SEASONAL VARIATION IN THE INFESTATION OF RODENTS WITH *IXODES RICINUS* (ACARI: IXODIDAE) AND PREVALENCE OF INFECTION WITH *BORRELIA BURGDORFERI* IN A RECREATION AREA

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Abstract - In woodlands of Western Europe, rodents like yellow-necked mice (*Apodemus flavicollis* Melchior) or bank voles (*Clethrionomys glareolus* Schreber) play an important role in the natural circulation of *B. burgdorferi* sensu lato. It was possible to catch a total number of 120 small mammals (only first capture) of four species, among them 62 voles and 52 mice and both were shown parasitized by uninfected and infected *Ixodes ricinus* immatures. The infestation of small mammals with larvae was highest in June and lowest in July/August. Our study also showed that the level of infestation with *I. ricinus* was lower in bank voles than in mice. The seasonal patterns of peak infestation by *I. ricinus* on *Apodemus* were bimodal with peaks in June and September. In contrast, voles showed a unimodal activity pattern with the highest larvae numbers in June. In general, the maximal abundance of larvae on rodents was observed in June. The infection rate of engorged larvae increased from spring to autumn. This behaviour was also detectable in questing nymphs of the same study area. Compared to *A. flavicollis*, bank voles had both a lower mean number of infesting larvae and a lower number of infected animals. The spirochetal agent of Lyme borreliosis occurred endemically in the study area, with *Apodemus flavicollis* being mainly participating when both investigated species of small mammals were compared. There is a high infection risk for men in the investigated recreation area. Due to the course of prevalence, the infection risk seems to be highest in summer and autumn.
Key words - Lyme borreliosis, epizootiology, mammals, reservoir

INTRODUCTION

Lyme borreliosis is a world-wide tick-borne zoonosis and is the most prevalent vector-borne disease in the Old and New World. The causative agent of Lyme disease, *Borrelia burgdorferi* Johnson, Schmid, Hyde, Steigenwalt et Brenner (Burgdorfer *et al.*, 1982) is transmitted by ticks of the *Ixodes ricinus* Linnaeus species complex. The natural maintenance of the Lyme disease-spirochete is based on transmission cycles involving vertebrates and ticks. In Europe, *B. burgdorferi* has been observed for example in the rodent species *Apodemus flavicollis* Melchior (yellow-necked mouse), *Apodemus sylvaticus* Linnaeus (wood mouse) and *Clethrionomys glareolus* Schreber (bank vole), which are also the natural hosts of the tick species *Ixodes ricinus* Linnaeus (Gern *et al.*, 1998),

The infection of man depends on the transmission through parasitizing *I. ricinus*, which is the only hard tick species in Central Europe that regularly parasitizes man. More data are neccessary for the effective surveillance and management of tick-borne pathogens in endemic areas of Thuringia. Therefore, the purpose of the present study was to investigate and analyse the relative abundance and seasonal activity periods of tick species collected from rodents and to estimate the seasonal course of the infection rate in vectors and carrier hosts in a recreation area in Thuringia.

MATERIALS AND METHODS

Study area

The study was carried out at a location in the Ilm-valley. The investigated area, a forest path, was at Bad Berka, about 500 m south-west of a major hospital near Weimar. The forest path, whose

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boundary is marked by a fir stand on the east side and a pin stand on the west side, is mainly covered with acidophilic plants and grasses, for example common wood sorrel *(Oxalis acetosella)*, common Saint-John's-wort (*Hypericum perforatum*), common speedwell (*Veronica officinalis*). On the western side, a small ditch and a dense stand of *Rubus* species are adjacent to a pine forest. Common birch (*Betula pendula*), red beech (*Fagus sylvatica*) and English oak (*Quercus robur*) as well as shrubs, e.g. common elder (*Sambucus nigra*), fly honey-suckle (*Lonicera xylosteum*) or blackthorn (*Prunus spinosa*) grow near the ditch. The occurrence of wood horsetail (*Equisetum sylvaticum*) indicated a high soil humidity.

