

B. burgdorferi in Switzerland¹

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Introduction

Borrelia burgdorferi is a spirochete which was first discovered in the U. S. A. (3, 5). Associated with ticks of the genus *Ixodes* in which it colonizes the intestine and rarely other organs, the spirochete is transmitted to humans and other vertebrates by a tickbite, apparently by regurgitation of the intestinal contents. In Europe its multiplication in man first causes a localized infection of the skin known as *Erythema Chronicum Migrans* (ECM). Then, depending on the patient, the spirochete may spread to numerous organs developing into a potentially crippling and even mortal disease. In the United States, the homologous symptoms were described by Steere et al. (12).

In America, *B. burgdorferi* is essentially transmitted by *Ixodes dammini* and in Switzerland by *Ixodes ricinus* (4, 6).

The preliminary results of our research were presented at the Assembly of the Swiss Society of Zoology in the Spring of 1985 (Aeschlimann et al.). Since then the research has been oriented in the following five directions:

1. To obtain in Switzerland clinical and epidemiological data for as many as possible of the patients contracting "Lyme Disease". We have made a survey by intermediacy of the consulting physicians.
2. To determine for the whole of the Swiss territory the geographical distribution and the percentage of the populations of *I. ricinus* infested with *B. burgdorferi* in relation to the development stages of the tick (larva, nymph, adult) but also in respect to the distribution of clinical cases indicated by the doctors.
3. To determine if altitude influences the rate of infection of ticks.
4. To see if other species of ticks are carriers of *B. burgdorferi* in our country.
5. To search for mammal hosts of *I. ricinus* as potential reservoirs for *B. burgdorferi*.

It should be noted here that certain clinical aspects associated with this spirochetosis in Switzerland have already been or will soon be published (7, 8, 11).

Materials and Methods

The sera sent by the participating doctors were tested by the indirect immunofluorescence technique described by Barbour et al. (3). An American strain of *B. burgdorferi* (L3/B32) as well as a Swiss strain (Sta 14/3a, isolated in our laboratory), were used as antigens.

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The sampling for the establishment of a distribution map of *B. burgdorferi* in Switzerland was carried out at three levels: 1) local, in a forest of the Swiss Plateau (the "Staatswald", 10) 2) regional, around Neuchâtel Lake, and finally 3) national, for the whole of Switzerland. On each of these levels, 20–25 locations were chosen arbitrarily for sampling of 100 *I. ricinus* nymphs per location. The adults of other tick species were also investigated: *Dermacentor reticulatus* in the region of Basle, *D. marginatus* in the Valais and Ticino as well as *Haemaphysalis punctata* in the Valais.

The ticks were collected by flagging (2) and were transported in glass tubes containing air with a high relative humidity. The *Borrelia* are detected by direct immunofluorescence (5) either of smears of adult intestines or of whole smears of immatures.

The search for possible reservoirs was carried out by trapping small mammals in the Staatswald, a forest with a high percentage of infected ticks. *I. ricinus* nymphs and larva from a laboratory colony (control – noncarriers of spirochetes) were fed on the captured animals. After the ticks had molted, they were analysed by IF in order to detect a possible infectious bloodmeal. Blood samples were also taken by cardiac puncture from fresh roe-deer carcasses. The red blood cells were cultured in Kelly's modified media (4) containing antibiotics (neomycin and 5-fluorouracyl). After 4–5 weeks of incubation at 37°C, the cultures were examined on a black field microscope to observe whether spirochetes were present and had multiplied.

Results

During the 15 month period of our survey, 1700 physicians were contacted. More than 800 cases were reported, 350 of which were confirmed clinically and serologically by IF, using *B. burgdorferi* as the antigen. These cases came from all of Switzerland (Fig. 1) and the various clinical manifestations observed are indicated in Table 1.

At first sight, when mapped out, the serologically positive cases furnish a good estimation of the locations contaminated by *B. burgdorferi* (Figs. 1–4). The cases of *Erythema chronicum migrans* (ECM, Fig. 2), as well as those of *Acrodermatitis chronica atrophicans* (ACA, Fig. 3) are regularly distributed on the Swiss Plateau. It is true that the distribution obtained is influenced by that of human populations and also that of the doctors contacted, but it is astonishing to note that it superimposes almost exactly over the distribution of *I. ricinus* in Switzerland (2). It does not appear at the national level that a relationship exists between the different geographic strains of *B. burgdorferi* and the nature of the clinical manifestations observed in the patients.

