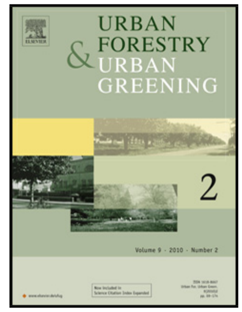
Risk of tick-borne zoonoses in urban green areas: a case study from Turin, northwestern Italy

DOI:10.1016/j.ufug.2021.127297

(Article begins on next page)

Journal Pre-proof



Risk of tick-borne zoonoses in urban green areas: a case study from Turin, northwestern Italy<!–<ForCover>Bellato A, Pintore MD, Catelan D, Pautasso A, Torina A, Rizzo F, Mandola ML, Mannelli A, Casalone C, Tomassone L, Risk of tick-borne zoonoses in urban green areas: a case study from Turin, northwestern Italy, *Urban Forestry and amp; Urban Greening*, doi: 10.1016/j.ufug.2021.127297</ForCover>–>

Alessandro Bellato (Methodology) (Software) (Validation) (Formal analysis) (Investigation) (Data curation) (Writing - original draft) (Visualization), Maria Domenica Pintore (Investigation) (Data curation) (Resources), Dolores Catelan (Methodology) (Software) (Formal analysis) (Investigation) (Writing - original draft) (Visualization), Alessandra Pautasso (Data curation), Alessandra Torina (Investigation), Francesca Rizzo (Investigation), Maria Lucia Mandola (Investigation), Alessandro Mannelli (Conceptualization) (Methodology) (Writing - review and editing), Cristina Casalone (Project administration) (Funding acquisition), Laura Tomassone (Conceptualization) (Methodology) (Investigation) (Supervision) (Writing - original draft)

PII: S1618-8667(21)00324-1

DOI: https://doi.org/10.1016/j.ufug.2021.127297

Reference: UFUG 127297

To appear in: *Urban Forestry & Urban Greening*

Received Date: 25 March 2021

Revised Date: 13 July 2021

Accepted Date: 8 August 2021

Please cite this article as: { doi: https://doi.org/

Risk of tick-borne zoonoses in urban green areas: a case study from Turin, northwestern Italy

Authors and affiliations:

Alessandro Bellato Department of Veterinary Sciences, University of Turin, L.go Paolo Braccini 2, 10095 Grugliasco, Italy;

Maria Domenica Pintore:Experimental Zoo-prophylactic Institute of Piedmont Liguria and Aosta Valley, V. Bologna 148, 10154 Torino, Italy;

Dolores Catelan Department of Statistics, Computer Science, Applications "G. Parenti", University of Florence, V.le Morgagni 59, 50134 Firenze (FI) Italy;

Alessandra Pautasso Experimental Zoo-prophylactic Institute of Piedmont Liguria and Aosta Valley, V. Bologna 148, 10154 Torino, Italy;

Alessandra Torina Experimental Zoo-prophylactic Institute of Sicily, V. Gino Marinuzzi 3, 90129 Palermo, Italy;

Francesca Rizzo Experimental Zoo-prophylactic Institute of Piedmont Liguria and Aosta Valley, V. Bologna 148, 10154 Torino, Italy;

Maria Lucia Mandola Experimental Zoo-prophylactic Institute of Piedmont Liguria and Aosta Valley, V. Bologna 148, 10154 Torino, Italy;

Alessandro Mannelli Department of Veterinary Sciences, University of Turin, L.go Paolo Braccini 2, 10095 Grugliasco, Italy;

Cristina Casalone Experimental Zoo-prophylactic Institute of Piedmont Liguria and Aosta Valley, V. Bologna 148, 10154 Torino, Italy;

Laura Tomassone Department of Veterinary Sciences, University of Turin, L.go Paolo Braccini 2, 10095 Grugliasco, Italy;

Abstract

Ticks are increasingly reported in urban and peri-urban areas, such as city parks or peri-urban forests, across Europe. Land use changes, like the transformation of natural ecosystems into residential or recreational areas, and the restoration of natural areas connectivity for biodiversity purposes, facilitate human contact with these vectors. We evaluated the infestation by Ixodid ticks and their infection by zoonotic agents in two natural reserves (La Mandria and Stupinigi) in the urban area of Turin, northwestern Italy, near to densely inhabited suburbs and consistently frequented by people. We monthly performed dragging in 15 sites during a three-year period (2012-2014).
Over 4000 ticks were collected, belonging to the species: *Ixodes ricinus*, *I. acuminatus*, *Dermacentor marginatus*, *D. reticulatus*, and *Haemaphysalis concinna*. The tick burden in La Mandria, a park very rich in wildlife, was higher than in Stupinigi, which is surrounded by a grid of roads and cultivated fields. The occurrence of *I. ricinus* nymphs was mainly related to habitat diversity and seasonal variation but weakly influenced by wildlife presence. Conversely, the density of *I. ricinus* nymphs

were effectively estimated by vegetation coverage, micro-climatic conditions, and the presence of large wildlife. Molecular analyses showed the infections by *Borrelia burgdorferi* s.l. (24.1%), *Anaplasma phagocytophilum* (2.9%), and Spotted Fever Group rickettsiae (30.4%) in *I. ricinus*. Tick-borne encephalitis virus, that is considered so far absent in northwestern Italy, was not detected. In La Mandria, the risk of encountering one *I. ricinus* nymph infected by *Borrelia burgorferi* s.l. in 100 m^2 was 59.1% (95% Confidence Interval [CI]: 36.3-83.0), and 71.3% (95%CI: 44.5-93.0) for *Rickettsia* spp.

Educational programs on personal protection and surveillance on tick-borne diseases should be enhanced to face the threat posed by tick vectors in green urban landscapes.

Keywords: Tick-borne pathogens; Ixodidae; Borrelia burgdorferi s.l.; SFG rickettsiae; pathogen co-occurrence; peri-urban green areas

Introduction

Tick-borne diseases (TBD) have been increasing their burden in Europe, especially Lyme borreliosis and tick-borne encephalitis (TBE), which are transmitted by the sheep tick *Ixodes ricinus* (Medlock et al., 2013; Süss, 2011). This vector is expanding its geographical range and has been increasingly reported in urban green areas, where the likelihood of exposure to tick bites may be high for human beings and companion animals (Rizzoli et al., 2014).

In European urban areas, changes in land use, agriculture and city expansion have changed the landscape in the last decades (Antrop, 2004; EEA, 2017). Reforestation, agricultural and socio-demographic factors, as well as wildlife management, have increased land patchiness in peri-urban areas, in which several animal species can move and survive (Snep et al., 2006; Sprong et al., 2018). In these fragmented habitats, wild ungulates serve as feeding hosts for ticks and bring them close to inhabited areas (Rizzoli et al., 2014; Selmi et al., 2018), while small-sized vertebrates such as rodents, shrews, birds and lizards can play a role as tick-borne pathogens reservoir (Amore et al., 2007; Craine et al., 1995; Hubálek et al., 1996; Humair et al., 1998; Olsén et al., 1995; Randolph and Storey, 1999). In addition, several urban and peri-urban green areas have been experiencing the anthropogenic introduction of alien mammal species, such as chipmunks, that contribute to the emergence of tick-borne pathogens (Marchant et al., 2017).