Small mammals and ticks

A combination of 8 Longworth live traps and 50 traps according to HALLE, baited with a mixture of oat flakes soaked in sunflower oil (Feldmann, 1997) were randomly set in our selected habitat at various sites and times during June, July, August and September 1997. The nesting box contained apple chips and pressed green fodder as sources of food. The traps were set during the day at a distance of 10 m in the vegetation along the forest path. There were always two traps per trap site, which were checked on the following morning and evening. The species of the captured rodents was identified, their sex and approximate age were determined and they got mark typical of the season. Afterwards, they were anesthetized with ether and examined for ticks.

All attached ticks were removed from hosts with forceps and the separated stages were maintained in transport vials with a few blades of grass inside. They were stored at + 6 ºC until they could be tested after one week. Blood was taken from the Plexus retroorbitalis. Immediately afterwards, the rodents were released at the point of capture.

Detection of *Borrelia burgdorferi*

The larvae were examined in pools of two by the indirect immunofluorescence assay (IFA) using a polyclonal antibody. Then it was possible to calculate the infection rate of the individual larvae with the formula $p = 1-^{2}/1-f$ according de Boer *et al.* (1993). The sera were tested for antibodies to B. burgdorferi by the indirect immuno-fluorescence assay using *B. burgdorferi* antigen (B 31) and antimouse IgG (H+L) FITC-conjugate (Labor Alomed/Rodolfzell). The samples were regarded as positive from a titre value of 1:80 onwards.

Statistical analysis

Rodent trapping data and tick infestation data of small mammals were analyzed with the t-Test according Student. Tick infection rates were compared using the Chi-Quadrat-Test. All statistical procedures were carried out with the statistical programme SPSS for windows.

RESULTS

Capture of small mammals

A total number of 120 rodents were trapped between June and September in 1997. Among the collected four kinds of rodents (Table 1) a mouse (*Apodemus flavicollis*) and a vole (*Clethrionomys glareolus*) were most abundant. Since other small mammals were relatively scarce, they are not taken into consideration in the following. On the whole, the role *C. glareolus* was far more abundant than any other rodent. In spring, however, the number of yellow-necked mice (*A. flavicollis*) was clearly higher than that of bank voles (*C. glareolus*). This ratio, however, become inverted in summer with the largest number of captured animals.

In both species, the proportion of male animals was clearly higher than that of female ones at all periods of trapping. The number of captured young animals was highest in summer, both in *C. glareolus* (25%) and in *A. flavicollis* (52%).

Table 1. Small mammals capture in Bad Berka in 1997 (only first capture).

Season	Period	A. flavicollis	C. glareolus	M. agrestis	S. araneus
spring	20/6 - 28/6	20	6	0	0
summer	23/7- 31/8	25	48	2	1
autumn	25/9 - 26/9	7	8	2	2
Σ		52	62	2	3

Ticks from rodents

Between June and September in 1997 the captured rodents were examined for tick infestation. The mean percentage of infested rodents is clearly higher in *A. flavicollis* with 93% than in *C. glareolus* with 59% (p>0.001). A total number of 287 ticks were available, all of which belonged to *I. ricinus*. Larvae were far more abundant than nymphs. 94.77% of the attached *I. ricinus* were larvae (n = 272) and 5.23% nymphs (n = 15). The overall ratio of larval to nymphal *I. ricinus* in parasitized mice and voles was 18:1. Adults were not found in small mammals.

