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Comparison of Urinary Bladder and Ear Biopsy Samples for Determining Prevalence of *Borrelia burgdorferi* in Rodents in Central Europe

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PCR was used to compare urinary bladder and ear biopsy samples from four European species of wild rodents for the presence of *Borrelia burgdorferi* sensu lato. From 60 paired comparisons of bladder and ear biopsy samples, the PCR results were concordantly positive or negative in 43 samples (71.7%). Of the 17 which differed, 14 bladder samples were positive and ear samples were negative while the converse occurred for three samples. Thus ear biopsy samples led to a significantly lower estimate of infection than bladder biopsy samples. This suggests that the use of ear biopsy samples in epidemiological studies of *B. burgdorferi* in Central European rodents is likely to lead to underestimates of the prevalence.

Rodents have been implicated as being the main natural reservoirs of *Borrelia burgdorferi* sensu lato in Central Europe (1, 6, 9, 13). Thus in order to elucidate the natural ecology of the disease, it is necessary to have accurate estimates of *B. burgdorferi* prevalence in these hosts. Ear biopsy sample is a useful method for determining this prevalence as it does not require the removal of hosts from the population and thus overcomes ethical problems involved with sacrificing potentially rare animals which may also be protected by government regulations. The most commonly used alternative, taking material from the urinary bladder or other internal organs, involves sacrificing the mouse (2–4).

American studies suggest that the ear punch method is reliable for detecting *B. burgdorferi* infections in laboratory hamsters and mice, and it has been used with success for detecting natural infection in the white-footed mouse, *Peromyscus leucopus* (4, 11, 16). However, data presented by Matuschka et al. (12) show that ear biopsy samples were not able to detect infection in *Apodemus flavicollis* and *Clethrionomys glareolus* individuals collected in the Kraichgau district of North Baden, Germany, although nymphs which had molted from larvae feeding on these hosts were infected.

Recent studies have also shown that the different genospecies of *B. burgdorferi* sensu lato may be differently distributed in host tissue. In a Japanese investigation, *Borrelia afzelii* was most frequent in the bladders compared with "group IV" organisms in the ears (14). Humair et al. (5) suggest that *B. afzelii* is the only genospecies prominent in the ears of the more common Central European rodents. This would imply that epidemiological data collected only by ear biopsy samples are likely to underestimate the prevalence of *Borrelia* infection as rodents are also known to harbor the other genospecies, *Borrelia burgdorferi* sensu stricto and *Borrelia garinii* (7).

In this work, we compare the prevalence estimates of both ear and bladder biopsy samples from four common Central European rodent species belonging to two subfamilies (Murinae and Microtinae) by PCR in which the primers detect all of the known genospecies of *Borrelia* from this area.

Trapping of rodents was carried out in eight different localities in the Kraichgau district of North Baden (see reference 9 for details). Rodents were live-trapped (wire traps; dimensions, 15 by 5 by 6 cm; mesh, 1.5 by 0.8 cm) during summer 1994. Traps were modified by providing a wooden housing to prevent exposure of the trapped mice prior to collection. Traps were baited with muesli (a mixture of oat flakes, cornflakes, hazelnuts, and raisins) combined with either tinned sardines or peanut butter. They were set at a distance from one another of 5 to 7 m directly alongside rodent burrows on rodent tracks or fallen tree trunks, under hedges, or in dense vegetation. They were distributed between 1600 and 1700 and collected from 0800 on the following morning, after which they were returned to the laboratory for processing.

Captured rodents were killed with pentobarbital (Narcoren) and dissected under sterile conditions to remove the urinary bladders. The urinary bladders were cut in half, and one half (approximately 3 by 3 mm by 0.2 mm thick) was used for PCR. In addition, ear biopsy samples (approximately 2 by 2 mm by 0.4 mm thick) were removed from each animal with flame-sterilized scissors. Samples were kept frozen (−30°C) until testing.

Detection of *B. burgdorferi* DNA was performed by PCR with the primer and probe system of Schwartz et al. (15), which amplifies a 259-bp fragment from the 23S rRNA gene. Composition of the reaction mixture, PCR amplification, and the detection of PCR products were exactly as described previously for the testing of urine samples (10), except that the following cycling profile was used: initial denaturation at 95°C for 2 min followed by 45 cycles of denaturation at 95°C for 45 s, annealing at 50°C for 60 s, extension at 72°C for 40 s, and final extension at 72°C for 2 min.

