

Lyme Borreliosis Spirochetes and Spotted Fever Group Rickettsiae in Ixodid Ticks from Pianosa Island, Tuscany Archipelago, Italy

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Abstract

A study on tick fauna and tick-borne pathogens was undertaken in Pianosa, an island in the Tuscany Archipelago that constitutes an important stopping and nesting point for migratory birds. Ticks were removed from feral cats and a few terrestrial birds, and host-seeking ticks were collected by dragging. A total of 89 ticks were found on animals: 57 *Ixodes ventalloi* Gil Collado, 1936 and 32 *Ixodes acuminatus* Neumann, 1901. Host-seeking ticks were 354 *Hyalomma* spp. larvae and 18 *Hyalomma* spp. adults, identified as *Hyalomma marginatum* C.L. Koch, 1844 ($n=11$) and 7 *Hyalomma detritum* Schulze, 1919 ($n=7$). A sample of adult ticks was subjected to molecular analyses to look for *Rickettsia* spp. and *Borrelia burgdorferi* sensu lato (s.l.). Sequence analysis of the 5S–23S intergenic spacer region and *OspA* gene of *B. burgdorferi* s.l.–positive samples showed the presence of *Borrelia spielmanii* ($n=3$; 3.7%, 95% confidence interval [CI] 0.08–10.4) and *Borrelia valaisiana* ($n=13$; 13.6%, 95% CI 7.0–23.0) in *Ixodes* ticks from cats and terrestrial birds. *Ixodes* spp. were also infected by *Rickettsia helvetica* ($n=19$; 23.4%, 95% CI 14.7–34.2). Finally, we detected *Rickettsia aeschlimannii* in 3 out of 12 host-seeking *Hyalomma* spp. adults tested (25%, 95% CI 5.5–57.2). Our study shows the presence of several tick-borne pathogens in Pianosa. *Hyalomma* spp. and *Ixodes* ticks other than *I. ricinus* seem to be involved in their epidemiological cycle, and birds could contribute to the pathogen dispersal along their migration routes. This is the first finding of *B. spielmanii* in Italy. We hypothesize the involvement of peridomestic rodents or hedgehogs in its maintenance in Pianosa.

Key Words: Pianosa island—*Ixodes* spp.—*Hyalomma* spp.—*Borrelia spielmanii*—*Rickettsia aeschlimannii*.

Introduction

RICKETTSIAE OF THE SPOTTED FEVER GROUP (SFG) and spirochetes in the *Borrelia burgdorferi* sensu lato (s.l.) complex are emerging threats in Europe. Along with the rickettsiae and borreliae that have been recognized as pathogenic for decades, an increasing number of species are being discovered and identified as human pathogens (Labruna et al. 2011, Stanek and Reiter 2011). For example, *Borrelia spielmanii* was isolated in the first decade of the 21st century in Lyme disease patients with erythema migrans (Fingerle et al. 2008). Among SFG rickettsiae, *Rickettsia aeschlimannii* was first characterized in Africa in 1997, but is now considered the causative agent of rickettsiosis in many areas inhabited by its tick vector *Hyalomma* spp. (Matsumoto et al. 2004).

Migratory birds could be partly responsible of the heterogeneous distribution of tick-borne pathogens in Europe, because they are considered responsible for the dissemination of ticks in their migration routes and are recognized reservoir hosts of some agents (Kjelland et al. 2010).

The island of Pianosa is an important stopping and nesting site for avian species because of its history, which has made it unspoiled and scarcely inhabited for many years. Within a project aiming at capturing feral cats, which constituted a threat for migrating birds, ticks were collected from the felines and from terrestrial birds. Moreover, host-seeking ticks were collected by dragging. Tick identification and molecular analyses were then undertaken to describe the tick fauna of Pianosa and the presence of tick-borne pathogens.

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Materials and Methods

Study area

The island of Pianosa (N 42° 34' 53", E 10° 4' 34") is part of Tuscany Archipelago in the Tyrrhenian Sea, Tuscany region, Italy. Pianosa has an area of 10.2 km², is plain, and its highest point reaches about 30 meters above sea level. From 1858, it was the seat of an agricultural penal colony. For this reason, and thanks to the establishment of a National Park (Parco Nazionale dell'Arcipelago Toscano) in 1996–1997, Pianosa remained unspoiled by human presence and activities for many years. Since the closure of the prison in 1997, the island is almost uninhabited, and only daily guided tours are possible for tourists during the summer season.

The vegetation is characterized by typical Mediterranean species, such as junipers (*Juniperus communis*, *J. oxycedrus* and *J. phoenicea*), mastik trees (*Pistacia lentiscus*), rosemaries (*Rosmarinus officinalis*), cists (*Cistus incanus*, *C. monspeliensis*), myrtle (*Myrtus communis*), helichrysum (*Helichrysum litor-eum*), and lavender (*Lavandula stoechas*) (Colom et al. 2004). The fauna are represented by small mammals, such as rodents, hedgehogs (*Erinaceus europaeus*), Kuhl's pipistrelles (*Pipistrellus kuhlii*), and wild hares (*Lepus europaeus*). The black rat (*Rattus rattus*), Norway rat (*Rattus norvegicus*), and house mouse (*Mus musculus domesticus*) were accidentally introduced to the island, and wild hares were introduced for hunting (Agnelli 1998, Angelici et al. 2009). Cats (*Felis catus*) live as wild predators; they were abandoned by humans after the closure of the penal colony and represent a threat for land and marine birds (seagulls and shearwaters in particular). The avifauna, both local and migratory, are abundant in Pianosa. Among others, colonies of Audouin's gull (*Ichthyæetus audouinii*), Cory's shearwater (*Calonectris diomedea*), Mediterranean shearwater (*Puffinus yelkouan*), and European shag (*Phalacrocorax aristotelis desmarestii*) are present. Moreover, a large population of pheasants (*Phasianus colchicus*) and red partridges (*Alectoris rufa*) inhabits the island, and several marine and terrestrial species spend their winter there.