Compared to forests, urban and peri-urban areas are still less favourable habitats for ticks. However, in the past few years, several studies reported ticks and TBD in city parks and urban forests across Europe (Hansford et al., 2017; Kowalec et al., 2017; Kubiak et al., 2019; Lejal et al., 2019). Ticks were also reported in peri-urban parks in north (Olivieri et al., 2017), central (Mancini et al., 2014) and southern Italy (Torina et al., 2018). In Italy, TBD are deemed rare; however, their incidence is likely to increase due to the geographical expansion of ticks. In particular, Piedmont region, northwestern Italy, has been experiencing a recent increase in tick burdens in mountain areas (Garcia-Vozmediano et al., 2020b; Pintore et al., 2015), and the appearance of new species in peri-urban areas, such as *Dermacentor reticulatus* in Turin hillside (Garcia-Vozmediano et al., 2020a).

In this study, we analysed the distribution of Ixodid ticks and their infection by selected TBD agents (*Borrelia burgdorferi s.l.*, *Rickettsia* spp., *Anaplasma* spp., and Tick-borne Encephalitis Virus) in two peri-urban parks located near to densely inhabited suburbs of Turin, in order to evaluate risk factors influencing the occurrence of ticks and their abundance, and consequently the risk of park visitors being bitten by an infected tick.

Materials and Methods

Study area.

The study was carried out in two peri-urban parks located within 10 km from the centre of Turin city (2,259,223 inhabitants in the metropolitan area; National Institute of Statistics, https://www.istat.it/it/), Piedmont, northwestern Italy. They belong to the 'Royal Parks' (http://www.parchireali.gov.it/) since they were hunting reserves of the Royal Family in the 19th century. The parks differ in the habitat characteristics and abundance of wildlife populations, but they are both consistently frequented by people.

La Mandria Regional Park, a 3,600-ha natural reserve bounded by a 30 km long perimeter wall, is bordered by fifteen municipalities, and more than 220,000 inhabitants live nearby or inside the park itself. The Park is highly frequented, with up to 10,000-tourists peaks during weekends. The vegetation, characterized by broad-leaf deciduous woods alternating with pastures, resembles the former lowland forest as it would appear before the agricultural revolution. Various exotic tree species were introduced decades ago, such as white pine (*Pinus strobus*), swamp Spanish oak (*Quercus palustris*) and red oak (*Quercus rubra*), which exist concurrently with autochthonous broadleaf tree and artificial poplar (*Populus* spp.) implants. Large populations of wildlife used to inhabit the enclosed area, including more than 200 red deer (*Cervus elaphus*), around 60 fallow deer (*Dama dama*) and 20 roe deer (*Capreolus capreolus*). Wild boars (*Sus scrofa*) are abundant; in the past few years, at least 600 wild boars have been killed each year with the purpose of eradicating the species inside the park. The consistency of the wild populations reported here are updated to 2021, but given the characteristics of the park, these populations have likely remained stable over the years. Mustelids, rodents, lagomorphs and sciurids, as well as birds and reptiles, are present. No domestic ruminants live inside the park area, while some horses are held in paddock or stable, with rare contacts with wildlife.

Only a small portion of Stupinigi Regional Park is enclosed near the royal castle. The Park has rather homogeneous habitat characteristics typical of the Po valley environment. It is characterized by woods of ashes (*Fraxinus excelsior*), hazels (*Corylus avellana*), hornbeams (*Carpinus betulus*) and oaks (*Quercus robur*), alternating with cultivated fields and farms. It covers a 1,750-ha area near to highly inhabited suburbs of Turin and three other densely populated towns. Wildlife populations are not regularly monitored, but 29 mammal species are reported as well as birds and reptiles. At the time the study was conducted, roe deer and wild boars were estimated as numerically small and with discontinuous presence, because of the intense human activity and car traffic in the area. Recently, their number has been increasing (e.g. over a hundred roe deer are estimated by park rangers) and the presence of wolves has been reported too. Only a portion of the park is frequented by people, who remain mainly on footpaths.

Tick collection.

To include different habitats, preferential sampling was performed, meaning that sampling transects were selected based on suggestions from park rangers. The coordinates of the transects' exact position were obtained using a portable GPS device GARMIN eTrex10 (GARMIN Ltd., Milan, Italy). In La Mandria, eight transects including both free-access and restricted-to-public areas were selected, while seven were chosen in Stupinigi, where no restricted areas were present. In each transect, questing ticks were collected by dragging 1 m^2 white cotton cloth over the ground vegetation for 100 m. The transects were sampled six times a year since April 2012 to October 2014. The sampling sessions were at least 15 days apart from each other. The sampling was not performed in Stupinigi in July and August 2012 due to logistic reasons, and in both areas in April 2013 because of adverse weather conditions. The ticks collected were preserved in 70% ethanol and subsequently identified using taxonomic keys (Manilla et al., 2005). Due to the location, sampling was usually performed later in Stupinigi (6.2 ± 0.2 hrs; measured as hours since the sunrise) than in La Mandria (4.5 ± 0.1 hrs).

Laboratory analysis.

Total nucleic acid extraction was performed individually from *I. ricinus* nymphs and adults, with Nucleospin RNA II kit (MACHEREY-NAGEL GmbH & Co., Neumann-Neander Str. 6–8, 52355 Dueren, Germany). *Borrelia burgdorferi* s.l. infection was investigated by a PCR protocol targeting the 16S rRNA gene (Marconi and Garon, 1992) and the 5S-23S intergenic spacer region (Rijpkema et al., 1995). *GtlA* and *OmpA* genes (Eremeeva et al., 2003) were targeted for *Rickettsia* spp. detection, and the 16S rRNA gene for *Anaplasma* spp. (Rymaszewska and Adamska, 2011). For the search of Tick-Borne Encephalitis virus (TBEV) RNA, a Real Time PCR specific for a portion of the 3' NC region (Rudenko et al., 2004) was carried out. Positive samples were identified to the species level by DNA sequencing. Due to economic constraints, only *I. ricinus* were subjected to laboratory analyses.

Meteorological data collection.
At each transect at the time of sampling, they were measured the temperature, the relative humidity at 1.5 m in height (air) and at soil height using a HI9564 thermo-hygrometer (HANNA instruments®, Milan, Italy).
To determine the average temperature and relative humidity of the 15 days before the sampling date, the historical meteorology database of the Regional Agency for Environment Protection (ARPA Piemonte, https://www.arpa.piemonte.it/) was consulted. The nearest thermo-hygrometric stations were “Venaria La Mandria” (alt. 347 m a.s.l.; WGS 84/UTM zone 32N, X: 386790 Y: 5003396; 4,818 m from the furthest sampling site of La Mandria) and “Torino Vallere” (alt. 239 m a.s.l.; WGS 84/UTM zone 32N, X: 395514 Y: 4985692; 8,145 m from the furthest sampling site of Stupinigi).
As synthetic index of temperature and relative humidity, the saturation deficit (SD) of both the air and the soil at the time of sampling was calculated, as well as its average of the 15-days-before-sampling (15DbS). SD was calculated using the following equation by Randolph and Storey (1999):

$$SD = \left(1 - \frac{RH}{100}\right) * 4.9463e^{0.0621T}$$

where *RH* is the relative humidity measured as percentage, and *T* is the temperature measured in degrees Celsius (°C). SD ranges from 0 to infinite and it is measured in mmHg.

