

## BORRELIA BURGdorFERI IN TICKS OF THE CANTON TESSIN (SWITZERLAND)

V. MISEREZ, L. GERN, A. AESCHLIMANN

*Institute of Zoology, University of Neuchâtel, Chantemerle 22, CH-2000 Neuchâtel, Switzerland.*

*Abstract.* In the northern Alps it is hard to find an *Ixodes ricinus* population which does not harbour *Borrelia burgdorferi*. The infection rates range from 5 to 34% for adults and nymphs to 3.1% for larvae. This study shows that the infection rates on the southern side of the Alps are similar, at 25% for adults, 36.2% for nymphs and 3.2% for larvae. With respect to tick species other than *I. ricinus*, we did not succeed in finding any spirochetes in *Dermacentor marginatus*, *Haemaphysalis punctata*, *Ixodes hexagonus*, *Rhipicephalus sanguineus*.

*Key words:* *Borrelia burgdorferi*, ticks, Switzerland.

### INTRODUCTION

Since the isolation of *Borrelia burgdorferi* (agent of Lyme borreliosis) in Switzerland by Barbour *et al.*, in 1983, from *Ixodes ricinus* ticks, other studies have revealed the presence of this microorganism in ticks originating from different parts of Europe (Doby *et al.*, 1985; Aeschlimann *et al.*, 1986; Wilske *et al.*, 1987).

To date, no Swiss populations of *I. ricinus* which do not harbour *B. burgdorferi* infection have been found. Average infection rates of both nymphs and adults on the Swiss Plateau and in the Jura and Wallis valleys, range from 5 to 34% (Aeschlimann *et al.* (1986). The larvae are much less infected with 3.1% of unfed larvae showing infection (Zhioua *et al.*, 1988).

This is the first time that the ticks in the Canton Tessin have been studied in this way, although the clinical study of Clara *et al.*, (1985) suggested that *B. burgdorferi* was present in this region when they described a human case of Lyme borreliosis in the Muggio Valley (Mendrisiotto). Moreover, a serological study of Tessin inhabitants showed that 9.1% of the people presented significant titers of antibodies against *B. burgdorferi* (Miserez *et al.*, in preparation). Moreover, cases of Lyme borreliosis have been described in Italy since 1983 (Cacciapuotì, 1988) and *B. burgdorferi* was isolated from *I. ricinus* nymphs in 1987 (Cinco *et al.*, 1988).

Our objective was to estimate the situation concerning *B. burgdorferi* infections of ticks in the Tessin.

## METHODS

*Capture of ticks*

Free-living ticks were collected by flagging, a method which has been described by Aeschlimann *et al.* (1972). Six surveys were organised between March and September of 1987. In this way, 19 stations were visited at least once (Fig. 1).

Ticks attached to hosts were collected from goats, sheep, cows, dogs and cats. These animals were surveyed in four areas: in Crotta (station 10)