Tick collection

Ticks were collected on vertebrate hosts and by dragging the vegetation, within the framework of the LIFE Natura Project LIFE04NAT/IT/000172, co-financed by European Union and region of Tuscany (2004–2007). One of the project aims was eliminating wild cats from the island by sterilizing and releasing them on the Elba Island.

Ticks were collected from trapped cats and from 3 terrestrial birds found dead/dying on the island footpaths—1 common pheasant (*Phasianus colchicus*), 1 red partridge (*Alectoris rufa*), and 1 water rail (*Rallus aquaticus*). Dragging was performed on the same transects used to trap wild cats, which were randomly chosen after dividing the island in 6 areas and considering existing footpaths. Four 1-km length transects per area were selected, and dragging was carried out by 2 operators, using a 1-square meter cotton cloth. The operators stopped at each 10 meters approximately to check the cloth for tick presence. Tick collection was performed in October, 2006 (on-the-host and host-seeking ticks collection), and August, 2007 (dragging only); sampling periods were chosen based on convenience (logistic support on the island by the personnel of LIFE Natura project). A total of 7 dragging

transects were covered in October, 2006, and 24 in August, 2007. Arthropods were stored in 70% ethanol and subsequently identified using keys by Manilla (1998), Estrada-Peña et al. (2004), and Cringoli et al. (2005).

Molecular analyses

Tick DNA was extracted by using the QIAGEN DNeasy tissue kit (Qiagen GmbH, Hilden, Germany). The infection by *B. burgdorferi* s.l. was investigated by a PCR protocol targeting a 337-bp fragment of the intergenic spacer (IGS) region included between genes coding for the 5S and 23S subunits of ribosomal RNA (Rijpkema et al. 1995). For better characterization, positive samples were submitted to the amplification of a 800-bp fragment of the outer surface protein A (*OspA*) gene. In detail, 5 µL of DNA extract were put in 25-µL reactions containing 10X CoralLoad PCRBuffer PCR (Qiagen), 0.03 mM concentrations of the forward primer *OspAf* (Grego et al. 2007; 5'-ATGAAAAAATATTTATGGGAATA-3') and of the reverse primer *OspAr_gen* (5'-ATTCTCCYTATTTYAAAGCG-3'), 200 µM of deoxyribonucleotide triphosphates (dNTPs), and 1 U/reaction of *Taq* DNA polymerase (Qiagen). Cycling conditions included denaturation at 94°C for 3 min; 40 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min; and a final extension at 72°C for 10 min. DNA from *Borrelia afzelii* (Nancy strain) and *B. garinii* (BL3 strain) was used as positive controls in each PCR run. The *OspA* reverse primer was designed based on the alignment of *B. burgdorferi* s.l. reference sequences available on GenBank and the sequences database obtained from previous studies.

The presence of rickettsial DNA in ticks was investigated as described in Tomassone et al. (2010): a first PCR assay targeted the citrate synthase gene (*gltA*); positive samples were further tested to detect the *ompA* gene, specific for the SFG rickettsiae. In each PCR run, DNA from *Rickettsia conorii* was used as a positive control.

RNase- and DNase-free water and nucleic acid extracts from sure negative samples were used as negative controls to determine any possible cross-reactivity or contamination; 1 negative control every 5 field samples was included in each PCR run.

Amplified products were analyzed by electrophoresis in 2.5% agarose and visualized by staining with 0.1% ethidium bromide. Amplicons were purified using ExoSAP-IT PCR Clean-up Kit (GE Healthcare Limited, Chalfont, UK) and sent to an external service (MacroGen Inc., Amsterdam, The Netherlands) for automatic sequencing. Sequences were analyzed by Chromas 2.0 software (Technelysium, Helensvale, Australia) and submitted to BLAST (<http://blast.ncbi.nlm.nih.gov/>) to identify similarities to known sequences and, therefore, to classify pathogens at the species level.

Prevalence of tick species and PCR-positive results were calculated, with 95% exact binomial confidence intervals (95% CI), by using R software (R Development Core Team, 2008).

Phylogenetic analysis

Sequences obtained from positive samples were aligned with reference sequences available in GenBank using ClustalW (Thompson et al. 1997) and were imported into PAUP* (ver. 4.0b10; Swofford, 2003). The model of molecular evolution was estimated using a hierarchical likelihood ratio test approach and the Akaike information criterion (Akaike 1973) implemented in the computer program ModelTest ver. 3.7

(Posada and Crandall 2001). The phylogenetic trees and the statistical support for clades were created using the Bayesian methods implemented in MrBayes (ver. 3.1.1; Ronquist and Huelsenbeck 2003). A Markov chain Monte Carlo search for 1,000,000 generations using 2 runs with 4 chains (temperature=0.05) was performed, and results were represented as a 50% majority rule consensus tree. The pairwise genetic distance comparisons were calculated by the Nei-Gojobori method (Nei and Gojobori 1986).

Results

A total of 426 ticks were collected during fieldwork. On-the-host ticks were all adults ($n=89$): 57 *Ixodes ventralloii* Gil Collado, 1936, and 32 *Ixodes acuminatus* Neumann, 1901. Host-seeking ticks were 354 *Hyalomma* spp. larvae, which were identified at the genus level, and 18 *Hyalomma* spp. adults, namely *Hyalomma marginatum* C.L. Koch, 1844 ($n=11$) and *Hyalomma detritum* Schulze, 1919 ($n=7$) (Table 1). Eleven *Hyalomma* larvae were collected in October, 2006 (0.16 specimens per 100-meter dragging), while in August, 2007, we gathered 343 larvae (1.43 specimens/100 meters) and the 18 adults (0.075/100 meters).

The PCR for *B. burgdorferi* s.l. and *Rickettsia* spp. detection was performed on 81 *Ixodes* spp. ticks. The *Rickettsia* spp. PCR assay was also carried out on 12 host-seeking *Hyalomma* spp. adults. The remaining *Ixodes* and *Hyalomma* specimens were kept in the laboratory as reference specimens.