Remote-sensing data collection.
Images captured by USGS Landsat 8 Operational Land Imager and Thermal Infrared Sensor, red (band 4, wavelength: 0.64-0.67, resolution: 30m x 30m) and Near Infra-Red (NIR) bands (band 5, wavelength: 0.85-0.88, resolution: 30m x 30m), or USGS Landsat 4 and 5 Thematic Mapper, (band 3, wavelength: 0.63-0.69, and band 4, wavelength: 0.76-0.90, resolution: 30m x 30m) were obtained from USGS (U.S.A. Department of Interior, https://earthexplorer.usgs.gov). The images were imported in the Geographic Information System (GIS) software QGIS version 3.4.4 - Madeira (QGIS Project, https://qgis.org/en/site/), using the following coordinates reference system: World Geodetic System 1984 (WGS 48) Universal Transverse Mercator (UTM), zone 32N. Only those having a cloud coverage lower than 20 % have been selected. Since Landsat images were already geocoded, no further geometric correction was needed.
To calculate daily NDVI for each sampling location, the *raster calculator* tool was used, applying the formula (Rouse et al., 1973):

$$NDVI = \frac{(NIR - Red)}{(NIR + Red)}$$

where *NIR* and *Red* refer to bands 4 and 5 of Landsat 8, or 3 and 4 of Landsat 4-5, respectively. The units of measurement are simplified, hence NDVI is a raw number.
The image chronologically closest to each sampling date was used. For every transect, the NDVI monthly average was calculated averaging daily NDVI of the same period (± 10 days) in subsequent years, with the underlying assumption that no significant variation of vegetative coverage occurred. Late spring NDVI is calculated as the mean of May and June records.

GIS analysis.
To analyse geographic data, QGIS version 3.4.4 – Madeira was used. Transects’ and meteorologic stations’ coordinates were added manually. All the other layers (administrative boundaries, water, and vegetation items) were obtained from the Territorial Reference Database of Piedmontese Bodies (BDTRE, https://www.geoportale.piemonte.it/cms/bdtre)

Statistical analysis.

Descriptive statistics

For each site, descriptive statistics are reported (range, median, mean, and standard deviation). Odds Ratio (OR) and Incidence Density Ratio (IDR) are evaluated to compare ticks' occurrence and abundance, respectively.

The density of each tick species and stage was evaluated, but only *I. ricinus* nymphs were analysed throughout because of their abundance and importance for public health.

Univariate analysis

The occurrence of *I. ricinus* ticks in the two parks was compared using logistic regression.

Overall, 23 variables (see Supplementary material, table 1) were evaluated individually: the association between each variable alone and the occurrence of *I. ricinus* nymph was evaluated using univariate logistic regression; the association of the same 23 variables and *I. ricinus* nymphs' density was evaluated with univariate Poisson regression.

Multivariate analysis

Due to the high degree of correlation among variables some of them were excluded, and only eight were maintained based on their biological plausibility and their raw effect estimated by univariate analysis. Given the hierarchical structure of the data, the model was fit with sites and parks as double level random effects. In order to adjust for the calendar period, the median cubic spline with knots every 20 days was included. Then, the final set of covariates was selected by stepwise backward selection based on the log-likelihood comparison to the saturated model.

For *I. ricinus* nymphs' occurrence, a logistic Generalized Linear Model (GLM, family: binomial) was fit, including vegetation type, phytotype, tree species, traces of large wildlife, late-spring NDVI, air- and soil-height SD at sampling, and average daily SD of 15DbS as covariates. Site and park are included as random effects.

For *I. ricinus* nymphs' density, a negative binomial GLM was fit to adjust for overdispersion, including vegetation type, phytotype, tree species, traces of large wildlife, late-spring NDVI, air- and soil-height SD at sampling, and average daily SD of 15DbS as covariates. Site and park are included as random effects as well.

TBP prevalence

The mean prevalence of infected *I. ricinus* ticks is reported for each TBP alone and for coinfection, which was defined as the simultaneous infection of the same subject by two or more TBPs. Prevalence is reported at Park level along with 95% Confidence Intervals (CI).

Acarological risk

The probability of release of almost one infected *I. ricinus* in 100 m^2 was determined by Acarological Risk (AR) as described in Mannelli et al. (2003):

$$AR = 1 - e^{-(pT)}$$

where p is the observed proportion of infected *I. ricinus* by a specific pathogen, and T is the mean number of *I. ricinus* collected in 100 m^2. It was calculated using counts of nymphs infected by the TBD pathogens (TBP), as well as coinfected ticks. To predict point estimates and 95% confidence intervals of acarological risk an intercept-only Generalized Estimating Equation (GEE, family: negative binomial) model was used, with site as grouping variable.

In the abstract we selected two out of the eight acarological risk estimates presented in the body of the text. Confidence intervals are valid until they are presented together with all the others, but not when they are selected to be presented independently because the uncertainty derived from selection process was not accounted for (Carugno et al., 2016). Therefore, we applied the appropriate correction as suggested by Benjamini and Yekutieli (2005).

Statistical analysis was performed using STATA 15.1 (Stata Corp LLC, 4905 Lakeway Drive College Station, Texas 77845 USA).

Results

Descriptive analysis

From March 2012 to October 2014, out of 234 transects sampled 180 (76.9%) were positive for the presence of ticks. Ticks were collected in 120 out of 136 transects (88.2%) in La Mandria while in 60 out of 98 (61.2%) in Stupinigi. Overall, 4371 ticks were gathered, of which 3886 (88.9%) were larvae, 445 (10.2%) nymphs, and 40 (0.9%) adults (20 females and 20 males). The total number of ticks in La Mandria (n=4017, average per transect: 29.5, 95%CI: 28.6-30.4) was higher than in Stupinigi (n=352, avg.: 3.6, 95%CI: 3.2 – 4.0). In particular, the density of larvae and of nymphs per transect were greater, while adult density did not differ (data not shown).

Five tick species were identified: *I. ricinus* (n=3156; 2777 larvae, 353 nymphs, 26 adults); *I. acuminatus* (3 nymphs); *Dermacentor marginatus* (n=35; 9 larvae, 23 nymphs, 3 adults); *D. reticulatus* (n=2 adults); *Haemaphysalis concinna* (n=1175; 1112 larvae, 67 nymphs, 6 adults).

Ixodes ricinus was recovered in 169 transects (72.2%, n=234), of which 115 (68.1%) in La Mandria. Thirteen transects (5.6%, n=234) were positive for *D. marginatus* presence (10 in La Mandria, 3 in Stupinigi), two (0.9%) for *D. reticulatus*, three (1.3%) for *I. acuminatus* (1 in La Mandria, 2 in Stupinigi), and 83 (35.5%) for *H. concinna* (81 in La Mandria, 2 in Stupinigi).

