The efficacy of co-feeding as a means of maintaining 
Borrelia burgdorferi: a North American model system

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ABSTRACT: Although research on co-feeding as a means of maintaining tick-borne pathogens has focused chiefly on viruses, recent interest has been directed toward the importance of this phenomenon in maintaining the Lyme disease spirochete, Borrelia burgdorferi. In the current study, an experimental model was developed to determine under what conditions immature co-feeding ticks exchange B. burgdorferi using the principal North American vector (Ixodes scapularis) and reservoir (Peromyscus leucopus) species. Experiments conducted with the density of ticks likely to be encountered in nature (8 nymphs & <40 larvae) demonstrated that no co-feeding larvae became infected; in contrast, horizontal transmission infected 30-64% of test larvae. Only the highest densities of ticks (40 nymphs & >200 larvae) produced infected larvae (5%) upon co-feeding of larvae and nymphs. An important role for co-feeding in the ecology of Lyme disease in North America has yet to be established. Journal of Vector Ecology 26 (2): 216-220. 2001.

Keyword Index: Borrelia burgdorferi, ticks, co-feeding.

INTRODUCTION

The phenomenon wherein a pathogen passes from an infected arthropod vector to a non-infected vector while they feed simultaneously on the same host has received much recent attention. This event has been referred to as pathogen transfer during “co-feeding”. Since ixodid ticks feed on hosts for several days, optimizing the chance for transfer of pathogens during co-feeding, this phenomenon has been of special interest in the field of tick-borne diseases. Co-feeding is of key importance in the maintenance of several tick-borne viruses; Thogoto virus was the first pathogen in which co-feeding of ticks received intense research interest. In a series of elegant experiments, it was demonstrated that Thogoto virus (an orthomyxo-like virus) was passed from tick to tick even though the host never became viremic, and this process was termed “non-viremic” transmission (Jones et al. 1987, 1990). Moreover, non-viremic transmission is facilitated by the presence of tick saliva at the feeding site; this was termed “salivary activated transmission” (Jones et al. 1989, 1992). In addition to Thogoto virus, co-feeding was found to be an efficient means of transfer from tick to tick of the important human pathogen Tick-borne encephalitis virus (TBE). TBE virus, in the family Flaviviridae, was found to pass from infected adult Ixodes ricinus to uninfected nymphs while feeding on the same guinea pig (Labuda et al. 1993), as well as yellow-necked field mice and bank voles (Labuda et al. 1996).

Although research on co-feeding as a means of maintaining tick-borne pathogens has focused chiefly on viruses, recent interest has been directed toward the importance of this phenomenon in maintaining the Lyme disease spirochete, Borrelia burgdorferi (Randolph et al. 1996). Larval I. ricinus acquired infection with B. burgdorferi sensu stricto when feeding on white mice within capsules containing infected nymphs (Gern and Rais 1996). Similarly, B. burgdorferi was passed from infected to uninfected female I. scapularis when these ticks were fed within ear bags on rabbits (Piesman et al. 1998). Co-feeding nymphal I. scapularis also passed infection with B. burgdorferi among themselves when feeding on gerbils (Patrican 1997). Surprisingly, I. ricinus ticks co-feeding on sheep became infected with B. burgdorferi even though sheep are refractory to infection themselves (Ogden et al. 1997). It has been suggested in Europe that the principal importance of co-feeding on Lyme disease ecology is to extend the range of vertebrate hosts that contribute significantly to the
maintenance of spirochetes in nature (Randolph et al. 1996).

In regions of the northeastern United States that are hyperendemic for Lyme disease, the white-footed mouse, *Peromyscus leucopus*, serves as the principal reservoir host for *Borrelia burgdorferi* (Donahue et al. 1987). In an endemic site in Massachusetts, 46% of larval *I. scapularis* feeding on naturally infected *P. leucopus* acquired infection with *B. burgdorferi* (Mather et al. 1989). An important component of the extremely high reservoir competence of *P. leucopus* for *B. burgdorferi* is the timing of larval and nymphal feeding. In New England, nymphal *I. scapularis* peak feeding occurs in May-June, while larval peak feeding does not occur until August-September (Piesman and Spielman 1979). This “reverse” pattern in which nymphs transmit infection to white-footed mice in early summer and larvae acquire infection from mice several weeks to months later is thought to optimize the reservoir competence of infected rodents in the northeastern U.S. (Spilman et al. 1985, Wilson and Spielman 1985, Yuval and Spielman 1990). Some observers have noticed that there is a smaller peak of larvae in May-June when nymphs are also feeding (Carey et al. 1980, Main et al. 1982). This so-called bimodal pattern of larval feeding would place larvae and nymphs on rodents at the same time. Moreover, in the midwestern U.S., larvae also demonstrate a strong bimodal feeding pattern and the majority of both larvae and nymphs may feed simultaneously on hosts during the early summer (Godsey et al. 1987, Mannelli et al. 1994, Jones and Kitron 2000). Co-feeding might be an important auxiliary to traditional horizontal transmission of *B. burgdorferi* from tick to rodent to tick in the midwestern U.S. Accordingly, we developed an experimental model to determine under what conditions immature co-feeding ticks exchange *B. burgdorferi* using the principal North American vector (*I. scapularis*) and reservoir (*P. leucopus*) species.