Overall, 16 *Ixodes* spp. were positive to *B. burgdorferi* s.l. 5S-23S IGS PCR (19.7%; 95% CI 11.7–30.1): 12 *I. ventralloii*, and 4 *I. acuminatus*. The sequences clustered into two clades defined by reference strains of *B. spielmanii* and *Borrelia valaisiana* (Fig. 1). The pairwise genetic distances varied from 0 to 0.034 among the *B. spielmanii* sequences and from 0 to 0.027 among the *B. valaisiana* sequences. Positive ticks were taken from 3 different cats and from the red partridge (*B. spielmanii*), water rail, and pheasant (*B. valaisiana*). Results are detailed in Table 1. The borrelia *OspA* gene could be amplified only from 9 *Ixodes* spp. positive to 5s–23s IGS. The dendrogram based on the *ospA* gene (Fig. 2) showed that the *B. valaisiana* sequences belong to the subgroup I strain (Wang et al. 2000; Ryffel et al. 2003); the pairwise genetic distances varied from 0 to 0.023. Only one *ospA* *B. spielmanii* sequence was obtained.

The *Rickettsia gltA* gene was detected in 20 *Ixodes* spp. (35.8%; 95% CI 25.5–47.2) collected from cats and terrestrial birds. Nine *I. ventralloii* and 10 *I. acuminatus* were found infected by *Rickettsia helvetica*, whereas *Rickettsia* sp. IRS3 was detected in 1 *I. ventralloii* from a cat. Positive ticks were collected from 8 different cats. Co-infection by *B. valaisiana* and *R. helvetica* was detected in 1 *I. acuminatus* from a cat and 1 *I. ventralloii* from the pheasant, whereas *B. spielmanii* was detected together with *R. helvetica* in 1 *I. acuminatus* collected on the red partridge. Finally, 3 out of the 12 adult host-seeking *Hyalomma* examined were positive to both *gltA* and *OmpA* rickettsial genes (25%, 95% CI 5.5–57.2), and *Rickettsia aeschlimannii* was identified.

Discussion

A variety of tick-borne zoonotic agents were detected in our study in Pianosa. *B. spielmanii* is reported for the first time in Italy. This spirochete is characterized by a focal distribution in Europe (Fingerle et al. 2008) and is considered to be more host specific than other *B. burgdorferi* s.l. genospecies, being

TABLE 1. NUMBER OF ON-THE-HOST AND OFF-HOST ADULT TICKS COLLECTED IN PIANOSA (OCTOBER 2006 AND AUGUST 2007), BY SOURCE (ANIMAL HOST/VEGETATION) AND TICK SPECIES, WITH RESULTS OF THE PCR TARGETING *BORRELIA BURGDORFERI* S.L. AND SFG *RICKETTSIAE*

Tick source	Tick species	No. tested	No. positive to <i>B. burgdorferi</i> s.l. (prevalence, 95%CI) and genospecies (n)	No. positive to SFG <i>Rickettsiae</i> (prevalence, 95%CI) and species (n)
Cat (<i>Felis catus</i> ; n = 11)	<i>Ixodes ventralloii</i> (n = 42)	38	2 (5.3, 0.6–17.7), <i>B. spielmanii</i> (1) and <i>B. valaisiana</i> (1)	7 (18.4, 7.7–34.3), <i>R. helvetica</i> (6) and <i>Rickettsia</i> sp. IRS3 (1)
Common pheasant (<i>Phasianus colchicus</i> ; n = 1)	<i>Ixodes acuminatus</i> (n = 28)	24	3 (12.5, 2.6–32.4), <i>B. spielmanii</i> (1) and <i>B. valaisiana</i> (2)	8 (33.3, 15.6–55.3), <i>R. helvetica</i>
	<i>Ixodes ventralloii</i> (n = 11)	11	9 (81.8, 48.2–97.7), <i>B. valaisiana</i>	2 (18.2, 2.3–51.8), <i>R. helvetica</i>
Red partridge (<i>Alectoris rufes</i> ; n = 1)	<i>Ixodes acuminatus</i> (n = 2)	2	0	0
	<i>Ixodes ventralloii</i> (n = 1)	1	0	0
Water rail (<i>Rallus aquaticus</i> ; n = 1)	<i>Ixodes acuminatus</i> (n = 2)	2	1 (50.0, 1.25–98.7), <i>B. spielmanii</i>	2 (100, 15.8–100), <i>R. helvetica</i>
	<i>Ixodes ventralloii</i> (n = 3)	3	1 (33.3, 0.8–9.1), <i>B. valaisiana</i>	1 (33.3, 0.8–9.1), <i>R. helvetica</i>
Vegetation	<i>Hyalomma marginatum</i> (n = 11)	8	na	2 (25, 3.2–65.1), <i>R. aeschlimannii</i>
	<i>Hyalomma detritum</i> (n = 7)	4	na	1 (25.0, 0.6–8.1), <i>R. aeschlimannii</i>

CI, Confidence interval; SFG, spotted fever group; na, not applied.