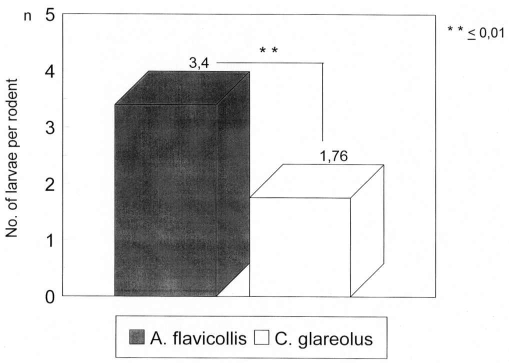

Figure 1. Mean numbers of *I. ricinus* larvae per rodent parasitizing on *A. flavicollis* and *C. glareolus* at Bad Berka, Thuringia, between June and September 1997

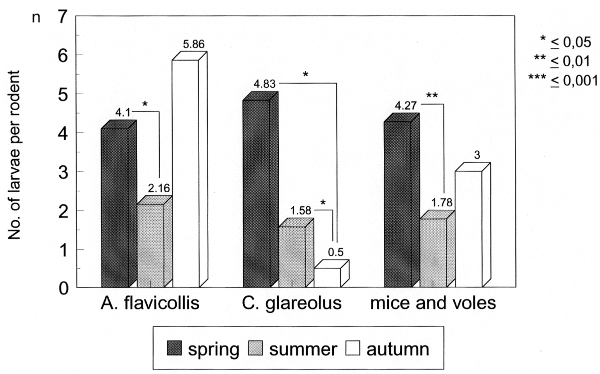

Figure 2. Seasonal occurrence of *I. ricinus* larvae on rodents trapped in Bad Berka, Thuringia, June through September 1997

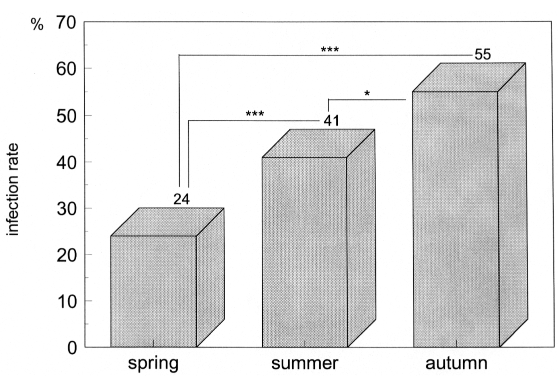

Figure 3. Seasonal distribution of mean prevalence of *B. burgdorferi* in fed larvae derived from rodents trapped in Bad Berka, Thuringia, 1997

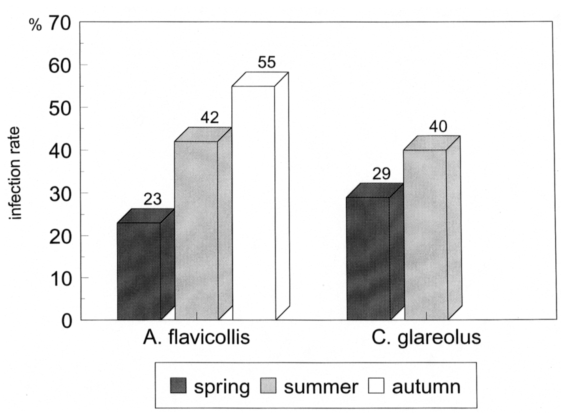

Figure 4. Seasonal distribution of *B. burgdorferi* infection rates in fed larvae derived from rodents trapped in Bad Berka, Thuringia, 1997

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At Bad Berka, *Apodemus* mice harboured greater numbers of *I. ricinus* ticks than bank voles. This was true both for larvae and nymphs (Fig. 1). The mean number of larvae collected on *A. flavicollis* was significantly higher than that on *C. glareolus*. A mean of 3.4 larval ticks were found on parasitized mice and 1.8 on voles. Male yellow-necked mice harboured greater numbers of *I. ricinus* larvae than females especially in spring and summer, whereas the opposite was true for *C. glareolus*. Young voles and mice tended to be infested by a smaller number of *I. ricinus* larvae than female or male voles and mice.