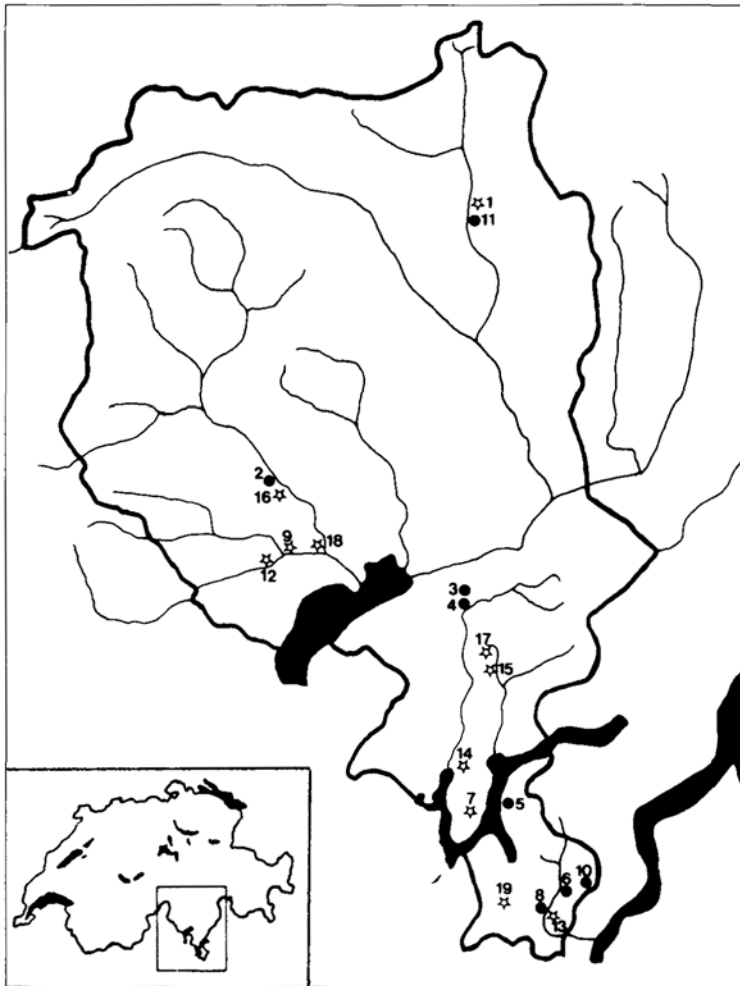


Fig. 1. Geographical distribution of *I. ricinus* in stations of the Canton Tessin. ● Presence of *I. ricinus*; ☆ Absence of *I. ricinus*. See also Table 3.

(50 goats), in Odogno (station 15) (52 goats, 5 sheep and 2 dogs), in Tremona (station 19) (20 cows, 3 sheep, 4 dogs and 2 cats) and in Stinche (station 17) (30 goats and 1 dog). In addition, ticks were also removed from dogs and cats originating in Bironico (stations 3 and 4).

#### *Culture of spirochetes*

To isolate *B. burgdorferi* from *I. ricinus* we used the BSK II medium (Barbour *et al.*, 1984), to which we added 50 µg/ml Fosfocin and 50 µg/ml Rimactan, in order to inhibit the development of other bacteria. Cultures were maintained at 35°C for 10 days and then examined.

#### *Direct immunofluorescence test (DIF)*

We used the DIF test to detect *B. burgdorferi* in ticks. The conjugate was prepared according to the method described by Peacock *et al.* in 1970.

## RESULTS

#### *Capture of ticks*

A total of 398 *I. ricinus* were captured, both free living and fixed to hosts: 88 adults, 94 nymphs and 216 larvae (Table 1).

TABLE 1 - Infection rate of *I. ricinus* in the Tessin.

Stage	Collected ticks	Infected ticks	%
Adults	88	22	25
Nymphs	94	34	36.2
Larvae	216	7	3.2
Total	398	63	

Four females were found on hosts, i.e. one on a goat in Crotta and the other three on dogs in Bironico. The 394 remaining ticks were captured by flagging.

Apart from the above, 21 ticks belonging to other tick species were collected either free living or attached to hosts, i.e. 4 *Dermacentor marginatus* (1 free and 3 on hosts), 1 *Haemaphysalis punctata* (free), 4 *I. hexagonus* (on hosts) and 12 *Rhipicephalus sanguineus* (all on hosts) (Table 2).

The most important capture of *I. ricinus* was executed in June, the majority of the ticks having been collected in 2 stations: Bironico 2 and Bruzella (Table 3).

TABLE 2 - Infection rate of tick species other than *I. ricinus*.

Tick species	Collected ticks	Infected ticks
<i>D. marginatus</i>	4	None
<i>H. punctata</i>	1	None
<i>I. hexagonus</i>	4	None
<i>Rb. sanguineus</i>	12	None

#### Isolation of *B. burgdorferi*

To isolate *B. burgdorferi* from *I. ricinus*, 49 adult ticks collected in June and 7 in September were dissected. The spirochetes were observed in 5 cultures. In one of the cultures the spirochetes were not motile. Only one strain could be maintained in the laboratory (NE131). No spirochetes could be isolated from tick species other than *I. ricinus*.

#### DISCUSSION

In 1987, 396 free living ticks (394 *I. ricinus*, 1 *D. marginatus*, 1 *H. punctata*) and 23 ticks attached to hosts (4 *I. ricinus*, 3 *D. marginatus*, 4 *I. hexagonus*, 12 *R. sanguineus*) were captured in the Tessin between April and September. If we compare these results with those obtained by Aeschlimann *et al.* in 1967 and 1980 (Aeschlimann *et al.*, 1968; 1986), two features were observed.

- 1) Very few ticks were collected from hosts in 1987, whereas Aeschlimann *et al.* (1968; 1986) found the majority of their ticks on animals, both in 1967 and in 1980.
- 2) In 1987, no flock was infested (except one goat on which we found only one *I. ricinus*), whereas 2 years previously, flocks were systematically covered with ticks according to the goatherds.

Otherwise, the number of free-living ticks, captured by flagging, was not very important and 12/19 of stations visited were free of ticks altogether, even though they had been chosen according to the required conditions for *I. ricinus*.