There was high variability in the density of *I. ricinus* nymphs among sites and parks (Table 1). In La Mandria, all but one sites were infested at least once every year. Conversely in Stupinigi, only half of the sites were infested by *I. ricinus* nymphs, and their occurrence was inconstant (< 30% of the times). In La Mandria, the odds of observing at least one *I. ricinus* nymph was two times larger than that in Stupinigi (OR = 2.1, 95%CI: 1.2-3.7). Accordingly, the density of *I. ricinus* nymphs was two and a half times greater (IDR = 2.5, 95%CI: 1.9-3.1).

Univariate analysis

Phytotype, tree species and vegetation type differed among the sites and the parks. The odds of occurrence of *I. ricinus* nymphs were 50% higher in the woods compared to the forest-pasture mixed environment (OR = 1.5, 95%CI: 1.1-2.1), as well as their median density which was higher in the woods than in the mixed environment (Figure 2). Conversely, the mean density of *I. ricinus* nymphs was lower in wooded transects (1.4 nymphs/100 m^2, 95%CI: 1.2-1.6) than in mixed environment ones (1.8 nymphs/100 m^2, 95%CI: 1.5-2.1).

The most represented tree species in our study areas were cherry tree (n=167 transects), hornbeam (n=65), and oak (n=62). More frequent in La Mandria, the hornbeam was positively associated with both density (IDR = 1.3, 95%CI: 1.1-1.6) and occurrence (OR = 3.0, 95%CI:1.6-5.6) of *I. ricinus* nymphs. Some tree species were not present in both parks; for example, the white pine exclusively characterized site 03 of La Mandria, where the count of *I. ricinus* nymphs was far above the average.

Traces of large wildlife were more frequently observed in La Mandria (77.9 ± 3.6%) than in Stupinigi (57.1 ± 5.0%) and they were associated with greater occurrence (OR = 2.3, 95%CI:1.3-4.1) and higher density (IDR = 2.3, 95%CI: 1.7-3.0) of *I. ricinus* nymphs (Figure 2).

The air saturation deficit (SD) differed between parks (La Mandria: 10.1 ± 0.3; Stupinigi: 11.8 ± 0.4), and from SD at soil-height (La Mandria: 7.5 ± 0.3; Stupinigi: 8.6 ± 0.4). An association was observed between SD and both the occurrence of *I. ricinus* nymphs ($OR_{air\text{-}height}$ = 1.1, 95%C: 0.9-1.5; $OR_{soil\text{-}height}$ = 1.1, 95%CI: 0.8-1.4) and their density ($IDR_{air\text{-}height}$ = 1.0, 95%CI: 0.9-1.2; $IDR_{soil\text{-}height}$ = 1.1, 95%CI: 1.0-1.2).

On the other hand, the hours since the sunrise were not associated neither with the presence, nor with the density of nymphs of *I. ricinus*.

Regarding seasonality, we observed an increase in the occurrence and density of *I. ricinus* nymphs between April and May, then they decreased from May to September. Compared to May, the decline of occurrence was significant from July to October, while the density was significantly lower in all months. The lowest point was in August, when the presence and density were lower not only than in May, but also from the previous month.

The average, minimum and maximum daily SD of the 15DbS were higher in Stupinigi compared to La Mandria (Table 3). The average of the previous fifteen days of the daily SD had a strong positive effect on the occurrence and density of *I. ricinus* nymphs. By contrast, the average of daily minimum and maximum SD had a significantly negative association with the occurrence and density (Table 3).

Late spring NDVI was higher in La Mandria than in Stupinigi, and it was not associated with the occurrence of *I. ricinus* nymphs (OR = 0.3, 95%CI: 0.0-3.2). A negative association with nymphs density was observed (IDR = 0.3, 95%CI: 0.1-0.6).

Multivariate analysis

For the occurrence of *I. ricinus* nymphs, the final random-effects GLM (family: binomial) included late-spring NDVI, average daily SD of the 15DbS, and traces of large wildlife. The random effect of the transects was included, whence it explained part of data variability, while that of parks was excluded. The final model for the occurrence of *I. ricinus* nymphs showed a decrease in their prevalence when average daily SD and late spring NDVI were high. High values of late spring NDVI (>0.7) were associated to the absence of nymphs. Elevated values of average daily SD (>7mmHg) had a strong negative association to the presence of nymphs too, while a positive association was observed for values below 2 mmHg. The traces of large wildlife were associated to a 70% increase of *I. ricinus* nymphs' occurrence.

For the density of *I. ricinus* nymphs, the final negative binomial GLM was fit including late-spring NDVI, average daily SD of the 15DbS, and traces of large wildlife, too. Both level of random effect, namely park and transects, were excluded because the other covariates explain most of the variability of the data. The presence of wildlife traces was associated to an average increase of 2.2 times in the density of nymphs. A lower density was associated to high values of late-spring NDVI, as well as to elevated values of average daily SD (Table 4).

TBP prevalence.

Overall, 379 *I. ricinus* (n=353 nymphs, n=26 adults, of which 13 females and 13 males) were tested for *Borrelia burgdorferi* s.l., *Rickettsia* spp., *Anaplasma* spp. and TBEV. Prevalence of infected *I. ricinus* ticks varied among parks for all TBP tested and for coinfected ticks too. (Table 5).

The prevalence of *Borrelia burgdorferi* s.l. infection was 24.0% (95%CI: 19.7-28.3). Adults (42.3 %, 95%CI: 23.3-61.3) were more infected than nymphs (22.7%, 95%CI: 18.3-27.0). Infection increased from April to June, then decreased to 0 % in September, and was higher in La Mandria than in Stupinigi (OR = 3.93, 95% C.I.: 1.91-8.07). No association was observed between the prevalence of *Borrelia burgdorferi* s.l. infection and NDVI (OR = 0.04, 95% C.I.: 0.00-47.65) or traces of large wildlife (OR = 0.90, 95% C.I.: 0.55-1.49). Out of 91 samples positive to *Borrelia burgdorferi* s.l., 59 (64.8%) were identified as *B. lusitaniae*, 12 (13.2%) as *B. afzelii*, 3 (3.3%) as *B. garinii*, 1 (1.1%) as *B. burgdorferi* s.s.. Genospecies of 16 (17.6%) positive samples could not be identified.

Rickettsia spp. infected the 30.6% (95%CI: 26.0-35.2) of *I. ricinus* individuals. Nymphs (31.0%, 95%CI: 27.1-36.9) were more infected than adult ticks (11.5%, 95%CI: 0.0-23.8). Also, prevalence of *Rickettsia* spp. infection was higher in La Mandria than Stupinigi (OR = 5.65, 95% C.I.: 2.96-10.80), while it was not associated with NDVI (OR = 0.01, 95% C.I.: 0.00-1.24), nor with traces of large wildlife (OR = 0.94, 95% C.I.: 0.61-1.43). Out of 116 samples positive to *Rickettsia* spp., 110 (94. 8%) were identified as *R. monacensis*, 4 (3.5%) as *R. helvetica*, and 2 (1.7%) could not be identified to species level.

Anaplasma spp. was detected in 2.8% of *I. ricinus* nymphs (95%CI: 1.1-4.6), and in 3.8% of adults (95% CI: 0.0-11.2). All eleven positive ticks were collected in La Mandria. Out of them, 4 (36.4%) were identified as *A. phagocytophilum*, while the species of the remaining samples could not be identified.