Seasonal activities

The seasonal variation in the number of larvae on small mammals was highest in spring (June) and lowest in summer (July/August) (Fig. 2). In contrast, the mean number of larvae on rodents decreased continuously from spring to summer in voles, with the smallest number of them being registered in autumn.

Tick infection rates

The examination of ticks for spirochete infection showed that the overall infection prevalence increased significantly from spring to autumn (Fig. 3). The mean infection rate of *I. ricinus* larvae was lowest in spring (23.7%), higher in summer (41.0%), and highest in autumn (55.3%). This was mainly true for mice whereas the infection rate of larvae collected from voles in spring and summer remainde relatively the same (Fig. 4). Compared to female rodents, male yellow-necked mice had a higher number of infected larvae at all times, which was not that consistent in the larvae collected from bank

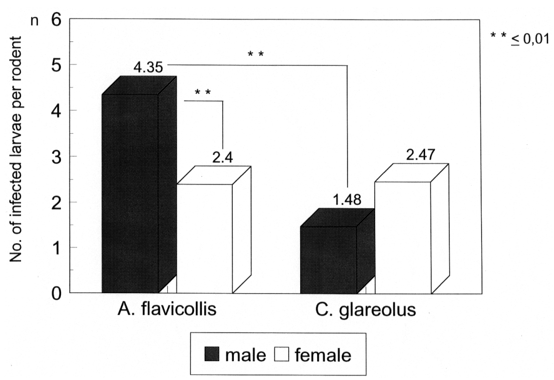

Figure 5. Mean number of infected *I. ricinus* larvae on *A. flavicollis* and *C. glareolus* trapped in Bad Berka, Thuringia, from June to September 1997

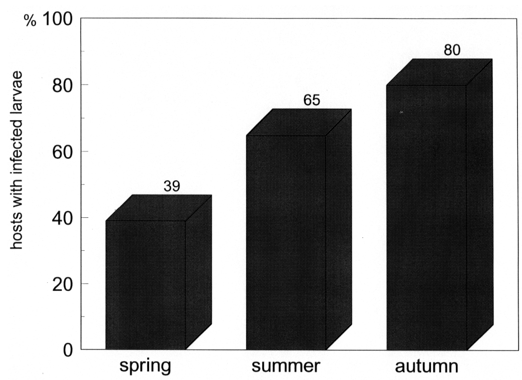

Figure 6. Seasonal distribution of small mammals trapped in Bad Berka, Thuringia, parasitizing by infected *I. ricinus* larvae, 1997

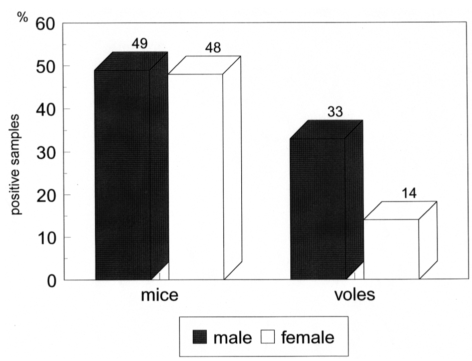

Figure 7. Prevalence of antibodies to *B. burgdorferi* in *A. flavicollis* and *C. glareolus* trapped in Bad Berka, Thuringia, from June to September 1997

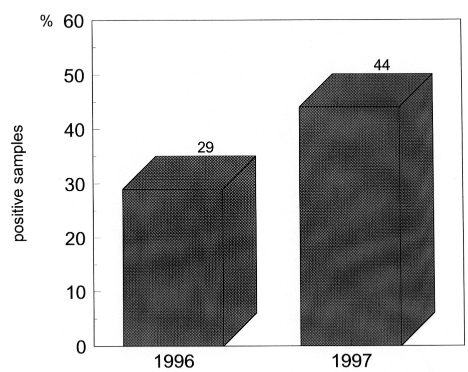

Figure 8. Prevalence of antibodies to *B. burgdorferi* in *A. flavicollis* and *C. glareolus* trapped in Bad Berka, Thuringia, from June to September 1996 and 1997

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vole (Fig. 5). The seasonal distribution of small mammals parasitized by infected *I. ricinus* larvae increased both in mice and voles (Fig. 6). A higher proportion of rodents captured in autumn was shown to harbour infected ticks than did those captured in spring and summer.