Biopsy samples from rodent urinary bladders and ear snips were digested for 2 h at 56°C in 40 μl of lysis buffer (50 mM KCl, 10 mM Tris, 1.5 mM $MgCl_2$, 1% Triton X-100, 200 μg of proteinase K per ml). The supernatant of each tissue digest was transferred to a fresh tube, 20 μl of a 20% Chelex suspension (chelating resin Chelex 100; Bio-Rad Laboratories, Richmond, Calif.) was added, and the sample was boiled for 10 min. Ten

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TABLE 1. Pairwise comparisons of *B. burgdorferi* infections in rodents[a]

Sp.	No. tested	No. bladder +/ear +	No. bladder +/ear −	No. bladder −/ear +	No. bladder −/ear −
Murinae					
A. flavicollis	15	0	5	1	9
A. sylvaticus	11	1	3	0	7
Subtotal	26	1	8	1	16
Microtinae					
C. glareolus	19	7	2	1	9
M. arvalis	15	5	4	1	5
Subtotal	34	12	6	2	14
Total	60	13	14	3	30

[a] Bladder +/ear + and bladder −/ear − indicate consistently positive or negative PCR results for bladders and ears, respectively. Bladder +/ear − indicates a positive bladder but negative ear, and bladder −/ear + indicates a negative bladder and positive ear.

microliters of the supernatant of each prepared sample was added to the PCR.

The PCR products were analyzed by electrophoresis on 8% polyacrylamide gels, subsequently transferred to nylon membranes, and hybridized at 47°C with the ^{32}P-labeled oligonucleotide FS1 (15). These steps were performed as described previously (8). The strength of the signals for both urinary bladder and ear biopsy samples covered the same range. Eleven (40.7%) and seven (43.8%) showed weak reactions, and 16 (59.3%) and 9 (56.2%) showed moderate to strong reactions.

Four species of rodents were captured. These were *A. flavicollis* and *Apodemus sylvaticus* belonging to the subfamily Murinae and *C. glareolus* and *Microtus arvalis* belonging to the subfamily Microtinae. From 60 paired comparisons of bladder and ear biopsy samples, the PCR results were concordantly positive or negative in 43 samples (71.7%). Of the 17 which differed, 14 bladder samples were positive and ear samples were negative while the converse occurred for three samples. Thus ear biopsy samples yielded significantly less positive results than bladder biopsy samples (assuming a 1:1 ratio between the two categories of nonmatching pairs; $\chi^2_1 = 7.12$, $P < 0.01$). Data for all species show this tendency (Table 1), although data for the four individual species are not sufficient for statistical evaluation.

The comparison of murine with microtine rodents by double positive biopsy sample pairs and pairs showing different results indicates that there is significantly less correspondence in murine rodents than in microtine rodents (Fisher's exact test; $P = 0.017$).

Our data indicate that estimates of the prevalence of *B. burgdorferi* in Central European rodents can differ depending on the biopsy method used. Urinary bladder biopsy samples proved more sensitive in detecting the pathogen than ear punch biopsy samples. This suggests that significant differences occur in the densities of pathogens in bladder and ear tissue. These are unlikely to be accounted for by the minor differences in the sizes of the biopsy specimens which were taken to conform with current practices.

These results contrast with those from studies in the United States, where several reports show ear biopsy samples to be of almost equal sensitivity to bladder biopsy samples for determining the natural prevalence of *B. burgdorferi* in *P. leucopus* populations (4, 16). The reasons for this difference are not known but may involve either host-dependent factors or differences between genospecies of *B. burgdorferi*.

The use of ear biopsy samples in Central European epidemiological studies can therefore significantly underestimate the prevalence of *B. burgdorferi* in the common rodents which are known to act as natural hosts for this pathogen. This result corroborates data presented by Matuschka et al. (12) for *A. flavicollis* and *C. glareolus*. The other two common rodent species, *A. sylvaticus* and *M. arvalis*, which were also analyzed in the study by Matuschka et al. (12), were not found to harbor *B. burgdorferi* either by the ear punch biopsy sample or by xenodiagnosis.

Although our sample size is relatively small, the significant difference in the number of mismatched pairs between murine and microtine rodents suggests that taxa can play a role in the detectability of infection by different methods of analysis. Such taxonomic dependence could explain the differences in detectability of *B. burgdorferi* in European rodents and *P. leucopus* in America (12, 16) as well as the different distribution of *B. afzelii* in European and Japanese rodents (5, 14).

It has become increasingly clear that the model involving *B. burgdorferi* sensu lato, *Ixodes ricinus*, and rodents in the natural ecological cycle of Lyme disease in Central Europe is too simplistic and that the true complexity of the system is only now beginning to be understood. For this understanding to be developed, the accuracy of the various analytical procedures used to accumulate epidemiological data must be checked for each tissue type, for each host species, and for each genospecies of *B. burgdorferi* in order to prevent biases which may later be hard to detect and remove.

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