More epidemiological details are given in Table 2.

ECM may occur in all age groups, but is slightly more frequent in women than in men. This difference is particularly evident for ACA.

Only 32% of the patients remember having been bitten by an arthropod, often unidentified. This may be explained by the very small size of the vector. The nymphs are approximately 2 mm long and the larva are less than one mm.

Close to 38% of the patients frequent the forest. Thus, the distance "home-place of tickbite", determined in 50 cases, averaged 17 km: it was less than 10 km in almost 70% of these 50 cases.

Forty percent have a dog, often a carrier of ticks. At present, in Switzerland, there exists one dog for every 17 inhabitants. Consequently, the dog probably represents risk factor for contracting the *Burgdorfer* spirochete in the case where he pushes his master to frequent the biotopes of *I. ricinus* more often, for example during a walk.

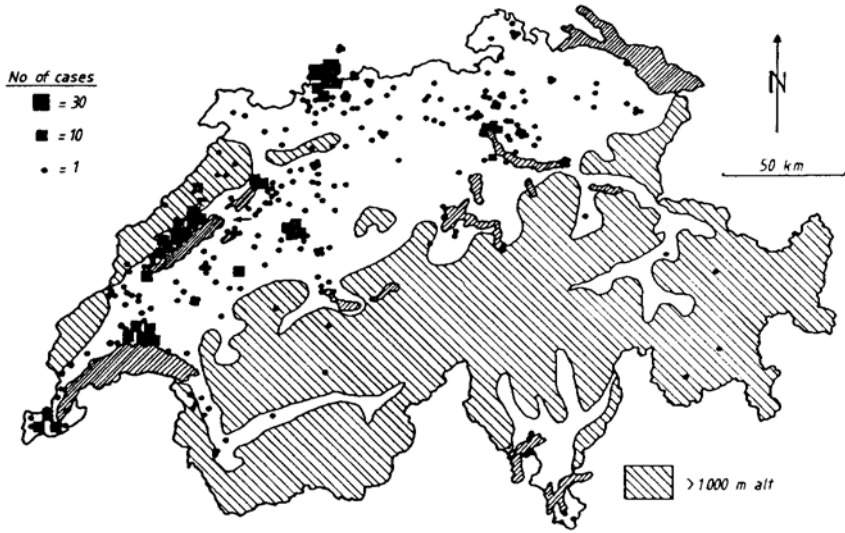


Fig. 1. Distribution of patients from whom sera were tested (→ Staatswald).

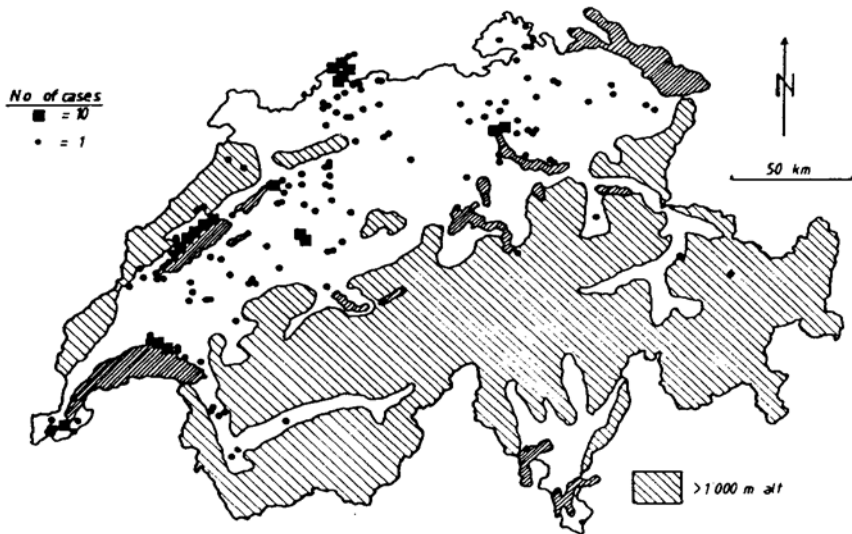


Fig. 2. Distribution of ECM patients serologically positive.

