a cumulative total of 53 infected female I. dammini for 36 h failed to become infected, as did three rabbits exposed to infected ticks for 24 h.

The efficacy of skin biopsy and needle aspiration for detecting B. burgdorferi was compared (table 2). All 7 infected rabbits were positive on both skin biopsy and needle aspiration. Overall, the proportions of skin biopsies (21/28, 75%), BSK aspirates (9/12, 75%), and PBS aspirates (10/13, 76%) that produced spirochetes in culture were remarkably similar.

Discussion

Although 50% of female I. dammini are naturally infected with B. burgdorferi, and these ticks frequently bite people living in hyperendemic Lyme disease regions, only occasional cases of Lyme disease have been reported during the peak period of adult I. dammini questing [10]. The results of the present study and a previous study on nymphal I. dammini [5] suggest that these ticks must be attached for ≥2 days to efficiently transmit spirochetes. Apparently, the diminutive (<2 mm) nymphal stage is often able to escape detection and feed on human hosts for 2 days and transmit B. burgdorferi, while the adults are routinely detected and removed before the minimum interval required for transmission. Systematic collections of I. dammini removed from residents of Westchester County (NY), are available [11]. A previously described scutal index of engorgement duration [12] should be applied to nymphal and adult I. dammini to determine when during the feeding process each stage is detected and removed.

Given the numerous difficulties encountered with standardization of serologic tests for Lyme disease [13], rapid and sensitive methods for the isolation of B. burgdorferi from patients are urgently needed. Although B. burgdorferi has been isolated from blood, synovial fluid, and cerebrospinal fluid [14], these tissues are not particularly rich in spirochetes. In contrast, numerous isolates have been obtained from human skin [14, 15]. The sensitive needle aspiration method used in this study to isolate spirochetes from rabbits may be a useful alternative to punch biopsies for the isolation of B. burgdorferi from human skin.

Table 1. Association between attachment duration of adult female Ixodes dammini and transmission of Borrelia burgdorferi to rabbits.

<table>
<thead>
<tr>
<th>Note</th>
<th>Rabbit</th>
<th>Duration of tick attachment (h)</th>
<th>No. ticks infected/no feeding</th>
<th>Culture results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>&gt;120*</td>
<td>3/18</td>
<td>+</td>
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<tr>
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<td>4</td>
<td>20</td>
<td>&gt;120*</td>
<td>8/9</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>&gt;120*</td>
<td>5/9</td>
<td>+</td>
</tr>
</tbody>
</table>

NOTE. NT = not tested.
* Ticks feeding for >120 h fed to repletion.

References

Pneumococcal Soft-Tissue Infections: Possible Association with Connective Tissue Diseases


Streptococcus pneumoniae is not a well-recognized cause of soft-tissue infections. In <4 years, 12 cases of pneumococcal soft-tissue infection were identified through discussions with infections disease subspecialists in the Philadelphia area. Principal sites of involvement included skin and fascia, tongue, epiglottis, thyroid, brain, and breast. Pneumococcal bacteremia was documented in six cases (50%); in three of these, pneumococci were also cultured from the involved soft tissues. In the cases in which bacteremia was not demonstrated, pneumococci were isolated from the infected sites. Six patients had connective tissue diseases, of which five were diagnosed as systemic lupus erythematosus. Four of these patients were receiving corticosteroids when their infections developed. Two additional patients were HIV-seropositive intravenous drug users. S. pneumoniae may be a more important cause of soft-tissue infections than previously appreciated, especially in patients with connective tissue diseases.

Methods

Patients with pneumococcal infections primarily involving soft tissues were identified through formal and later informal discussions among infectious disease subspecialists working in the Philadelphia area. Cases were accepted for this report if soft-tissue infection was the sole or major clinical manifestation and pneumococci were isolated from blood or the involved soft tissue. Pneumococci were identified by the routine methods used in the clinical microbiology laboratories of each hospital.

Results

In <4 years, we collected 12 cases of soft-tissue infection due to S. pneumoniae (table 1). Six patients (50%) had demonstrable pneumococcal bacteremia. Pneumococci were also isolated from the relevant soft tissues in three of those with bacteremia. In all patients whose blood cultures were sterile, pneumococci were cultured from the involved soft-tissue sites. Gram's stains of pus obtained from the sites of infection typically demonstrated gram-positive diplococci without other bacteria.

The pneumococcal origin of these soft-tissue infections seems certain in 11 of the 12 cases. Whether pneumococci were