



Borrelia miyamotoi and other tick-borne pathogens in a natural area recently colonized by ticks in the Western Alps, Italy



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Introduction

In Europe, a geographical expansion of Ixodid ticks is being observed in mountain areas.

In the Susa valley, Piedmont region, western Italian Alps, ticks were very rarely found in the past, not even on wild ungulates (Rossi and Meneguz, 1989). Now tick bites are increasingly reported by people visiting the area and three cases of Lyme disease were officially reported in a naturalist guide and two forestry workers during the spring-summer 2016.

We then started a tick collection, in order to explore the diversity, abundance and distribution of ticks in the different areas of the park and determine the prevalence of tick-borne pathogens (TBP).

Results

All ticks collected in 2016 were identified as *I. ricinus* (1562 larvae, 918 nymphs and 32 adults).

Ticks were not encountered above 1750m a.s.l.. The most infested sites were woods below 1400 m altitude (Table 1).

A sample of 365 nymphs was tested for TBP. We found *B. burgdorferi* s.l. in 9.0% of the nymphs; the identified genospecies were *B. afzelii* and *B. garinii*. We also detected Spotted Fever Group rickettsiae in 11.0% of the sample, namely *R. helvetica* and *R. monacensis*. *A. phagocytophilum* infected 1.9% of the nymphs, and we could identify three samples as ecotype I (Jahfari et al., 2014). Details of infection by altitude are shown in Table 1. Finally, *B. miyamotoi* was detected in two nymphs (0.5%).



Fig.1. Natural parks belonging to the Cozie Alps protected area, Susa valley, Turin province, Piedmont region, Italy (<http://www.parchialpicozie.it/>). In green, the study area (natural park 'Gran Bosco di Salbertrand'), with, on the right, its location in Italy.

Altitudinal belt (m a.s.l.)	% of sites infested by <i>I.r.</i> nymphs (No. of study sites)	Mean no. of <i>I.r.</i> nymphs / 100m dragging in infested sites	No. <i>I.r.</i> nymphs tested by PCR (total no. of collected nymphs)	% infection <i>B.burgdorferi</i> s.l. in <i>I.r.</i> nymphs (no. positives)	% infection <i>Rickettsia</i> spp. in <i>I.r.</i> nymphs (no. positives)	% infection <i>A.phagocytophilum</i> in <i>I.r.</i> nymphs (no. positives)
950-1200	83% (18)	6.9	135 (329)	12.6 (17)	11.8 (16)	1.5 (2)
1201-1400	100% (8)	8.3	130 (342)	10.8 (14)	10.0 (13)	2.3 (3)
1401-1600	85.7% (7)	4.6	92 (131)	2.2 (2)	11.9 (11)	2.2 (2)
1601-1800	42.9% (7)	1.6	8 (8)	0	0	0
>1800	0% (5)	-	-	-	-	-

Table 1. Nymphs of *I. ricinus* (*I.r.*) collected in the dragging sites and their infection by TBP, by altitude. High Susa valley, May-September 2016.

Materials and methods

Tick collection was carried out in the regional park 'Gran Bosco di Salbertrand' (Fig.1). Wild ungulates, that were re-introduced in the '60s, are very abundant in the area, in particular red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), chamois (*Rupicapra rupicapra*) and wild boars (*Sus scrofa*).

The 45 sites were located at 950-1880 m above sea level (a.s.l.) and were characterized by different vegetation (Fig. 2). Ticks were collected from May to October, 2016, every two weeks. Tick collection is continuing in 2017.

A sample of *Ixodes ricinus* nymphs was analyzed by PCR to evaluate the infection prevalence by the following TBP: *Borrelia miyamotoi* (flagellin gene), *Borrelia burgdorferi* s.l. (*OspA*, flagellin and IGS), *Rickettsia* spp. (*gltA* and *OmpA* genes), *Anaplasma phagocytophilum* (*msp2* gene), *Neorhlichia mikurensis* (*groEL* gene).



Fig.2. Examples of dragging sites in the high Susa valley, Western Italian Alps: a. leisure area, Scots pines (*Pinus sylvestris*), 1074 m; b. humid area with common reeds (*Phragmites australis*), 1078 m; c. footpath with ashes (*Fraxinus excelsior*), 1160m; d. European larch (*Larix decidua*) wood, 1324 m; e. Norway spruce (*Picea abies*) wood, 1697 m; f. silver fir (*Abies alba*) and arolla pine (*Pinus cembra*) wood, 1880 m.



Fig. 3. A *D. marginatus* male questing in the park area (photo: S. Molino).

Discussion

Our data confirm the presence of *I. ricinus* at up to 1750 m a.s.l. in the Italian side of the Western Alps. Several TBP are also present, posing a risk to the tourists and workers in the regional park. The finding of *B. miyamotoi*, which was recently recognized to cause disease in humans, is the first report in Italy, and deserves further studies to evaluate its distribution and prevalence. Investigations are ongoing, to monitor the possible altitudinal increase of ticks, to identify high-risk areas for tick bites and transmission of TBP. Preliminary results of the 2017 tick collection highlighted the presence of rare *I. ricinus* nymphs up to the highest dragging sites. Moreover, *Dermacentor marginatus* (Fig. 3) ticks have been collected, and we identified *Rickettsia slovaca*, the agent of tick-borne lymphadenopathy, in some specimens.

Susa valley was almost 'tick free' a decade ago. Together with the park personnel, we are carrying out educational activities to raise awareness on this new health threat and inform on the importance of preventing tick bites.