Concurrent infection by at least two pathogens was observed in 43 (11.3%, 95%CI: 8.2-14.5) *I. ricinus* ticks (39 nymphs, 2 females and 2 males). Out of them, 2 (4.7%) were infected by all three pathogens, 36 (83.7%) by *B. burgdorferi* s.l. and *Rickettsia* spp., 3 (7.0%) by *B. burgdorferi* s.l. and *Anaplasma* spp., and 2 (4.7%) by *Rickettsia* spp. and *Anaplasma* spp. *B. lusitaniae* and *R. monacensis* were the pathogens most frequently associated (n=23). Among the *Rickettsia* genus, only *R. monacensis* was observed in coinfected ticks. La Mandria had a prevalence of coinfected ticks higher than Stupinigi (Table ~~3~~5).

All *I. ricinus* ticks tested for Tick-Borne Encephalitis virus resulted negative.

Acarological risk.
Raw estimated acarological risk was 45.3% (95%CI: 30.0-64.0) for *I. ricinus* nymphs infected by *B. burgdorferi* s.l. and 56.2% (95% C.I.: 37.7-76.4) for nymphs infected by *Rickettsia* spp.; it was lower for nymphs infected by *Anaplasma* spp. (AR = 7.3%, 95%CI: 3.0-17.0) and for coinfected nymphs (AR = 26.5%, 95%CI: 16.1-41.7). For all TBP and for coinfected nymphs, the estimated acarological risk was higher in La Mandria than in Stupinigi (Table 6).

Discussion

Our study describes the presence of Ixodid ticks and TBD agents in two natural parks located near the city of Turin and characterized by different ecosystems and ecological features.
Ticks and TBP were more abundant in La Mandria than in Stupinigi, and the difference is possibly explained by the diverse recent history of the parks. In fact, *I. ricinus* was deemed absent or very rare in the whole Turin province until the years 2000; however, its infestation on animals in La Mandria dates back to the 1980s at least, when Rossi and Meneguz (1989) highlighted its presence only inside this park and not in other study areas. We hypothesize that ticks have been introduced in La Mandria from other areas of Europe through the restocking of wildlife, finding here suitable conditions for their maintenance. This peculiarity persisted for years, since the park was enclosed and surrounded by roads and towns, with scarce possibility for wild ungulates to disperse. Thereby, tick infestation burdens in La Mandria are notoriously high, as it was confirmed by the results of our study.
The sampling site 03 recorded the highest density of *I. ricinus* nymphs. The site was characterized by white pine as the only tree species and no underwood; hence it could appear unsuitable for tick survival. However, this site has been chosen as resting place by red deer and fallow deer (park rangers, personal communication), thanks to the good sunlight exposure, the proximity of water streams, and to the distance from areas highly frequented by park visitors; this could explain the high tick density. Conversely, the site 04 -the closest site to 03, recorded the lowest tick density and prevalence of the whole park. Site 04 is an artificial implant of poplars, directly exposed to sunlight in the summer, surrounded by open pastures and close to a road; it is probably too dry and disturbed, and park rangers reported it as seldom frequented by large animals.
Stupinigi Park reflects the typical environment of peri-urban green areas, with cultivated fields, busy roads, and relative low presence of large wildlife. Human disturbance in this area likely limits the populations of wild ungulates, and therefore ticks abundance.
Wildlife frequentation thus seems an important factor for explaining the difference in tick burdens between sites. Indeed, the traces of wildlife have been identified as a useful parameter to estimate the risk of infestation (Rosà et al., 2018). Vertebrates are essential for ticks' maintenance and dispersal, and for the maintenance of disease cycles.
Trout Fryxell et al. (2015) stated that, in some environments, habitat and vegetation characteristics are not enough to predict tick presence, even if considering plant evenness, canopy openness and plant diversity. Nevertheless, tree species can be considered as indicators of the habitat since they are bound to suitable micro-climatic conditions, and they play a key role in modifying soil micro-climate as well. In our study, a positive association of trees with *I. ricinus* density was observed for hornbeam, as previously described in Italy (Bisanzio et al., 2008; Ceballos et al., 2014), while a negative association occurred for oaks.
Our findings indicate forests as the most suitable habitat for maintaining the ticks' populations, since tick occurrence was higher in wood sites than in ecotonal zones. On the other hand, the nymphs' density reached greater values in ecotonal zones than in forests, which could be explained by the inconstant co-existence of various hosts in narrow areas, leading to an amplification of the number of ticks (Mazurkiewicz and Rajska-Jurgiel, 1987).

The density of *I. ricinus* nymphs is related to many variables that vary with calendar period. It peaked in spring and followed the same trend described in other geographical areas in Turin province (Garcia-Vozmediano et al., 2020b). Since the main issue for ticks is avoiding dehydration, the questing behaviour is affected by variables related to air moisture, which are conveniently measured by remote sensing, and can be used to describe an homogeneous climate niche at small scale (Estrada-Peña et al., 2013). Among these variables, saturation deficit is deemed a good predictor of the probability of collecting *I. ricinus* specimens. In our study, except for extreme condition of frosting temperature or strong dryness, saturation deficit was more associated with the density of *I. ricinus* nymphs than to their occurrence. Thus, it is plausible to hypothesize that SD affects the duration of questing behaviour more than the ticks' survival. Very rainy spring months are usually associated with high ticks' density; in 2013, we indeed registered the highest values of nymphal density, following heavy rains that hindered the first dragging session. Hence, it can be assumed that meteorological conditions may play a role not immediately, but lagged, as previously reported (Ehrmann et al., 2017; Kjær et al., 2019; Perret et al., 2000). In this study, only measures lagged by 15 days were evaluated, since this was considered a sensible time interval.

The number of hours of light also varies with seasons, and the time of the day has been related to tick activity (Perret et al., 2003). However, we did not observe any association between small variations in hours of light and the density of *I. ricinus* nymphs.

Ixodes ricinus is deemed to be the main disease vector in Europe (Medlock et al., 2013). It was the most abundant species collected in both study areas and was infected by zoonotic bacteria. We identified three *B. burgdorferi* s.l. genospecies that cause Lyme disease in humans, and one whose pathogenicity is suspected (*B. lusitaniae*). The diversity of genospecies indicates the possible involvement of different vertebrate species as reservoir (mainly birds for *B. garinii*, rodents for *B. afzelii* and *B. burgdorferi* s.s., and lizards for *B. lusitaniae*; Mannelli et al., 2012). Also, *Ixodes ricinus* was infected by SFG rickettsiae, namely *R. monacensis*, the agent of Mediterranean spotted fever–like cases, and *R. helvetica*, responsible of uneruptive tick bite fever (Parola et al., 2013). Finally, we detected *A. phagocytophilum*, the agent of granulocytic anaplasmosis, which rarely cause clinical disease cases in Europe (Matei et al., 2019). Around 11% of *I. ricinus* were coinfected by at least two pathogens; this constitutes a risk for public health, since people infected by multiple pathogens may experience more severe and acute illness, with atypical clinical symptoms (Swanson et al., 2006).

The prevalence of infection by the three TBP is in line with those reported in Piedmont region (Garcia-Vozmediano et al., 2020b; Pintore et al., 2015), which suggests that it remained quite stable in the last ten years. The importance of historical data series in vector-borne zoonoses lies precisely in the ability to compare the prevalence of infestation and infection over time.