**MATERIALS AND METHODS**

**Ticks**

The ticks used in these experiments originated from *I. scapularis* females collected from Bridgeport, Connecticut. The F1 generation of ticks from this colony were checked for transovarial transmission of spirochetes and none were observed. F2 immature ticks were used in the experiments described in this study. Ticks were held at 21°C in saturated humidity when not feeding on hosts. Batches of nymphs infected with the B31 strain of *B. burgdorferi* were reared as previously described (Piesman 1993). Briefly, larvae were allowed to feed on outbred white mice exposed to infected nymphs 1 month previously. Larvae were held until molting to nymphs. Our B31 infected colonies consistently have had a high infection rate. To verify that the majority of colony B31 ticks used as infected nymphs in these experiments were indeed infected, we cultured replete nymphs 10-12 days after feeding. Of the 290 replete nymphs cultured, 205 (71%) were infected.

**Rodents**

The rodents used in these experiments were female *P. leucopus* obtained from the University of South Carolina *Peromyscus* Genetic Stock Center. They were obtained as 4-week old mice and were exposed to ticks within a month of arrival.

**Tick Feedings**

Larval and nymphal ticks were allowed to feed *ad libitum* on all hosts. Hosts were anesthetized with ketamine prior to tick exposure. Ticks were counted (to the best of our ability) as they were placed on the rodents. Rodents were placed in plastic cages contained within a water moat. Replete ticks were allowed to crawl out of the cage and into the moat where they were collected. Ticks were collected for up to 1 week after attachment.

**Borrelia culture**

Ticks were disinfected for 20 min in Wescodyne (Amsco Medical Products; Erie, PA) and for 20 min in 70% alcohol. The ticks were then placed in glass homogenizers containing 0.5 ml of culture medium and homogenized. The homogenate was decanted into 5 ml snap cap tubes filled (except for a minimal air space) with culture medium. The culture medium used was Barbour-Stoenner-Kelly (BSK) medium, prepared as previously described (Sinsky and Piesman 1989). Test larvae were allowed to molt to nymphs before they were homogenized and cultured. To be certain that each *P. leucopus* was infected during the course of the experiment, white-footed mice were sacrificed after exposure to larvae and an ear biopsy sample and urinary bladder were cultured in BSK. All 45 mice used in these experiments produced positive spirochete cultures. BSK cultures were held at 33-34°C and examined weekly for live spirochetes under darkfield microscopy (at 400X magnification) for up to 4 weeks.

**RESULTS**

The initial experiment was designed to compare the efficacy of co-feeding to standard horizontal tick-rodent-tick transmission. In this experiment, 8 B31 infected nymphs were placed on each mouse, and a standard number of larvae (30-40) placed on the mice either
simultaneously with the nymphs, or several weeks thereafter. Replete larvae were collected and allowed to molt to nymphs before being cultured. Of the 157 larvae that fed simultaneously with infected nymphs, none acquired infection with *B. burdorferi* (Table 1). In contrast, nearly two-thirds of larvae feeding 2 weeks after nymphs became infected (64%); more than one third of the larvae became infected when feeding 4 weeks after nymphs (37.3%), and less than one third of the larvae became infected when feeding after 8 weeks (30%) (Chi-square=164.5; P<0.01).

The subsequent experiment was designed to see if the density of ticks on mice influenced the efficacy of co-feeding. Variable numbers of B31 colony infected nymphs and xenodiagnostic larvae were placed on mice simultaneously. With up to 32 nymphs placed on individual mice and 50-100 larvae, transfer of infection from nymphs to larvae was extremely rare; only 1 out of >500 larvae acquired infection with *B. burgdorferi* (Table 2). At higher densities of ticks, however, transfer of infection in co-feeding ticks was more frequent. With 40 infected nymphs and >200 larvae placed on each animal, 10/200 (5%) of larvae acquired infection (Chi-square= 21.9; P<0.01).