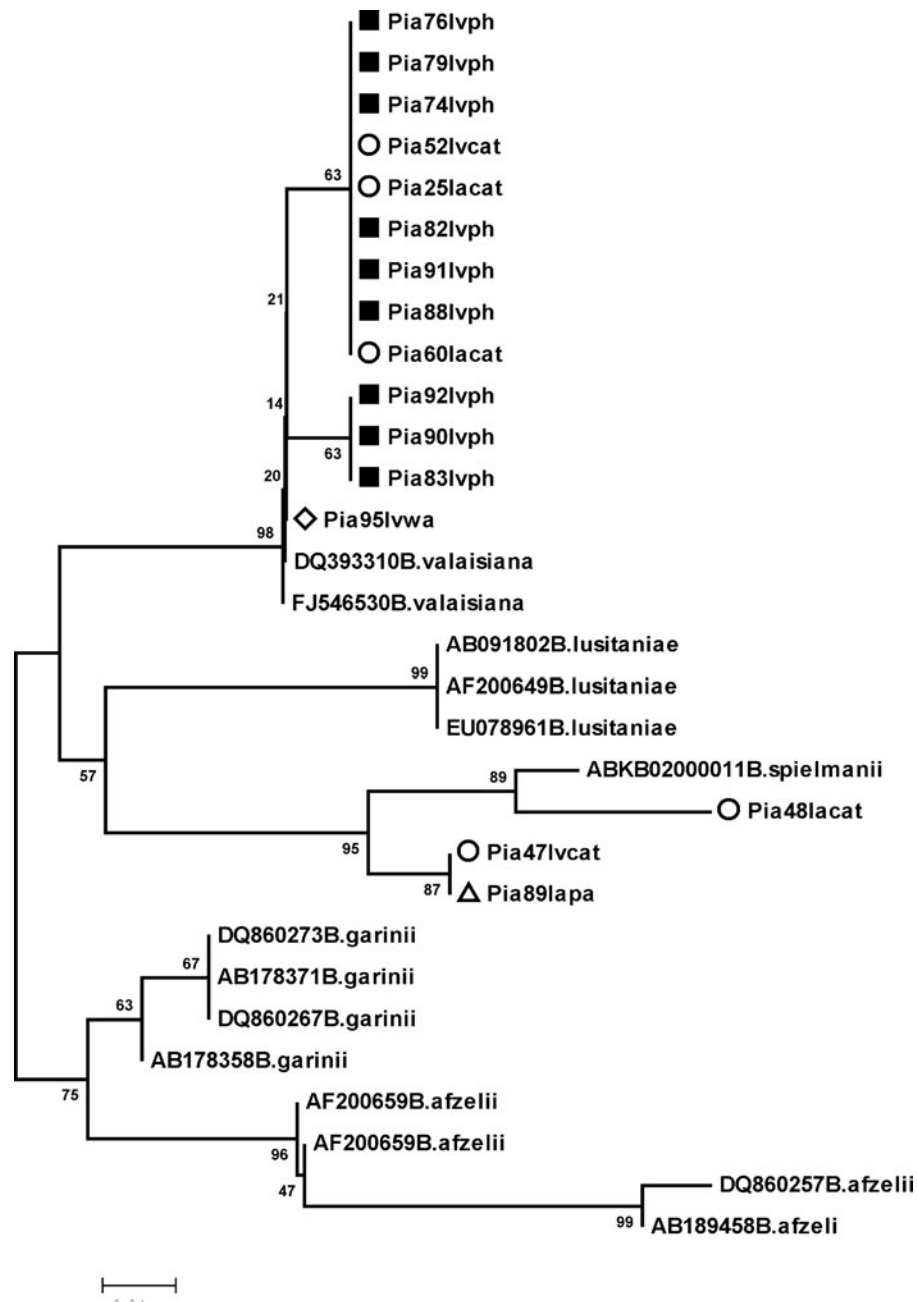


FIG. 1. Phylogenetic tree based on the 5S–23S intergenic spacer (IGS) of Pianosa *B. burgdorferi* s.l. sequences and National Center for Biotechnology Information (NCBI) reference sequences. Pianosa sequences are highlighted by a square (positive ticks from pheasants), a circle (from cats), a rhombus (from water rail), or a triangle (from red partridge). Iv, *I. ventalloi*; Ia, *I. acuminatus*; cat, tick collected on a cat; ph, on pheasant; wa, on water rail; pa, on partridge. Posterior probability values are reported below branches. Bar, 0.01 substitutions per site.

strictly associated with the garden dormouse (*Eliomys quercinus*; Richter et al. 2004). Dormice are not reported in Pianosa (Angelici et al. 2009); thus, we can hypothesize the maintenance of *B. spielmanii* by resident alternative hosts such as peridomestic rodents or hedgehogs. Indeed, mice (*Apodemus sylvaticus*, *Apodemus flavicollis*, *M. musculus*) and Norway rats were shown to be competent hosts for *B. spielmanii*, although being less efficient reservoirs than dormice (Richter et al. 2011). Moreover, Skuballa et al. (2007, 2012) detected *B. spielmanii* in tissues from *E. europaeus* and in *Ixodes ricinus*

ticks subjected to xenodiagnostic experiments on hedgehogs, showing the possible role of these animals as a reservoir.

B. valaisiana also infected our tick sample. This borrelia, occasionally detected in Lyme disease patients (Strle and Stanek 2009), is considered to be associated with avian species (Kurtenbach et al. 2002). Indeed, we detected it in over 80% *I. ventalloi* collected from the pheasant (Table 1, Figs. 1 and 2). Because pheasants have been shown to be reservoir hosts of *B. valaisiana* (Kurtenbach et al. 1998), it is possible that ticks were infected either during the blood meal on the examined