Fortunately, our tick sample was negative to TBE virus, showing that the virus likely did not reach the area by 2014. Nevertheless, continuous surveillance is needed because of the severity of TBE, and considering that the disease is endemic in North-eastern Italy and has spread in Switzerland in the past few years (Casati Pagani et al., 2019).

Due to *I. ricinus* willingness to bite humans, and to the fact that Lyme borreliosis –transmitted by *I.ricinus*– is the main zoonosis reported in Piedmont (https://www.seremi.it/), we only analysed this tick species for TBD agents. However, the other species collected can pose a health threat to visitors too, since they are recognized vectors of several viruses (including TBEV) and rickettsiales, and they are reported biting humans (Estrada-Peña et al., 2017). *Haemaphysalis concinna,* the second most collected species, adapts to a variety of habitats and animal hosts (Estrada-Peña et al., 2017). *Dermacentor* spp., that we collected in small numbers, are open-country tick species and they are considered emerging vectors in Europe (Rubel et al., 2016). *Dermacentor marginatus* is rather common in northern Italy, where adults are mainly associated to wild boar (Garcia-Vozmediano et al., 2020a). We also collected two *D. reticulatus* specimens; this species is typical of central Europe, where it is reported in urban and suburban areas (Rubel et al., 2016). Our findings confirm its recent occasional collection south of the Alps, including in northern Italy and Turin hillside (Garcia-Vozmediano et al., 2020a).

The acarological risk estimates the probability of finding at least one infected *I. ricinus* nymph in 100 m^2 by considering the environment alone. Therefore, it overestimates the risk of being bitten by an infected nymph unless it is combined with a measure of human exposure (Millet et al., 2019). Acarological risk was worrisome in La Mandria, but most of the dragging sites were in wooded areas restricted to the public. This means that the risk is overestimated for the general population, but likely correct for the rangers who frequent those areas daily. On the other hand, none of the dragging sites in Stupinigi were restricted to public, but the tick burden and the infection rate were lower, with a consequent smaller hazard. Accordingly, in recent years forest rangers reported more and more tick bites in La Mandria but not in Stupinigi. This also indicates the particular risk of developing TBD in conditions of high exposure to ticks, like working in woodlands; assessing the risk for occasional visitors is more difficult, due to different behaviours that affect the exposure to ticks and the fruition of the park.

Conclusion

Climate and environmental changes, and an increase in wildlife populations, have been shown to affect tick abundance, and favour the introduction of tick vectors in new areas, as well as the appearance of new tick species (Medlock et al., 2013).

Peri-urban areas are ever-increasingly affected by vector expansion (Grochowska et al., 2020), thanks to interventions on the urban green to mitigate urban heat islands and restore biodiversity (Braks et al., 2019). Green areas within and around the cities exert a positive effect on the quality of life and the citizens' health (Cetin, 2015). The COVID-19 pandemic not only revealed the necessity to make cities more liveable, but also the danger of the close interaction with wild animal species. So, the risk of exposure to wildlife-associated pathogens should be considered when planning at the urban scale. For example, Piedmont Region is planning requalification projects in Stupinigi to remove most of the traffic, which will make this protected area more "natural": such projects will likely favour an increase of the tick density in the years to come.

In the light of the increasing frequentation of urban and peri-urban green spaces, educational programs on personal protection are of key importance, in order to avoid tick bites and pathogen transmission (Braks et al., 2019). Although TBD remain quite rare diseases in Italy and other parts of Europe, the geographical expansion of ticks and the increasing number of reported tick bites highlight the need of enhancing surveillance and risk-communication programs.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors are very grateful to the Royal Parks' personnel for the logistic support, in particular to P.Stocco and L.D'Angelo. We thank S.Bessone for helping in fieldwork. This work was carried out in the framework of the Post-graduate Master Course in Epidemiology of the University of Turin.

CRediT authorship contribution statement

A.Bellato: methodology, software, validation, formal analysis, investigation, data curation, writing - original draft, visualization; M.D.Pintore: investigation, data curation, resources; D.Catelan: methodology, software, formal analysis, investigation, writing - original draft, visualization; A.Pautasso: data curation; A.Torina, F.Rizzo, M.L. Mandola: investigation; A.Mannelli:

conceptualization, methodology, writing - review & editing; C.Casalone: project administration; funding acquisition; L.Tomassone: conceptualization, methodology, investigation, supervision, writing - original draft.

References

European Environmental Agency (EEA), 2017. Landscapes in transition - An account of 25 years of land cover change in Europe. EEA Report No. 10/2017, ISSN 1977-8449. https://doi.org/10.2800/81075

Alonso-Carné, J., García-Martín, A., Estrada-Peña, A., 2016. Modelling the Phenological Relationships of Questing Immature Ixodes Ricinus (Ixodidae) Using Temperature and NDVI Data. Zoonoses Public Health 63, 40–52. https://doi.org/10.1111/zph.12203

Amore, G., Tomassone, L., Grego, E., Ragagli, C., Bertolotti, L., Nebbia, P., Rosati, S., Mannelli, A., 2007. Borrelia lusitaniae in Immature Ixodes ricinus (Acari: Ixodidae) feeding on common wall lizards in Tuscany, central Italy. J. Med. Entomol. 44, 303–307. https://doi.org/10.1093/jmedent/44.2.303

Antrop, M., 2004. Landscape change and the urbanization process in Europe. Landsc. Urban Plan., Development of European Landscapes 67, 9–26. https://doi.org/10.1016/S0169-2046(03)00026-4

Benjamini, Y., Yekutieli, D., 2005. False Discovery Rate–Adjusted Multiple Confidence Intervals for Selected Parameters. J. Am. Stat. Assoc. 100, 71–81. https://doi.org/10.1198/016214504000001907

Bisanzio, D., Amore, G., Ragagli, C., Tomassone, L., Bertolotti, L., Mannelli, A., 2008. Temporal variations in the usefulness of normalized difference vegetation index as a predictor for Ixodes ricinus (Acari: Ixodidae) in a Borrelia lusitaniae focus in Tuscany, central Italy. J. Med. Entomol. 45, 547–555. https://doi.org/10.1603/0022-2585(2008)45[547:tvituo]2.0.co;2

Braks, M., Giglio, G., Tomassone, L., Sprong, H., Leslie, T., 2019. Making Vector-Borne Disease Surveillance Work: New Opportunities From the SDG Perspectives. Front. Vet. Sci. 6. https://doi.org/10.3389/fvets.2019.00232

Carugno, M., Consonni, D., Randi, G., Catelan, D., Grisotto, L., Bertazzi, P.A., Biggeri, A., Baccini, M., 2016. Air pollution exposure, cause-specific deaths and hospitalizations in a highly polluted Italian region. Environ. Res. 147, 415–424. https://doi.org/10.1016/j.envres.2016.03.003

Casati Pagani, S., Frigerio Malossa, S., Klaus, C., Hoffmann, D., Beretta, O., Bomio-Pacciorini, N., Lazzaro, M., Merlani, G., Ackermann, R., Beuret, C., 2019. First detection of TBE virus in ticks and sero-reactivity in goats in a non-endemic region in the southern part of Switzerland (Canton of Ticino). Ticks Tick-Borne Dis. 10, 868–874. https://doi.org/10.1016/j.ttbdis.2019.04.006

Ceballos, L.A., Pintore, M.D., Tomassone, L., Pautasso, A., Bisanzio, D., Mignone, W., Casalone, C., Mannelli, A., 2014. Habitat and occurrence of ixodid ticks in the Liguria region, northwest Italy. Exp. Appl. Acarol. 64, 121–135. https://doi.org/10.1007/s10493-014-9794-y

Cetin, M., 2015. Using GIS analysis to assess urban green space in terms of accessibility: case study in Kutahya. Int. J. Sustain. Dev. World Ecol. 22, 420–424. https://doi.org/10.1080/13504509.2015.1061066

Craine, N.G., Randolph, S.E., Nuttall, P.A., 1995. Seasonal variation in the role of grey squirrels as hosts of Ixodes ricinus, the tick vector of the Lyme disease spirochaete, in a British woodland. Folia Parasitol. (Praha) 42, 73–80.