**DISCUSSION**

Co-feeding appears to play a more important role in the maintenance of tick-borne viruses, like TBE virus, than it does in the maintenance of Lyme disease spirochetes (Randolph et al. 1996, 1999, 2000). In specific foci, however, co-feeding may be important in the ecology of Lyme disease. There are areas in the U.K. where sheep are reported to feed the vast majority of all 3 stages of *I. ricinus*, and >20% of adult ticks are infected; yet, sheep do not appear to support systemic infection with *B. burgdorferi*, but rather serve as a vehicle to transfer infection among co-feeding ticks (Ogden et al. 1997). Sheep may actually not become infected with *B. burgdorferi* but allow co-feeding infection to take place, since their complement is only partially borreliacidal (Kurtenbach et al. 1998). Deer complement, on the other hand, is extremely borreliacidal. Deer complement may be so borreliacidal that even co-feeding infection of ticks on deer is pre-empted. Similarly, lizard complement has been found to be extremely borreliacidal (Kuo et al. 2000). Curiously, rodent complement seems to kill select genospecies of *Borrelia* in Europe (e.g. *B. garinii* and *B. valaisiana*) whereas bird complement kills other genospecies (e.g. *B. afzelii*) (Kurtenbach et al. 1998). The predominant genospecies in North America, *B. burgdorferi* sensu stricto, appears to be sensitive only to deer and lizard sera. Thus, infection via standard horizontal tick-host-tick and co-feeding appear to be

<table>
<thead>
<tr>
<th>No. infected nymphs</th>
<th>No. test larvae</th>
<th>No. mice</th>
<th>No. ticks(^2) infected/No. examined (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>50-100</td>
<td>4</td>
<td>0/120 (0%)</td>
</tr>
<tr>
<td>24</td>
<td>50-100</td>
<td>7</td>
<td>1/210 (0.5%)</td>
</tr>
<tr>
<td>32</td>
<td>50-100</td>
<td>6</td>
<td>0/180 (0%)</td>
</tr>
<tr>
<td>40</td>
<td>&gt;200</td>
<td>4</td>
<td>10/200 (5.0%)</td>
</tr>
</tbody>
</table>

\(^1\)Test larvae and infected nymphs were placed on mice simultaneously and allowed to feed *ad libitum*  
\(^2\)Test larvae were allowed to molt to nymphs before being examined for the presence of spirochetes.
possible routes of *B. burgdorferi* sensu stricto maintenance in both rodents and birds. The link between co-feeding, spirochete diversity, complement lysis and reservoir competence is complex and deserves further research attention.

In the current study, we have examined the dynamics of co-feeding in a reservoir that is highly competent. In various areas of the northeastern U.S., where Lyme disease is highly endemic, >25% of nymphs and >50% of adult *I. scapularis* are infected with *B. burgdorferi* (Maupin et al. 1991). In these endemic regions, *P. leucopus* is thought to be the principal reservoir (Levine et al. 1985, Donahue et al. 1987, Mather et al. 1989). Horizontal tick-rodent-tick transmission is clearly highly efficient in this cycle. In the field, 46% of larvae dropping off field infected *P. leucopus* became infected with *B. burgdorferi* (Mather et al. 1989). Our results are consistent with the concept that in highly endemic regions of the U.S., rodents serve to produce infected nymphal *I. scapularis* by infecting larvae via standard horizontal transmission. Co-feeding does not appear to be an important auxiliary leading to additional populations of infected ticks. Only the highest densities of co-feeding larval and nymphal ticks produced infection via co-feeding on *Peromyscus*. These densities, of 40 nymphs and >200 larvae, are much higher than those commonly observed in nature (Wilson and Spielman 1985, Deblinger et al. 1993). Co-feeding would have probably been much more efficient if the ticks were fed in capsules or ear bags, as performed in previous studies (Gern and Rais 1996; Piesman et al. 1998). An artificial feeding process, however, would not tell us much about the importance of co-feeding under natural conditions. In North America, co-feeding has not proved to be an efficient means of maintaining *B. burgdorferi* in highly reservoir competent species like *P. leucopus* and the American robin (*Turdus migratorius*) (Richter et al. 2000). An important role for co-feeding in the ecology of Lyme disease in North America has yet to be established.

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