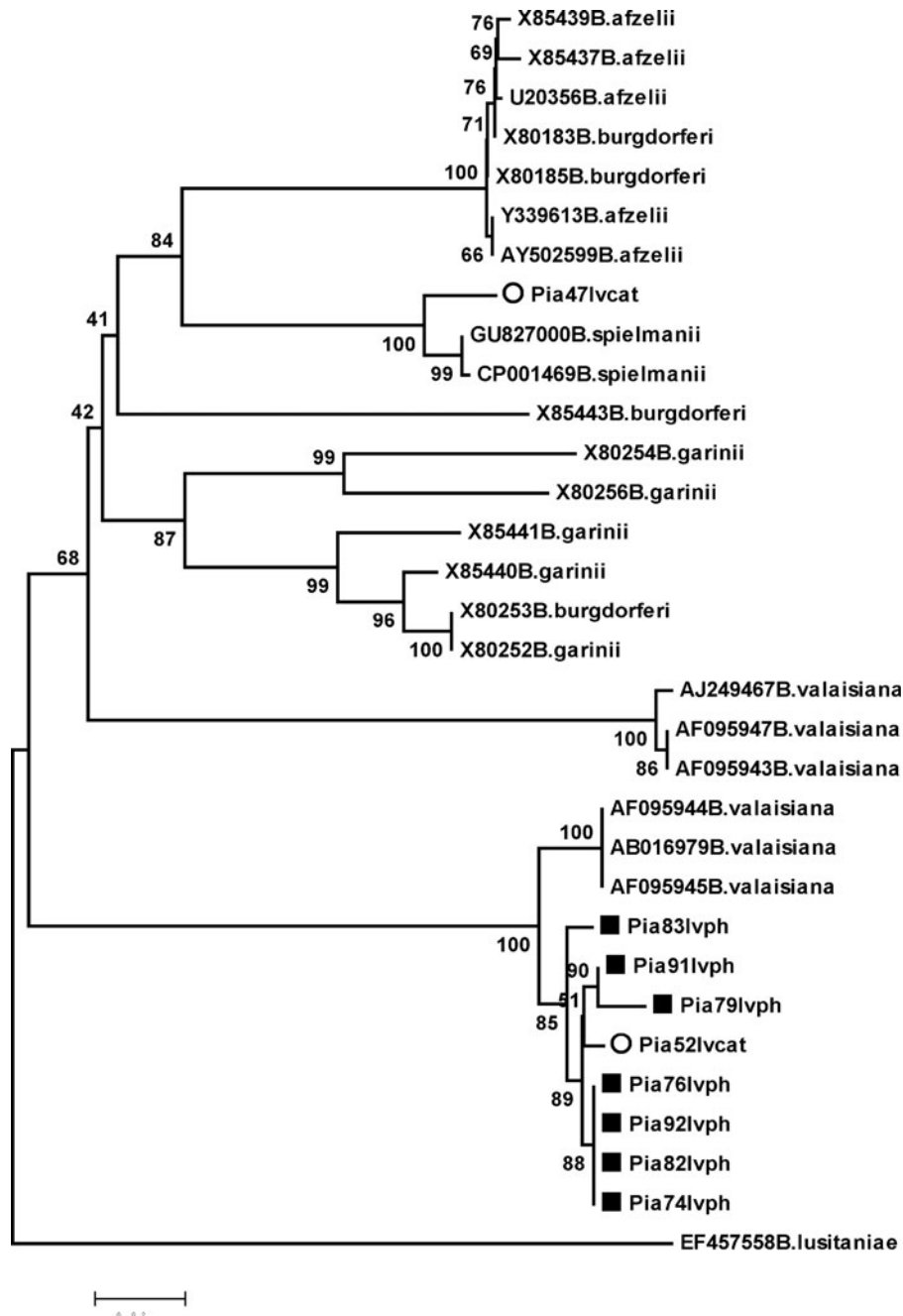


FIG. 2. Phylogenetic tree based on the *OspA* gene of Pianosa *B. burgdorferi* s.l. sequences and NCBI reference sequences. Pianosa sequences are highlighted by a square (positive ticks from pheasants) or a circle (positive ticks from cats). Iv, *I. ventralloii*; cat, tick collected on cat; ph, tick collected on pheasant. Posterior probability values are reported below branches. Bar, 0.02 substitutions per site.

host or on other pheasants in the previous tick stages. *B. valaisiana* was also detected in a *I. ventralloii* feeding on the water rail. This bird species was found infected by *B. burgdorferi* sensu stricto in a study in migrating birds in Slovakia (Schwarzová et al. 2006).

Apart from terrestrial birds, *Ixodes* ticks infected by borrelia were collected on felines. Cats, feral ones in particular, are exposed to *B. burgdorferi* s.l., but clinically apparent disease is not common. Their role as reservoir for this pathogen is unknown (Berrada and Telford 2009). Our results show a few

positive ticks from cats (8%), infected by *B. spielmanii* or *B. valaisiana* (Figs. 1 and 2), and we can thus hypothesize that these animals are not involved in the pathogen transmission in our study area.

The phylogenetic analysis of 5S–23S IGS showed the clus- terization of Pianosa samples in the *B. spielmanii* or *B. va- laisiana* groups; no associations between genetic variability and unique ticks species or hosts were observed. Focusing on the *B. valaisiana ospA* gene, this gene was conserved among our sequences. This may be due either to it being found in a

single tick species, or to a geographic conservation in the island (Qiu et al. 2002). Indeed, only *B. valaisiana* subgroup I was identified in Pianosa. In other geographic areas, *B. valaisiana* subgroups I and II co-exist, such as in the regions at the France–Germany borders (Margos et al. 2009). A possible explanation of the presence of a unique subgroup in our study area may be the single migratory route, from Africa to northern Europe, passing on the Tuscany Archipelago through Tunisia.

Lyme spirochetes were detected in *I. ventalloi* and *I. acuminatus*. Recently, *B. afzelii* infection in *I. acuminatus* was reported in Hungary (Rigó et al. 2011), but this is the first finding of *B. burgdorferi* s.l. in *I. ventalloi*. Although *B. burgdorferi* s.l. has been detected or isolated from several ticks, *Ixodes hexagonus* and *Ixodes uriae* are the unique recognized alternative vectors besides *I. ricinus* (Gern et al. 2008). However, no other *Ixodes* spp. species were collected in Pianosa, so our results suggest the involvement of *I. ventalloi* and *I. acuminatus* in the *B. burgdorferi* s.l. biological cycle. The same tick species may be involved in SFG rickettsiae maintenance, because they were found to be infected by *R. helvetica* and *Rickettsia* sp. IRS3. Positive ticks were collected from terrestrial birds and cats; however, the role of vertebrate hosts in the transmission cycle of *R. helvetica* and other rickettsiae is not known, and ticks are considered reservoir and vectors of the pathogens (Boretti et al. 2009). *Rickettsia* spp. were already reported in *I. ventalloi* collected from migrating birds in Cyprus (Ioannou et al. 2009), Spain (Márquez 2008; Márquez and Millán 2009), and Portugal (Santos-Silva et al. 2006).