Ehrmann, S., Liira, J., Gärtner, S., Hansen, K., Brunet, J., Cousins, S.A.O., Deconchat, M., Decocq, G., De Frenne, P., De Smedt, P., Diekmann, M., Gallet-Moron, E., Kolb, A., Lenoir, J., Lindgren, J., Naaf, T., Paal, T., Valdés, A., Verheyen, K., Wulf, M., Scherer-Lorenzen, M., 2017. Environmental drivers of Ixodes ricinus abundance in forest fragments of rural European landscapes. BMC Ecol. 17. https://doi.org/10.1186/s12898-017-0141-0

Eremeeva, M.E., Dasch, G.A., Silverman, D.J., 2003. Evaluation of a PCR Assay for Quantitation of Rickettsia rickettsii and Closely Related Spotted Fever Group Rickettsiae. J. Clin. Microbiol. 41, 5466–5472. https://doi.org/10.1128/JCM.41.12.5466-5472.2003

Estrada-Peña, A., D'Amico, G., Palomar, A.M., Dupraz, M., Fonville, M., Heylen, D., Habela, M.A., Hornok, S., Lempereur, L., Madder, M., Núncio, M.S., Otranto, D., Pfaffle, M., Plantard, O., Santos-Silva, M.M., Sprong, H., Vatansever, Z., Vial, L., Mihalca, A.D., 2017. A comparative test of ixodid tick identification by a network of European researchers. Ticks Tick-Borne Dis. 8, 540–546. https://doi.org/10.1016/j.ttbdis.2017.03.001

Estrada-Peña, A., Estrada-Sánchez, A., Estrada-Sánchez, D., de la Fuente, J., 2013. Assessing the effects of variables and background selection on the capture of the tick climate niche. Int. J. Health Geogr. 12, 43. https://doi.org/10.1186/1476-072X-12-43

Garcia-Vozmediano, A., Giglio, G., Ramassa, E., Nobili, F., Rossi, L., Tomassone, L., 2020a. Dermacentor marginatus and Dermacentor reticulatus, and Their Infection by SFG Rickettsiae and Francisella-Like Endosymbionts, in Mountain and Periurban Habitats of Northwestern Italy. Vet. Sci. 7. https://doi.org/10.3390/vetsci7040157

Garcia-Vozmediano, A., Krawczyk, A.I., Sprong, H., Rossi, L., Ramassa, E., Tomassone, L., 2020b. Ticks climb the mountains: Ixodid tick infestation and infection by tick-borne pathogens in the Western Alps. Ticks Tick-Borne Dis. 11, 101489. https://doi.org/10.1016/j.ttbdis.2020.101489

Grochowska, A., Milewski, R., Pancewicz, S., Dunaj, J., Czupryna, P., Milewska, A.J., Róg-Makal, M., Grygorczuk, S., Moniuszko-Malinowska, A., 2020. Comparison of tick-borne pathogen prevalence in Ixodes ricinus ticks collected in urban areas of Europe. Sci. Rep. 10, 6975. https://doi.org/10.1038/s41598-020-63883-y

Hansford, K.M., Fonville, M., Gillingham, E.L., Coipan, E.C., Pietzsch, M.E., Krawczyk, A.I., Vaux, A.G.C., Cull, B., Sprong, H., Medlock, J.M., 2017. Ticks and Borrelia in urban and peri-urban green space habitats in a city in southern England. Ticks Tick-Borne Dis. 8, 353–361. https://doi.org/10.1016/j.ttbdis.2016.12.009

Hubálek, Z., Anderson, J.F., Halouzka, J., Hájek, V., 1996. Borreliae in immature Ixodes ricinus (Acari:Ixodidae) ticks parasitizing birds in the Czech Republic. J. Med. Entomol. 33, 766–771. https://doi.org/10.1093/jmedent/33.5.766

Humair, P.F., Postic, D., Wallich, R., Gern, L., 1998. An avian reservoir (Turdus merula) of the Lyme borreliosis spirochetes. Zentralblatt Bakteriol. Int. J. Med. Microbiol. 287, 521–538.

Kjær, L.J., Soleng, A., Edgar, K.S., Lindstedt, H.E.H., Paulsen, K.M., Andreassen, Å.K., Korslund, L., Kjelland, V., Slettan, A., Stuen, S., Kjellander, P., Christensson, M., Teräväinen, M., Baum, A., Klitgaard, K., Bødker, R., 2019. Predicting and mapping human risk of exposure to Ixodes ricinus nymphs using climatic and environmental data, Denmark, Norway and Sweden, 2016. Eurosurveillance 24, 1800101. https://doi.org/10.2807/1560-7917.ES.2019.24.9.1800101

Kowalec, M., Szewczyk, T., Welc-Falęciak, R., Siński, E., Karbowiak, G., Bajer, A., 2017. Ticks and the city - are there any differences between city parks and natural forests in terms of tick abundance and prevalence of spirochaetes? Parasit. Vectors 10, 573. https://doi.org/10.1186/s13071-017-2391-2

Kubiak, K., Dziekońska-Rynko, J., Szymańska, H., Kubiak, D., Dmitryjuk, M., Dzika, E., 2019. Questing Ixodes ricinus ticks (Acari, Ixodidae) as a vector of Borrelia burgdorferi sensu lato and Borrelia miyamotoi in an urban area of north-eastern Poland. Exp. Appl. Acarol. 78, 113–126. https://doi.org/10.1007/s10493-019-00379-z

Lejal, E., Marsot, M., Chalvet-Monfray, K., Cosson, J.-F., Moutailler, S., Vayssier-Taussat, M., Pollet, T., 2019. A three-years assessment of Ixodes ricinus-borne pathogens in a French peri-urban forest. Parasit. Vectors 12. https://doi.org/10.1186/s13071-019-3799-7

Mannelli, A., Bertolotti, L., Gern, L., Gray, J., 2012. Ecology of Borrelia burgdorferi sensu lato in Europe: Transmission dynamics in multi-host systems, influence of molecular processes and effects of climate change. FEMS Microbiol. Rev. 36, 837–861. https://doi.org/10.1111/j.1574-6976.2011.00312.x

Mannelli, A., Boggiatto, G., Grego, E., Cinco, M., Murgia, R., Stefanelli, S., Meneghi, D.D., Rosati, S., 2003. Acarological risk of exposure to agents of tick-borne zoonoses in the first recognized Italian focus of Lyme borreliosis. Epidemiol. Infect. 131, 1139–1147. https://doi.org/10.1017/S0950268803001328

Marchant, A., Coupanec, A.L., Joly, C., Perthame, E., Sertour, N., Garnier, M., Godard, V., Ferquel, E., Choumet, V., 2017. Infection of Ixodes ricinus by Borrelia burgdorferi sensu lato in peri-urban forests of France. PLOS ONE 12, e0183543. https://doi.org/10.1371/journal.pone.0183543

Marconi, R.T., Garon, C.F., 1992. Development of polymerase chain reaction primer sets for diagnosis of Lyme disease and for species-specific identification of Lyme disease isolates by 16S rRNA signature nucleotide analysis. J. Clin. Microbiol. 30, 2830–2834.

