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A Study of the Different Modes of Transmission of *Borrelia burgdorferi* by *Ixodes ricinus**

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Summary

Only a small number of unfed *Ixodes ricinus* larvae collected in nature have been found to be infected by *Borrelia burgdorferi (Bb)*. Therefore transovarial transmission seems to be rare.

In contrast, an average of 30% of unfed nymphs and adults are infected. This finding shows that the ticks have become infected during an immature stage. It also provides an indication of the efficiency of the transstadial transmission.

The purpose of this study was to estimate the efficiency of the transovarian and transstadial transmissions. Different methods to infect the ticks in the laboratory were used.

Bb growth following to blood meal in unfed artificially infected females of *I. ricinus* was studied by a combination of the cryocut and direct immunofluorescence techniques. Some ticks (2/11) had spirochetes in their hemolymph 2 days after the beginning of the blood meal. *Bb* has been observed in the salivary glands of some ticks (6/51) and in the ovaries (5/51) from the third day of blood meal.

No spirochetes were detected in the rectal ampulla, so the possibility of transmission through spirochete-infected fecal material seems to be negligible.

Introduction

Recently several studies have been carried out on the evolution of *Borrelia burgdorferi (Bb)* in the American tick *Ixodes dammini*. In fact two modes of transmission of the spirochete from this vector to the host have been hypothesized:

1. a passage from the midgut through the hemolymph to the salivary glands and followed by an injection of the spirochete with the salivary secretions, proposed by Ribeiro *et al.* (5) and

2. an eventual regurgitation of the spirochetes with other gut contents during the blood meal, proposed by Burgdorfer *et al.* (1).

In contrast to *I. dammini*, very little is known about the evolution of *Bb* in *Ixodes ricinus*, the vicarian species of *I. dammini* in Switzerland. However, the presence of trans-stadial and trans-ovarian transmission of the spirochete has been demonstrated in this European tick species. (3, 4). The purpose of our study is:

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1. to determine the percentage of successful trans-stadial transmission of *Bb* in *I. ricinus*,

2. to study trans-ovarian transmission and finally

3. to obtain further knowledge of the evolution of *Bb* in *I. ricinus* during the blood meal, comparing our results with the known data on *I. dammini*.

Fig. 1. *Ixodes ricinus* feeding from capillary tubes (hypostome and chelicerae inserted into capillary tube).

Methods

Before starting the actual studies, it was necessary to develop new methods to infect the ticks artificially.

We first tried to infect ticks by feeding them on a mouse in which cultured spirochetes had been injected in the peritoneum. In a second experiment, we injected the spirochete culture either intraperitoneally or subcutaneously into healthy splenectomized mice. In a final attempt to infect ticks, we injected a culture of spirochetes subcutaneously but at the site of tick attachement. Since these three methods were inconclusive, we chose to infect our ticks by using a modified version of the capillary method of Burgdorfer (1957) (Fig. 1). This method allowed us to infect larvae, nymphs as well as adults. In addition, it was possible to infect ticks before, during and after the bloodmeal on the host.

For the study of trans-stadial transmission, the ticks were artificially infected using the capillary method either before taking their bloodmeal on rabbits or after dropoff. For the study of trans-ovarian transmission, females were infected in the same manner.

After successfull infections, we needed to develop a method to visualize the evolution of *Bb* in *I. ricinus*. We first infected females using the capillary method, and then allowed them to feed on healthy white rabbits. A number of ticks were removed daily to be frozen and then sectioned with a deepfreeze cutter. The sections (20 µm) were fixed on slides and stained by direct immunofluorescence.

The two spirochete strains used were an American one (B32) and a Swiss strain (Sta14).

Results

None of the ticks which fed on the mouse infected by intraperitoneal injection of the spirochete culture were infected. When splenectomized mice where used, 4% of the larvae and 14% of the nymphs were infected. Only after injecting the spirochete culture subcutaneously beneath the site of attachement were we able to obtain a moderate level of infected ticks (46%).

After infecting unfed ticks (thus before the bloodmeal) with a two hour meal on the capillary, 75.56% of the females, 79.37% of the nymphs and 66.67% of the larvae were still positive two days later. The weight of the tick increased 38.5%, 55.27% and 80%, respectively, compared to the initial weight of the tick.

TRANS-STADIAL TRANSMISSION

FIRST EXPERIMENT : TICKS INFECTED BEFORE THEIR BLOOD-MEAL

PASSAGE LARVAE-NYMPHS:	7/40	(17.5%)
PASSAGE NYMPHS-ADULTS:	14/47	(29.8%)

SECOND EXPERIMENT: TICKS INFECTED AFTER THEIR BLOOD-MEAL

PASSAGE LARVAE-NYMPHS:	3/12	(25.0%)
PASSAGE NYMPHS-ADULTS:	3/8	(37.5%)

Fig. 2. Results of the trans-stadial transmission.