To the best of our knowledge, we report for the first time *R. helvetica* in *I. acuminatus*. Also *R. helvetica* is commonly associated with *I. ricinus* ticks, but according to Santos-Silva et al. (2006), the presence of this pathogen in alternative ticks could indicate the existence of secondary maintenance cycles. *I. acuminatus* is a parasite of small rodents, insectivores, and hedgehogs (Boyard et al. 2008; Rigó et al. 2011). The *I. ventalloi* life cycle depends on wild rabbits (Millán et al. 2007; Márquez 2008), but it was reported on carnivores, including cats, rodents, hedgehogs, and birds such as the partridge (Beichel et al. 1996; Santos-Silva et al. 2006, 2011; Ioannou et al. 2009). Because both *Ixodes* species are known to infest humans (Gilot and Marjole, 1982), their possible role as zoonoses vectors can be speculated.

Likewise, *Hyalomma* ticks are well-known human parasites and can transmit a variety of diseases, including Crimean-Congo hemorrhagic fever (CCHF; Estrada-Peña and Jongejan 1999). Adults show an aggressive host-seeking behavior, and in our study area they were frequently found on the dragging operators' clothes. The small numbers of specimens obtained by dragging may be due to the limits of the method for collecting *Hyalomma* adults (Barandika et al. 2011). We detected *R. aeschlimannii* in host-seeking *H. marginatum* and *H. detritum*. This pathogen is characterized by a broad geographical distribution and is generally found in *Hyalomma* ticks from southern Europe (Croatia, Corsica, Cephalonia, Spain, Portugal, and Sicily; Beninati et al. 2005; Parola et al. 2005), North and sub-Saharan Africa (Beati et al. 1997; Mediannikov et al. 2010), and southwestern Russia (Shpynov et al. 2009). However, because of the long attachment on the hosts, *Hyalomma* immatures can be passively transported from the birds' wintering areas along migration routes (Jameson et al. 2012) and can spread rickettsiae to northern countries. For example,

R. aeschlimannii was recently detected in one *H. marginatum* from a bird migrating to Germany from Africa (Rumer et al. 2011).

In the light of the importance of the Tuscany Archipelago islands in the avian migratory routes, it would be interesting to clarify the role of avian species in the epidemiological cycles of both Lyme spirochetes and SFG rickettsiae in Pianosa. In fact, the involvement of wild birds in dispersing ticks and tick-borne pathogens in Europe, and their role as reservoir hosts, are well established. Migrating birds may indeed contribute to the dispersal of ticks and tick pathogens from areas where their biological cycles are maintained, either acting as a reservoir or being responsible for pathogen transmission to species of the local fauna (Olsén et al. 1995, Santos-Silva et al. 2006, Elfving et al. 2010, Hildebrandt et al. 2010, Kjelland et al. 2010, Dietrich et al. 2011, Hasle et al. 2011, Vollmer et al. 2011, Jameson et al. 2012). Contacts between different bird species in seabird colonies and the presence of relatively generalist tick vectors may indeed provide opportunities for pathogen transmission between different enzootic systems (Dietrich et al. 2011). For example, the admixture between marine and terrestrial strains of *B. garinii* was recently demonstrated (Gómez-Díaz et al. 2011). The changes in climate and environment may facilitate the establishment in new areas of infected tick species transported by birds (Rumer et al. 2011), so the study of avian migration patterns, and of the agents infecting the ticks from migratory birds, may be very useful for the surveillance and control of emerging tick-borne zoonoses.

In conclusion, our survey showed the presence of emerging zoonotic agents in the *B. burgdorferi* s.l. complex and SFG rickettsiae in Pianosa and indicated the potential vectorial role of *Hyalomma* and *Ixodes* spp. ticks. These results highlight the risk of human exposure to Lyme disease and rickettsiosis in Pianosa, and tourists and workers visiting the island should pay attention to tick bites.

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Author Disclosure Statement

No competing financial interests exist.

References

- Agnelli P. I mammiferi dell'isola di Pianosa. In: Museo Zoologico "La Specola" dell'Università degli Studi di Firenze (ed.). Studio tecnoscintifico sull'Isola di Pianosa. Rapporto interno per l'Ente Parco Nazionale dell'Arcipelago Toscano. 1998: 48–50.
- Akaike H. Information theory and an extension of the maximum likelihood principle. Proceedings of the Second International Symposium on Information Theory Budapest: Akademiai Kiado Petrov BN, Csaki, 1973; 267–281.
- Angelici FM, Laurenti A, Nappi A. A checklist of the mammals of small Italian islands. *Hystrix It J Mamm* 2009; 20:3–27.
- Barandika JF, SA Olmeda, MA Casado-Nistal et al. Differences in questing tick species distribution between Atlantic and continental climate regions in Spain. *J Med Entomol* 2011; 48:13–19.