The weather during 1987 certainly played a role in this situation, creating different conditions, being less favorable than usual. As a matter of fact, the first 3 months of 1987 proved to be rather cold and dry, whereas April was an especially mild month. May, June and July were colder and wetter than average. The temperature rose only during the last 3 months of the year (Swiss Meteorological Institute, Annual Tables).

Other species of ticks were also more rare. *D. marginatus* was found to be more abundant in the surveys carried out in 1967, 1980 and 1985 (Aeschlimann *et al.*, 1968; 1986; Walther-Maridor, 1986). *H. punctata* was not rare in 1967, although less abundant in 1980 and 1987. *R. sanguineus* was

TABLE 3 - Seasonal and geographical distribution of *I. ricinus* in stations of the Canton Tessin.

Station number and name	Altitude (m)	Coordinates	March	April	May	June	August	September	Total
1. Acquarossa	580	715,5/146,4	—	—	—	—	0	—	0
2. Aurigeno di fuori	305	698,9/120,8	0	1	1	—	0	—	2
3. Bironico 1	500	716,0/108,0	0	—	2	—	—	—	2
4. Bironico 2	590	716,5/107,8	—	—	45	45	4	9	103
5. Bissone	320	718,5/ 90,1	—	—	—	1	—	—	1
6. Bruzella	650	724,3/ 82,6	—	0	—	166	37	83	286
7. Carona	620	716,0/ 90,7	—	—	—	0	—	—	0
8. Castel S. Pietro	400	722,5/ 80,1	—	—	—	1	—	—	1
9. Cavigliano	300	698,5/115,4	—	—	0	—	—	—	0
10. Crotta	650	726,6/ 84,5	—	0	1	—	—	—	1
11. Dongio	500	716,1/144,6	—	—	—	2	0	—	2
12. Intraga	300	697,7/115,0	—	—	0	—	—	—	0
13. Morbio-Superiore	570	723,4/ 80,3	—	0	—	—	—	—	0
14. Muzzano	340	715,1/ 95,1	—	—	—	—	—	—	0
15. Odogno	780	718,0/104,8	—	0	0	—	—	0	0
16. Ronchi	340	699,3/120,0	0	—	—	—	—	—	0
17. Stinché	1080	717,1/106,0	—	—	0	—	—	0	0
18. Tegna	340	701,6/116,2	0	0	0	—	0	—	0
19. Tremona	600	718,0/ 82,0	—	—	—	—	0	—	0
Total			0	1	49	215	41	92	398

first recorded in the Tessin in 1980. In 1987, we found 12 of them attached to 4 different dogs. This tick species originates on the Mediterranean coast and was imported into Switzerland by dogs being brought home from abroad. This tick can survive in apartments and kennels but probably also outside during the summer months. Four *I. hexagonus* were collected from dogs and cats in 1987. The first mention of this tick in the Tessin was in 1987. All these tick species were free of *B. burgdorferi*.

#### *B. burgdorferi* tick infection

Epidemiological studies of ixodid ticks were conducted in different parts of Switzerland. Infections of *I. ricinus* occur at a rate of between 5% and 34% for nymphs and adults depending on the region (Aeschlimann *et al.*, 1986). Walther-Maridor (1986) mentioned an infection rate of nymphs of about 18% in the Wallis. In Staatswald (Bern), 25% of the adults and 28% of the nymphs were infected (Jeanneret, 1985). Infection rates of larvae are generally very low. Transovarial transmission of *B. burgdorferi* by *I. ricinus* seems to be rare (Monin *et al.*, 1989). Zhioua *et al.* (1988) examined several groups of *I. ricinus* larvae from different regions of Switzerland and found an average infection rate of 3.1%. This percentage can be considered as an approximation for all regions. It must be remembered that, to date, no population of *I. ricinus* has been found free of the *B. burgdorferi* infection.

With respect to tick species other than *I. ricinus*, we did not succeed in finding any spirochetes in them, as was already the case of an earlier survey carried out by Walther-Maridor (1986).

The infection rates of *I. ricinus* in the Tessin are 25% for adults, 36.2% for nymphs and 3.2% for larvae. These infection rates are very similar to those observed in the regions situated on the north side of the Alps (Aeschlimann *et al.*, 1986). We can conclude that *B. burgdorferi* infection in ticks is widespread in Switzerland, from east to west and from north to south, i.e. also in the Canton of Tessin, wherever *I. ricinus* is present.