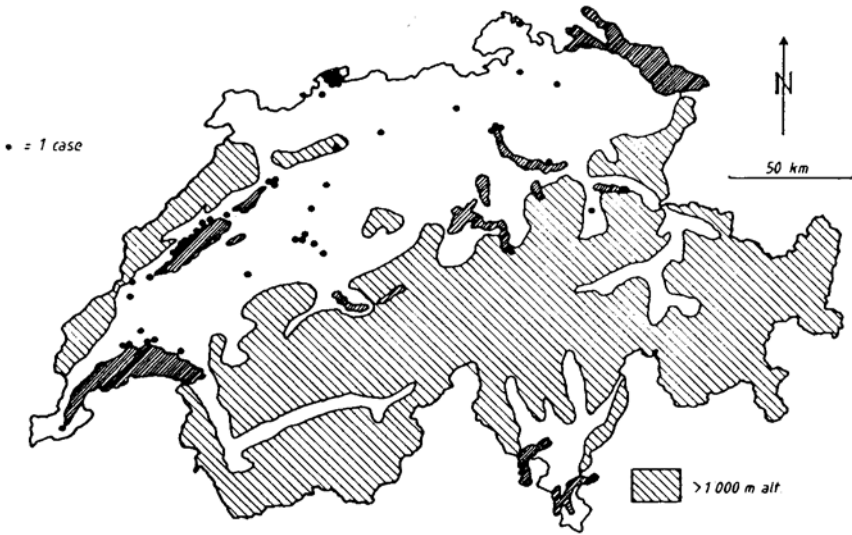


Fig. 3. Distribution of ACA patients serologically positive.

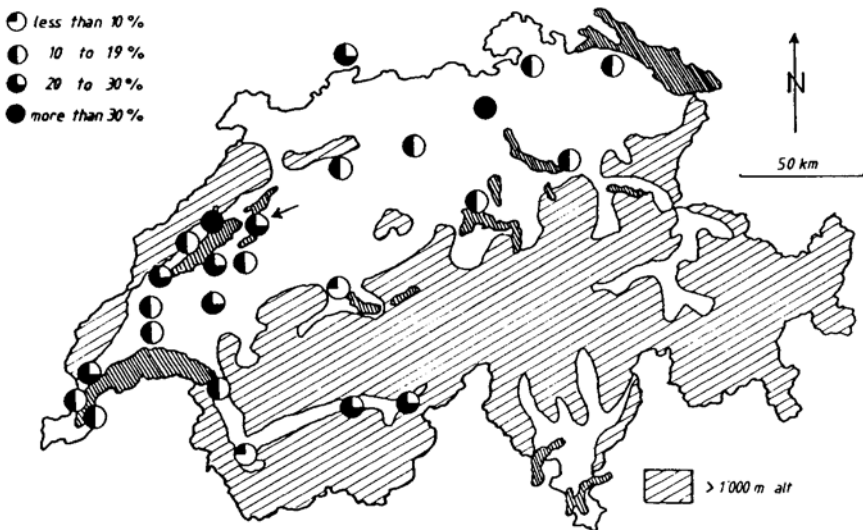


Fig. 4. Percent of *I. ricinus* infected by *B. burgdorferi* in Switzerland, calculated with lots of 100 nymphs (→ Staatswald).

CLINICAL MANIFESTATIONS		
	number	percent
E. C. M	158	45 %
NEUROLOGICAL Manifestations	88	25 %
A. C. A	52	15 %
ARTICULAR Manifestations	107	31 %
CARDIAC Manifestations	15	4 %
LOCALIZED ACHES	61	17 %

Table 1: Frequency of the diverse clinical manifestations on 350 serologically positive cases.

EPIDEMIOLOGY		
	No	
Number of positive cases	350	
Mean age	46 yrs	(4 - 90)
Sex ratio (σ/φ)	0,85	(160/188)
for A. C. A	0,44	(16/36)
Number of persons who remember having been bitten	112	32 %
by ticks	74	21 %
by "arthropods"	38	11 %
Frequent walks in the forest	134	38 %
place of work in the forest	21	6 %
Distance from home to place bitten < 10 km	32/47	68 %
Possess a domestic animal (dog)	90/202	45 %
wich regularly has ticks	49/202	29 %
Other cases in the family or neighbourhood	9/64	14 %

Table 2: Epidemiological data.