- Beati L, Meskini M, Thiers B, Raoult D. *Rickettsia aeschlimannii* sp. nov., a new spotted fever group rickettsia associated with *Hyalomma marginatum* ticks. *Int J Syst Bacteriol* 1997; 47: 548–554.
- Beichel E, Petney TN, Hassler D, Brückner M, Maiwald M. Tick infestation patterns and prevalence of *Borrelia burgdorferi* in ticks collected at a veterinary clinic in Germany. *Vet Parasitol* 1996; 65:147–155.
- Beninati T, Genchi C, Torina A, Caracappa S, et al. Rickettsiae in ixodid ticks, Sicily. *Emerg Infect Dis* 2005; 11:509–511.
- Berrada ZL, Telford SR. Burden of tick-borne infections on American companion animals. *Top Companion Anim Med* 2009; 24:175–181.
- Boyard C, Vourel G, Barnouin J. The relationships between *Ixodes ricinus* and small mammal species at the woodland-pasture interface. *Exp Appl Acarol* 2008; 44:61–76.
- Boretti FS, Perreten A, Meli ML, Cattori V, et al. Molecular Investigations of *Rickettsia helvetica* infection in dogs, foxes, humans, and *Ixodes* ticks. *Appl Environ Microbiol* 2009; 75: 3230–3237.
- Colom MR, Vaccari FP, Scartazza A, Brugnoli E, et al. Pianosa island: Structure, functioning and biodiversity of main ecosystems. *J Med Ecol* 2004; 5:31–40.
- Cringoli G, Iori A, Rinaldi L, Veneziano V, Genchi C. *Mappe Parasitologiche*. Napoli: Rolando Editore, 2005:308 pp.
- Dietrich M, Gómez-Díaz E, McCoy KD. Worldwide distribution and diversity of seabird ticks: implications for the ecology and epidemiology of tick-borne pathogens. *Vector Borne Zoonotic Dis* 2011; 11:453–470.
- Elfving K, Olsén B, Bergström S, Waldenström J, et al. Dissemination of spotted fever rickettsia agents in Europe by migrating birds. *PLoS One* 2010; 5:e8572
- Estrada-Peña A, Jongejan F. Ticks feeding on humans: A review of records on human-biting *Ixodoidea* with special reference to pathogen transmission. *Exp Appl Acarol* 1999; 23:685–715.
- Estrada-Peña A, Bouattour A, Camicas JL, Walker AR. *Ticks of Domestic Animals in the Mediterranean Region*. Ed. University of Zaragoza; 2004:131 pp.
- Fingerle V, Schulte-Spechtel UC, Ruzic-Sabljic E, Leonhard S, et al. Epidemiological aspects and molecular characterization of *Borrelia burgdorferi* s.l. from southern Germany with special respect to the new species *Borrelia spielmanii* sp. nov. *Int J Med Microbiol* 2008; 298:279–290.
- Gern L. *Borrelia burgdorferi* sensu lato, the agent of Lyme borreliosis: Life in the wilds. *Parasite* 2008; 15: 244–247.
- Gilot B, Marjolet M. Contribution à l'étude du parasitisme humain par les tiques (Ixodidae et Argasidae), plus particulièrement dans le sud-est de la France. *Méd Maladies Infect* 12:340–351.
- Grego E, Bertolotti L, Peletto S, Amore G, et al. *Borrelia lusitaniae* OspA gene heterogeneity in Mediterranean basin area. *J Mol Evol* 2007; 65:512–24518.
- Gómez-Díaz E, Boulonier T, Sertour N, Cornet M, et al. Genetic structure of marine *Borrelia garinii* and population admixture with the terrestrial cycle of Lyme borreliosis. *Environ Microbiol* 2011; 13:2453–2467.
- Hasle G, Bjune GA, Midthjell L, Røed KH, Leinaas HP. Transport of *Ixodes ricinus* infected with *Borrelia* species to Norway by northward-migrating passerine birds. *Ticks Tick Borne Dis* 2011; 2:37–43.
- Hildebrandt A, Franke J, Meier F, Sachse S, et al. The potential role of migratory birds in transmission cycles of *Babesia* spp., *Anaplasma phagocytophilum*, and *Rickettsia* spp. *Ticks Tick Borne Dis* 2010; 1:105–107.
- Ioannou I, Chochlakis D, Kasinis N, Anayiotos P, et al. Carriage of *Rickettsia* spp., *Coxiella burnetii* and *Anaplasma* spp. by endemic and migratory wild birds and their ectoparasites in Cyprus. *Clin Microbiol Infect* 2009; 15:158–160.
- Jameson LJ, Morgan PJ, Medlock JM, Watola G, Vaux AG. Importation of *Hyalomma marginatum*, vector of Crimean-Congo haemorrhagic fever virus, into the United Kingdom by migratory birds. *Ticks Tick Borne Dis* 2012 [Epub ahead of print].
- Kjelland V, Stuen S, Skarpaas T, Slettan A. *Borrelia burgdorferi* sensu lato in *Ixodes ricinus* ticks collected from migratory birds in Southern Norway. *Acta Vet Scand* 2010; 6:52–59.
- Kurtenbach K, Peacey M, Rijpkema SGT, Hoodless AN, et al. Differential transmission of the genospecies of *Borrelia burgdorferi* sensu lato by game birds and small rodents in England. *Appl Environ Microbiol* 1998; 64:1169–1174.
- Kurtenbach K, De Michelis S, Etti S, Schafer SM, et al. Host association of *Borrelia burgdorferi* sensu lato—the key role of host complement. *Trends Microbiol* 2002; 10:74–79.
- Labruna MB, Mattar S, Nava S, Bermudez S, et al. Rickettsioses in Latin America, Caribbean, Spain and Portugal. *Rev MVZ Córdoba* 2011; 16:2435–2457
- LIFE04NAT/IT/000172 Progetto Life Natura “Isole di Toscana: Nuove azioni per uccelli marini e habitat”. Available at http://ec.europa.eu/environment/life/project/Projects/index.cfm?fuseaction=home.showFile&rep=laymanReport&fil=LIFE04_NAT_IT_000172_LAYMAN.pdf/. Accessed October 31, 2012.
- Manilla G. *Acari, Ixodida (Fauna d'Italia 36)*. Bologna, Italy; Edizioni Calderoni, 1998.
- Margos G, Vollmer SA, Cornet M, Garnier M, et al. A new *Borrelia* species defined by multilocus sequence analysis of housekeeping genes. *Appl Environ Microbiol* 2009; 75:5410–5416.
- Márquez FJ. Spotted fever group *Rickettsia* in ticks from southeastern Spain natural parks. *Exp Appl Acarol* 2008; 45: 185–194.
- Márquez FJ, Millán J. *Rickettsiae* in ticks from wild and domestic carnivores of Doñana National Park (Spain) and surrounding area. *Clin Microbiol Infect* 2009; 15:224–226.
- Matsumoto K, Parola P, Brouqui P, Raoult D. *Rickettsia aeschlimannii* in *Hyalomma* ticks from Corsica. *Eur J Clin Microbiol Infect Dis* 2004; 23:732–734.
- Mediannikov O, Diatta G, Fenollar F, Sokhna C, Trape JF, Raoult D. Tick-borne rickettsioses, neglected emerging diseases in rural Senegal. *PLoS Negl Trop Dis* 2010; 4:pii e821.
- Millán J, Ruiz-Fons F, Márquez FJ, Viota M, et al. Ectoparasites of the endangered Iberian lynx *Lynx pardinus* and sympatric wild and domestic carnivores in Spain. *Med Vet Entomol* 2007; 21:248–254.
- Nei M, Gojoberi T. Simple methods for estimating the numbers of synonymous and nonsynonymous nucleotide substitutions. *Mol Biol Evol* 1986; 3:418–426.
- Olsén B, Jaenson TG, Bergström S. Prevalence of *Borrelia burgdorferi* sensu lato-infected ticks on migrating birds. *Appl Environ Microbiol* 1995; 61:3082–3087.
- Parola P, Paddock CD, Raoult D. Tick-borne rickettsiosis around the world: Emerging diseases challenging old concepts. *Clin Microbiol Rev* 2005; 18:719–756.
- Posada D, Crandall KA. Selecting the best-fit model of nucleotide substitution. *Syst Biol* 2001; 50:580–601.
- Qiu WG, Dykhuizen DE, Acosta MS, Luft BJ. Geographic uniformity of the Lyme disease spirochete (*Borrelia burgdorferi*) and its shared history with tick vector (*Ixodes scapularis*) in the Northeastern United States. *Genetics* 2002; 160: 833–849.