#### ACKNOWLEDGEMENTS

This work was supported by the Swiss National Research Foundation (3.975.87). We thank Dr. Manetti for his collaboration.

#### REFERENCES

- AESCHLIMANN A. (1972). *Ixodes ricinus*, Linné 1758 (Ixodoidea, Ixodidae), essai préliminaire de synthèse sur la biologie de cette espèce en Suisse. *Acta Trop.* 29: 321-340.
- AESCHLIMANN A., CHAMOT E., GIGON F., JEANNERET J. P., KESSELER D., WALTHER C. (1986). *Borrelia burgdorferi* in Switzerland. *Zentbl. Bakt. Hyg. A* 263: 450-458.
- AESCHLIMANN A., DIEHL P. A., EICHENBERGER G., IMMLER R., WEISS N. (1968). Les tiques (Ixodoidea) des animaux domestiques du Tessin. *Rev. Suisse Zool.* 75: 1039-1050.
- AESCHLIMANN A., SCHNEEBERGER S., PFISTER K., BURGENDORFER W., COTTY A. (1986). Données nouvelles sur les tiques Ixodides du canton du Tessin (Suisse) et sur la présence d'agents rickettsiens dans leur hémolymphe. *Annuaire Soc. Helv. Sc. Nat.* 1: 58-68.

- BARBOUR A. G. (1984). Isolation and cultivation of Lyme disease spirochetes. *Yale J. Biol. Med.* 57: 521-525.
- BARBOUR A. G., BURGDORFER W., HAYES S., PETER O., AESCHLIMANN A. (1983). Isolation of a cultivable spirochete from *Ixodes ricinus* ticks from Switzerland. *Curr. Microbiol.* 8: 123-126.
- CACCIAPUOTI B. (1988). Attuali conoscenze epidemiologiche della malattia di Lyme in Italia. Convegno Internazionale su *Malattie infettive dell'arco alpino*, Bolzano.
- CINCO M., BANFI E., TRAVISAN G. (1988). Primo isolamento in Italia di *Borrelia burgdorferi* della zecca comune, *Ixodes ricinus*, raccolta nel Carso triestino. Convegno Internazionale su *Malattie infettive dell'arco alpino*, Bolzano.
- CLARA F., RIVA A. (1985). A proposito di un caso di malattia di Lyme in Ticino: diagnosi, diagnosi differenziale, epidemiologia, batteriologia, clinica, sierologia e trattamento. *Tribuna medica Ticinese* 50: 432-436.
- DOBY J. M., ANDERSON J. F., COUATERMANACH A. (1985). Observation de spirochètes chez *Ixodes ricinus* en Bretagne. Note préliminaire. *Méd. Mal. Infect.* 10: 556-557.
- JEANNERET J. P. (1985). *Borrelia burgdorferi*, agent étiologique de la maladie de Lyme: infection naturelle d'*Ixodes ricinus* sur le Plateau suisse et transmission expérimentale sur des lapins. Travail de licence. Université de Neuchâtel. Faculté des Sciences. Institut de Zoologie.
- MISEREZ V., GERN L., AESCHLIMANN A. (In press.). Etude sérologique d'une population tessinoise.
- MONIN R., GERN L., AESCHLIMANN A. (1989). A study of the different modes of transmission of *Borrelia burgdorferi* by *Ixodes ricinus*. Lyme Borreliosis II. *Zbl. Bakt. Suppl.* 18. Gustav Fischer Verlag. Stuttgart, New York: pp. 14-20.
- PEACOCK M., BURGDORFER W., ORMSBEE R. A. (1971). Rapid fluorescent-antibody conjugation procedure. *Amer. Soc. Microbiol.* 3: 355-357.
- WALTHER-MARIDOR C. (1986). *Recherche du spirochète Borrelia burgdorferi chez les tiques Ixodes ricinus, Dermacentor marginatus et Haemaphysalis punctata en Suisse*. Travail de certificat. Université de Neuchâtel. Faculté des Sciences. Institut de Zoologie.
- WILSKE B., STEINHUBER R., BERGMEISTER H., FINGERLE V., SCHIERZ G., PREAC-MURSIC V., VANEK E., LORBEER B. (1987). Lyme Borreliose in Süddeutschland. *Deutsche Med. Wschr.* 112: 1730-1736.
- ZHILOUA E., MONIN R., GERN L., AESCHLIMANN A. (1988). Transmission transovarienne de *Borrelia burgdorferi*, agent étiologique de la maladie de Lyme, chez *Ixodes ricinus*. (Abstract). *Zentbl. Bakt.* 306: 293.