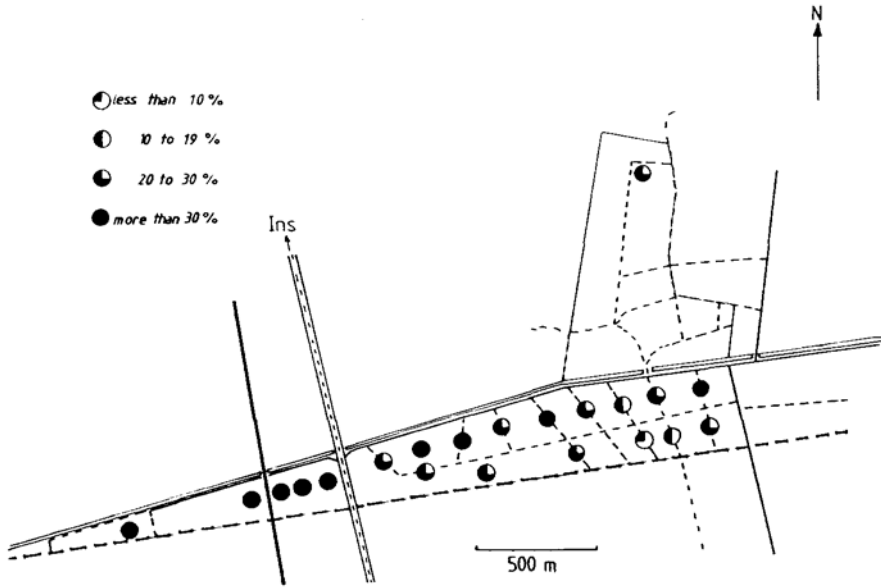


Fig. 5. Percent of infected *I. ricinus* in a forest (the Staatswald) of the Swiss Plateau, calculated with lots of 100 nymphs.

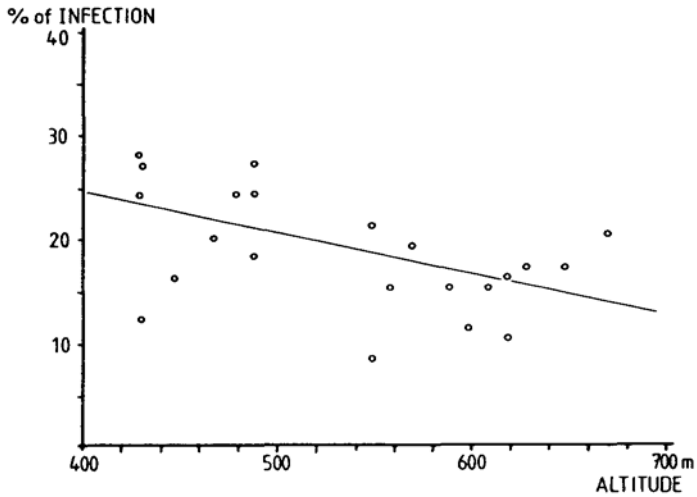


Fig. 6. Rate of infection of 22 lots of 100 *I. ricinus* nymphs in relation to the altitude where collected (regression line; $y = 0.035 \times 38.4$).

I. ricinus is abundant on the Swiss Plateau (mean alt. 600 m), rare between 1000 and 1500 m and absent at altitudes higher than 1500 m².

Figure 4 indicates the infection rates of lots of 100 *I. ricinus* nymphs collected in 25 forests with underbrush in Switzerland. *B. burgdorferi* was observed in each population examined, with an infection rate varying between 5 to 34%. Similar percentages were found in the adult males and females. In contrast, only few young larvae were infected, which proves in any case the existence of transovarian transmission. One can nevertheless, suppose that, in contrast to that which is observed in the case of *B. duttoni* and *Ornithodoros moubata* (1), this mode of transmission is too rare to be the only mode for maintaining *B. burgdorferi* in nature.

While the transovarian transmission appears inefficient, the transtadial transmission is effective at high rates from the larva to the nymph and from the nymph to the adult.