Serology

In 1997, 73 trapped rodents were tested for *B. burgdorferi* specific IgG - antibodies 1:80, which were considered to be seropositive. We were able to demonstrate in the serological investigations into mice sera that the level of positive serum samples was almost by 50 percent higher in *A. flavicollis* than in *C. glareolus* (Fig. 7), with 14 of the 73 positive sera showing titers of 1:160 or below (19.18 %). Two sera were found to have a titer of even 1:1280. According to the results of the serological investigations, the proportion of positive serum samples was clearly higher in mice than in voles. When the latest results are compared with those obtained in 1996 (the same habitat), the proportion of positive serum samples shows the tendency of being higher in 1997 than in 1996 (Fig. 8).

DISCUSSION

The ecology of *B. burgdorferi* s.l. in Europe involes one primary tick vector, *I. ricinus*, and a variety of vertebrate tick hosts, several of which are competent reservoirs for *B. burgdorfer*i s.l. The maintenance of *B. burgdorferi* in an ecosystem requires the presence of both suitable hosts for all tick stages and of tick hosts serving as spirochetal reservoirs by transmitting the bacterium from one tick generation to the next. To function as important reservoirs for *B. burgdorferi* vertebrate hosts must be abundant and be abundantly infested by larval and nymphal ticks.

Our results confirm that the study area near Weimar is a typical biotope for *I. ricinus*. Its plant communities with humid and acid conditions are most favorable for *I. ricinus* (Kurtenbach *et al.*, 1995; Gray *et al.*, 1998) The four micromammalian species trapped (*C. glareolus, A. flavicollis, M. agrestis* and *S. araneus*) were all parasitized by *I. ricinus* subadults. The mean percentage of infested rodents clearly differs between *A. flavicollis* and *C. glareolus*. On an average it amounts to 93 % in mice and to only 59 % in bank voles. Furthermore, it is known that greater numbers of *I. ricinus* larvae and nymphs were recorded on *Apodemus* mice than on bank voles. Similary to our findings, yellow-necked mice harboured greater numbers of *I. ricinus* larvae and male compared to female rodents generally harboured greater numbers of *I. ricinus* larvae (Humair et al.1993; Kurtenbach *et al.*, 1995; Tälleklint and Jaenson, 1997). According to Tälleklint and Jaenson (1997) and other authors in Europe *Apodemus* mice have larger home ranges than bank voles. In addition, males also have greater home ranges than females, in particular during the breeding seasons. Furthermore, an acqired resistance against feeding *I. ricinus* ticks has been reported for voles, but not for mice (Dizij and Kurtenbach, 1995).

The mean densities of *I. ricinus* larvae and nymphs infesting rodents in different areas vary from less than five to $\geq$ 30 larvae and from none to more than one nymph (Tälleklint and Jaenson, 1997). The infestation rates registered in our investigations are at the lower level of the data presented in the literature. In our opinion, the ratio of larval to nymphal *I. ricinus*, which, in comparison with other authors (Humair *et al.*, 1993; Kurtenbach *et al.*, 1995), is low in our investigations, is to be attributed to methodology. During the anaestetic, blood had to be taken, determinations had to be performed in the rodents, marks had to be fixed according to the season and all tick stages had to be collected. Therefore, there was not always enough time for a sufficiently long search for ticks on the rodent. Compared with the results of the year 1996 (obtained in the same habitat, but the animals were dead, not trapped alive) the infestation rates was significantly lower in 1997.