- R Development Core Team. R: A language and environment for statistical computing. 2008. Available at www.R-project.org/.
- Richter D, Schlee DB, Allgöwer R, Matuschka FR. Relationships of a novel Lyme disease spirochete, *Borrelia spielmani* sp. nov., with its hosts in Central Europe. *Appl Environ Microbiol* 2004; 70:6414–6419.
- Richter D, Schlee DB, Matuschka FR. Reservoir competence of various rodents for the Lyme disease spirochete *Borrelia spielmani*. *Appl Environ Microbiol* 2011; 77:3565–3570.
- Rigó K, Gyurancz M, Tóth AG, Földvári G. Detection of *Borrelia burgdorferi* sensu lato and *Anaplasma phagocytophilum* in small mammals and ectoparasites in Hungary. *Vector Borne Zoonotic Dis* 2011; 11:1499–1501.
- Rijpkema SG, Molkenboer MJ, Schouls LM, Jongejan F, Schellekens JF. Simultaneous detection and genotyping of three genomic groups of *Borrelia burgdorferi* sensu lato in Dutch *Ixodes ricinus* ticks by characterization of the amplified intergenic spacer region between 5S and 23S rRNA genes. *J Clin Microbiol* 1995; 33:3091–3095.
- Ronquist F, Huelsenbeck JP. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 2003; 19:1572–1574.
- Rumer L, Graser E, Hillebrand T, Talaska T, et al. *Rickettsia aeschlimannii* in *Hyalomma marginatum* ticks, Germany. *Emerg Infect Dis* 2011; 17:325–326.
- Ryffel K, Péter O, Dayer E, Bretz AG, Godfroid E. OspA heterogeneity of *Borrelia valaisiana* confirmed by phenotypic and genotypic analyses. *BMC Infect Dis* 2003; 3:14.
- Santos-Silva MM, Sousa R, Santos AS, Melo P, et al. Ticks parasitizing wild birds in Portugal: detection of *Rickettsia aeschlimannii*, *R. helvetica* and *R. massiliae*. *Exp Appl Acarol* 2006; 39:331–338.
- Santos-Silva MM, Beati L, Santos AS, De Sousa R, et al. The hard-tick fauna of mainland Portugal (Acari: Ixodidae): an update on geographical distribution and known associations with hosts and pathogens. *Exp Appl Acarol* 2011; 55:85–121.
- Schwarzová K, Betáková T, Neméth J, Mizáková A. Detection of *Borrelia burgdorferi* sensu lato and *Chlamydochlamydia psittaci* in throat and cloacal swabs from birds migrating through Slovakia. *Folia Microbiol (Praha)* 2006; 51:653–658.
- Shpynov S, Rudakov N, Tohkov Y, Matushchenko A, et al. Detection of *Rickettsia aeschlimannii* in *Hyalomma marginatum* ticks in western Russia. *Clin Microbiol Infect* 2009; 15:315–316.
- Skuballa J, Oehme R, Hartelt K, Petney T, et al. European hedgehogs as hosts for *Borrelia* spp., Germany. *Emerg Infect Dis* 2007; 13:952–953.
- Skuballa J, Petney T, Pfäffle M, Oehme R, et al. Occurrence of different *Borrelia burgdorferi* sensu lato genospecies including *B. afzelii*, *B. bavariensis*, and *B. spielmani* in hedgehogs (*Erinaceus* spp.) in Europe. *Ticks Tick Borne Dis* 2012; 3:8–13.
- Stanek G, Reiter M. The expanding Lyme Borrelia complex—clinical significance of genomic species? *Clin Microbiol Infect* 2011; 17:487–493.
- Strle F, Stanek G. Clinical manifestations and diagnosis of Lyme borreliosis. *Curr Probl Dermatol* 2009; 37:51–110.
- Swofford D. PAUP*: Phylogenetic analysis using parsimony (*and other methods), ver.4.0. Sunderland, MA, Sinauer Associates. 2003. Available at <http://paup.csit.fsu.edu/index.html/>.
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The CLUSTAL X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 1997; 25:4876–4882.
- Tomassone L, Conte V, Parrilla G, De Meneghi D. *Rickettsia* infection in dogs and *Rickettsia parkeri* in *Amblyomma tigrinum* ticks, Cochabamba Department, Bolivia. *Vector Borne Zoonotic Dis* 2010; 10:953–958.
- Vollmer SA, Bormane A, Dinnis RE, Seelig F, et al. Host migration impacts on the phylogeography of Lyme Borreliosis spirochaete species in Europe. *Environ Microbiol* 2011; 13:184–192.
- Wang G, van Dam AP, Dankert J. Two distinct ospA genes among *Borrelia valaisiana* strains. *Res Microbiol* 2000; 151:325–331.

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