In a forest (Staatswald), isolated in the middle of a cultivated area of the Swiss Plateau (Fig. 5), we have studied the infection rate of populations of *I. ricinus* separated into different small sectors. It is remarkable to note that the rates were not shown to be significantly different from one sector to another; the spirochete infects, in effect, more or less 20% of the nymphs and adults collected. This image does not resemble that of the virus of the Central Europe Tick Encephalitis (9), the distribution of which is more focused in the favorable biotope.

A preliminary study, during which we studied populations of *I. ricinus* nymphs collected at altitudes ranging from 400 to 700 m, permitted us to demonstrate that the infection rate of the vector decreased as the altitude increased (Fig. 6). Although infected ticks were found at altitudes as high as 1250 m (Kiental), one can hypothesize that the altitude, by the intermediacy of climatic conditions, plays a role in the development of *B. burgdorferi* in the vector. The species and the number of "reservoir" hosts available may also influence the infection of ticks. Complementary investigations are needed to verify this hypothesis.

At present, no spirochete has been discovered in *Dermacentor marginatus*, *D. reticulatus* and *Haemaphysalis punctata*, three species living sympatrically with *I. ricinus* on the same hosts. At the moment, we have no evidence that *B. burgdorferi* is associated with Ixodides other than *I. ricinus* in Switzerland while in the USA this spirochete has been found, in addition to *I. danmini*, in *I. pacificus*, *D. variabilis* and *Amblyomma americanum*.

One of the authors (D. Kessler) was recently able to infect healthy ticks on two species of field-mice of the genus *Apodemus* captured in the forest the "Staatswald". This result confirms the existence of wild "reservoir" hosts for *B. burgdorferi* (Table 3). In autumn of 1985, a strain of spirochete (*B. burgdorferi*?) was isolated from a roe-deer (*Capreolus capreolus*), killed in the Canton of Neuchâtel. Moreover, J.-M. Derscheid (Pers. comm. 1981) had observed spirochetes in the blood of 4 *Clethrionomys glareolus* from the Staatswald.

² However, two "high" altitude biotopes where *I. ricinus* completes its life cycle have been recently discovered, one in the Kiental (1250m: K. Pfister) and another in the Valais (1450m: A. Cotty). Some ticks of the Kiental were infected with *B. burgdorferi*.

N ^o	Species	<i>I. ricinus</i> found			<i>I. ricinus</i>		
		on the animal	molted	⊕ as nymphs	placed on host	recovered engorged	⊕ as adults
1	<i>Apodemus flavicollis</i> ♂	5 LL	—	—	10 NN	3	0
2	<i>A. flavicollis</i> ♀	13 LL	—	—	10 NN	2	0
3	<i>A. sylvaticus</i> ♀	27 LL	14	8	15 NN	6	5 [↙]
4	<i>A. flavicollis</i> ♂	43 LL	1	0	15 NN	14	5 [↙]
5	<i>A. flavicollis</i> ♂	9 LL	1	1	10 NN	2	0
6	<i>A. sylvaticus</i> ♀	3 LL	—	—	10 NN	1	0
7	<i>A. flavicollis</i> ♀	10 LL	—	—	10 NN	4	2 [↙]
8	<i>A. flavicollis</i> ♀	26 LL	8	4	10 NN	8	2 [↙]

Table 3: Experimental infections of uninfected nymphs on wild rodents.

Conclusions

1. Contrary to the idea still prevalent in the medical profession, the diseases associated with *B. burgdorferi* are frequent in Switzerland (ECM, ACA, other diseases with neurological, articular and cardiac manifestations etc.). During a 15 months survey (1984/85), 350 cases were indexed in this country.

2. *I. ricinus* is the vector of *B. burgdorferi*. Populations of this tick species are naturally infected with the spirochete at rates between 5 and 34%. Uninfected populations of this *Ixodes* have not yet been found in this country.

3. At present, no tick other than *I. ricinus* has been found to be a carrier of *B. burgdorferi* in Switzerland.

4. It has been possible to demonstrate experimentally that certain wild mammals (*A. sylvaticus* and *A. flavicollis*, perhaps also *C. glareolus* and *C. capreolus*) play a role as reservoirs for *B. burgdorferi*.

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