If the ticks were infected with a two hour meal on the capillaries after the blood meal, 100% of the ticks were still infected two days later. The weight increase represented 78% in the nymphs and 21.8% in the larvae.

Trans-stadial transmission

After the larval molt, in 7 of 40 ticks infected before the bloodmeal and in 3 of 12 ticks infected after the bloodmeal the spirochetes were present (Fig. 2). Successful transstadial transmission to the adult was obtained in 14 of 47 and 3 of 8 nymphs infected before and after feeding, respectively.

Trans-ovarian transmission

No positive larvae were obtained from females who had ingested spirochete culture (strain B32) before taking their bloodmeal (Fig. 3). However, in a second experiment, 3 of 7 females infected with the Swiss strain after their blood meal, produced about 50% positive larvae.

Evolution of Bb in I. ricinus (Fig. 4)

In females removed from the host after one day of feeding, the spirochetes were present only in the lumen of the midgut. None were observed in the hemolymph or in the pharyngial area.

On the second day of feeding, the spirochetes had migrated to the walls of the midgut. However, in two out of 11 cases, spirochetes were already present in the hemolymph.

After three days, many spirochetes were discovered in the hemolymph and sometimes in the salivary gland and in the ovary, indicating that a generalised infection is not necessary for spirochetes to be found in the hemolymh.

The number of spirochetes found in the salivary glands and in the ovaries increased on the fourth day.

After the fifth day, it was difficult to visualize the spirochetes in the midgut. This may be an indication that the spirochetes had changed their antigenic coat.

TRANS-OVARIAN TRANSMISSION

FIRST EXPERIMENT: FEMALES INFECTED BEFORE THEIR BLOOD-MEAL

0/15 POSITIVE

SECOND EXPERIMENT: FEMALES INFECTED AFTER THEIR BLOOD-MEAL

3/7 POSITIVES (42.9%) ----- THESE FEMALES PRODUCED 50%
POSITIVE LARVAE WITHIN EACH
EGG-LAYING; BUT ONLY AT THE
END OF THESE EGG-LAYINGS.

Fig. 3. Results of the trans-ovarian transmission.

In the ticks examined on the 3rd, 4th and 5th days of feeding, *Bb* was observed in the salivary glands of 6 of 51 ticks and in the ovaries of 5 of 51 ticks. No spirochetes were found in the rectal ampulla, thus indicating that transmission by spirochete-infected fecal material would seem improbable.

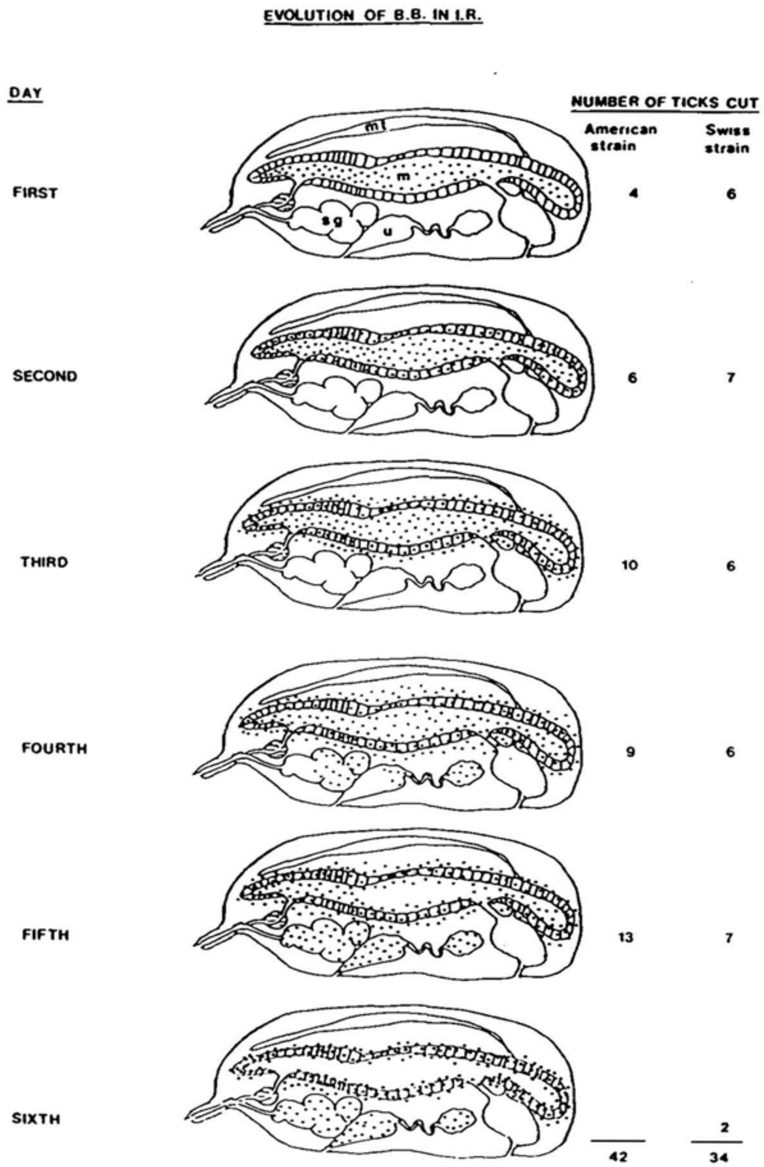
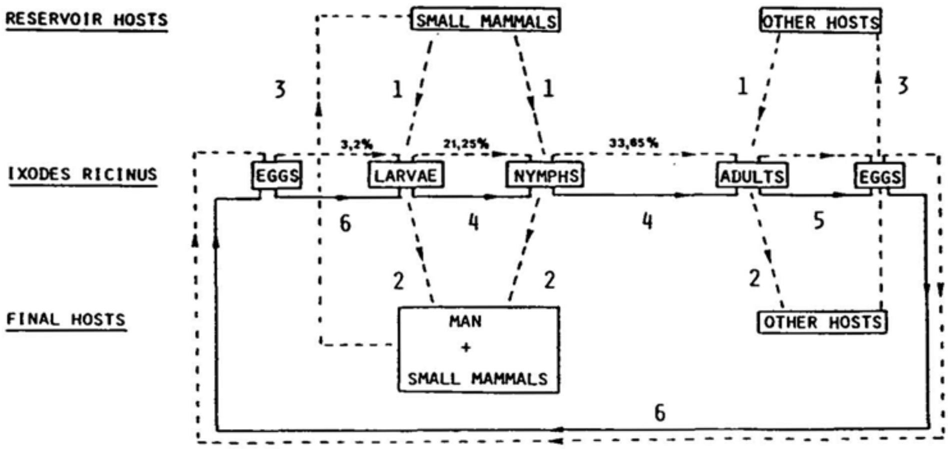