The seasonal patterns of the peak infestation with *I. ricinus* in mammals can be unimodal with a peak in May-July or August-September for the larvae or bimodal with peaks in May-June and August-September (Tälleklint and Jaenson, 1997). Similar patterns of activities were observed in our study area in 1997. Whereas voles showed a unimodale activity pattern, the seasonal patterns of the peak infestation with *I. ricinus* on Apodemus was bimodal with peaks in June and September. In general, the maximum abundance of larvae observed in our investigations was in June. The results demonstrate that mice and voles are important hosts for larval *I. ricinus* and that the seasonal patterns are very different in different species of rodents.

Rodents are know to play a role as amplifying hosts and one infective small mammal can give rise to a large number of infected larvae. We were able to demonstrate in this study that the role of rodents is seasonal. The infection rate of engorged larvae clearly increases from spring to autumn. This trend is mainly true for *Apodemus flavicollis*, with the seasonal distribution of small mammals parasitized by infected *I. ricinus* larvae being relatively even. The rodents captured in autumn, however, were shown to harbor more infected ticks than those captured in spring and summer. The fewest infected larvae were registered in mice and voles in spring.

According to Humair *et al.* (1993), rodents were mainly infective in later summer after the peak abundance of nymphs. Both in 1996 and in 1997, we found the major number of nymphs in summer and still clearly more in September than in June, a month, in which no nymphs parasitizing on mice and voles were found in both years. The consequence is an obvious increase of the number of infected larvae on rodents, which was also confirmed by our investigations. In contrast to the results presented in the literature, both the proportion of infected larvae and the prevalence of hosts with infected larvae is highest in September. This behaviour was also detectable in questing nymphs from the same habitat with the highest infection rate in autumn. Therefore, we are of the opinion that, due to the higher infection rates in autumn in spite of the far fewer questing ticks than in spring and summer, the risk of contracting the infection is higher for men in summer and autumn.

Not only the number of infesting ticks can change between different years in a locality (Kurtenbach *et al.*, 1995) but also the number of infective rodents. In connection with the results of the study concerning the determination of the distribution, abundance, seasonal activities and infection rates of ticks collected from the vegetation of the same area we assume that this is partly due to the abundance of nymphal *I. ricinus* ticks (Dorn *et al.*, 1997). In the case of too considerable a reduction of the density of nymphal stages on the vegetation, the transmission rate could be impaired.

Previous studies showed that the levels of an infestation with *I. ricinus* were lower in *C. glareolus* than in *Apodemus* spp. Although the frequency of the detection of infected larvae on mice and voles did not differ very much, the proportion of positive serum samples is clearly higher in *A. flavicollis* than in *C. glaveolus*. According to Dizij and Kurtenbach (1995) acquired resistance in *C. glareolus* to *I. ricinus* is a factor, which regulates both the natural ectoparasite burden and the spirochete transmission between vector ticks hosts. This would explain the clearly lower number of positive serum samples in voles. Since the acquired resistance in bank vole against *I. ricinus* impairs the spirochete transmission from vector ticks to hosts, the number of infective bank voles is clearly smaller than in *A. flavicollis* on the same site.

CONCLUSIONS

The results confirm that our study site is a typical biotope for *I. ricinus* and the spirochetal agent of Lyme borreliosis occurs endemically. Due to their abundance, *C. glareolus* and *A. flavicollis* are of greatest importance as host for ticks of the species *I. ricinus* in the investigated habitat, with yellow-necked mice playing the more important role when both investigated species of small mammals are compared.

Our studies also underline the importance of *I. ricinus* larvae in the ecology of *B. burgdorferi*. In spite of the extremely small number of parasitizing nymphs registered in our investigations, a large number of serologically positive mice and voles were detected in both experimental years. The seasonal pattern in the number of larvae on small rodents was highest in June and lowest in July/August and differed between mice and voles. The infection rate of engorged larvae clearly increases from spring to autumn. This behavior was also detectable in questing nymphs from the same habitat.

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There is a high infection risk for men in the investigated recreation area. Due to the course of prevalence, it seems to be higher in summer and autumn than in spring.

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