Fig. 4. Evolution of *Borrelia burgdorferi* in *I. ricinus*; mt: malpighian tubule, m: midgut, sg: salivary glands, u: uterus, ⊡ organs infected with *Bb*.

CYCLE OF BORRELIA BURGDORFERI IN IXODES RICINUS



EXPLANATIONS:

1) INFECTION OF TICKS BY RESERVOIR HOSTS
2) THE TICKS INFECT THE FINAL HOST
3) THE FINAL HOSTS CAN BECOME RESERVOIR HOSTS
4) TRANS-STADIAL TRANSMISSION (RESULTS OF THIS STUDY)
5) TRANS-OVARIAN TRANSMISSION (% OF INFECTED LARVAE COLLECTED IN THE NATURE)
6) DEVELOPMENT OF THE EGGS

--- CYCLE OF BORRELIA BURGDORFERI

—— CYCLE OF IXODES RICINUS

Fig. 5. Hypothetic cycle of *B. burgdorferi* in *I. ricinus*.

Discussion

We reconfirmed trans-stadial transmission of *Bb* in *I. ricinus* although an important loss of spirochetes was observed. This loss seemed to be greater during the passage from the larval stage to the nymphal stage than during the passage from the nymphal stage to the adult.

The difference between the first experiment, in which our ticks were infected before their blood meal, and the second, in which the ticks were infected after feeding, showed a loss of spirochetes during the blood meal following the infection by capillaries. Perhaps, however, the spirochetes changed their antigenic coat during blood meal of the vector. In this case, they would no longer react in the fluorescence test and thus no spirochetes would be observed in these ticks.

For the difference in the results obtained in the transovarian transmission when females are infected before or after the blood meal, several explanations are possible. Perhaps, the number of spirochetes in the tick before the blood meal was not high enough; in fact, the quantities of culture ingested was higher after the blood meal. Thus, more spirochetes had the possibility to survive until oviposition. As with trans-stadial transmission, there may have been a loss spirochetes during the blood meal. Or a change in the antigenic coat took place, which would explain a decrease in intensity of fluorescence as well as a "lack" of spirochetes. Trans-ovarial transmission appears to exist only if the infection occurs during the last part of the blood meal. Complementary studies need to be conducted to prove these hypotheses.

Using the results obtained in our laboratory and in the field, we have described a possible cycle of *Bb* in *I. ricinus* and in different hosts (Fig. 5).

We take the liberty to repeat and comment upon the conclusion given by Burgdorfer *et al.* (1) concerning the transmission of the spirochetes through ticks: "at present, we can only speculate that transmission of spirochetes during a tick's feeding process may occur via saliva by a tick with a generalised infection and/or by regurgitation of infected gut contents". Our conclusion is very much the same; however, we prefer to be more prudent when speaking of a generalised infection and reduce the importance of the role of regurgitation in transmission.

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