Matei, I.A., Estrada-Peña, A., Cutler, S.J., Vayssier-Taussat, M., Varela-Castro, L., Potkonjak, A., Zeller, H., Mihalca, A.D., 2019. A review on the eco-epidemiology and clinical management of human granulocytic anaplasmosis and its agent in Europe. Parasit. Vectors 12, 599. https://doi.org/10.1186/s13071-019-3852-6

Mazurkiewicz, M., Rajska-Jurgiel, E., 1987. Numbers, species composition and residency of a rodent community in forest and field-forest ecotones. Acta Theriol. (Warsz.) 32, 413–432. https://doi.org/10.4098/AT.arch.87-29

Medlock, J.M., Hansford, K.M., Bormane, A., Derdakova, M., Estrada-Peña, A., George, J.-C., Golovljova, I., Jaenson, T.G., Jensen, J.-K., Jensen, P.M., Kazimirova, M., Oteo, J.A., Papa, A., Pfister, K., Plantard, O., Randolph, S.E., Rizzoli, A., Santos-Silva, M.M., Sprong, H., Vial, L., Hendrickx, G., Zeller, H., Van Bortel, W., 2013. Driving forces for changes in geographical distribution of Ixodes ricinus ticks in Europe. Parasit. Vectors 6, 1. https://doi.org/10.1186/1756-3305-6-1

Millet, I., Ragionieri, M., Tomassone, L., Trentin, C., Mannelli, A., 2019. Assessment of the Exposure of People to Questing Ticks Carrying Agents of Zoonoses in Aosta Valley, Italy. Vet. Sci. 6. https://doi.org/10.3390/vetsci6010028

Oechslin, C.P., Heutschi, D., Lenz, N., Tischhauser, W., Péter, O., Rais, O., Beuret, C.M., Leib, S.L., Bankoul, S., Ackermann-Gäumann, R., 2017. Prevalence of tick-borne pathogens in questing Ixodes ricinus ticks in urban and suburban areas of Switzerland. Parasit. Vectors 10, 558. https://doi.org/10.1186/s13071-017-2500-2

Olivieri, E., Gazzonis, A.L., Zanzani, S.A., Veronesi, F., Manfredi, M.T., 2017. Seasonal dynamics of adult Dermacentor reticulatus in a peri-urban park in southern Europe. Ticks Tick-Borne Dis. 8, 772–779. https://doi.org/10.1016/j.ttbdis.2017.06.002

Olsén, B., Jaenson, T.G., Bergström, S., 1995. Prevalence of Borrelia burgdorferi sensu lato-infected ticks on migrating birds. Appl. Environ. Microbiol. 61, 3082–3087. https://doi.org/10.1128/AEM.61.8.3082-3087.1995

Parola, P., Paddock, C.D., Socolovschi, C., Labruna, M.B., Mediannikov, O., Kernif, T., Abdad, M.Y., Stenos, J., Bitam, I., Fournier, P.-E., Raoult, D., 2013. Update on tick-borne rickettsioses around the world: A geographic approach. Clin. Microbiol. Rev. 26, 657–702. https://doi.org/10.1128/CMR.00032-13

Perret, J.-L., Guerin, P.M., Diehl, P.A., Vlimant, M., Gern, L., 2003. Darkness induces mobility, and saturation deficit limits questing duration, in the tick Ixodes ricinus. J. Exp. Biol. 206, 1809–1815. https://doi.org/10.1242/jeb.00345

Perret, J.L., Guigoz, E., Rais, O., Gern, L., 2000. Influence of saturation deficit and temperature on Ixodes ricinus tick questing activity in a Lyme borreliosis-endemic area (Switzerland). Parasitol. Res. 86, 554–557. https://doi.org/10.1007/s004360000209

Pintore, M.D., Ceballos, L., Iulini, B., Tomassone, L., Pautasso, A., Corbellini, D., Rizzo, F., Mandola, M.L., Bardelli, M., Peletto, S., Acutis, P.L., Mannelli, A., Casalone, C., 2015. Detection of Invasive Borrelia burgdorferi Strains in North-Eastern Piedmont, Italy. Zoonoses Public Health 62, 365–374. https://doi.org/10.1111/zph.12156

Randolph, S.E., Storey, K., 1999. Impact of Microclimate on Immature Tick-Rodent Host Interactions (Acari: Ixodidae): Implications for Parasite Transmission. J. Med. Entomol. 36, 741–748. https://doi.org/10.1093/jmedent/36.6.741

Rijpkema, S.G., Molkenboer, M.J., Schouls, L.M., Jongejan, F., Schellekens, J.F., 1995. 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. 33, 3091–3095.

Rizzoli, A., Silaghi, C., Obiegala, A., Rudolf, I., Hubálek, Z., Földvári, G., Plantard, O., Vayssier-Taussat, M., Bonnet, S., Spitalská, E., Kazimírová, M., 2014. Ixodes ricinus and Its Transmitted Pathogens in Urban and Peri-Urban Areas in Europe: New Hazards and Relevance for Public Health. Front. Public Health 2, 251. https://doi.org/10.3389/fpubh.2014.00251

Rosà, R., Andreo, V., Tagliapietra, V., Baráková, I., Arnoldi, D., Hauffe, H.C., Manica, M., Rosso, F., Blaňarová, L., Bona, M., Derdáková, M., Hamšíková, Z., Kazimírová, M., Kraljik, J., Kocianová, E., Mahríková, L., Minichová, L., Mošanský, L., Slovák, M., Stanko, M., Špitalská, E., Ducheyne, E., Neteler, M., Hubálek, Z., Rudolf, I., Venclikova, K., Silaghi, C., Overzier, E., Farkas, R., Földvári, G., Hornok, S., Takács, N., Rizzoli, A., 2018. Effect of Climate and Land Use on the Spatio-Temporal Variability of Tick-Borne Bacteria in Europe. Int. J. Environ. Res. Public. Health 15. https://doi.org/10.3390/ijerph15040732

Rubel, F., Brugger, K., Pfeffer, M., Chitimia-Dobler, L., Didyk, Y.M., Leverenz, S., Dautel, H., Kahl, O., 2016. Geographical distribution of Dermacentor marginatus and Dermacentor reticulatus in Europe. Ticks Tick-Borne Dis. 7, 224–233. https://doi.org/10.1016/j.ttbdis.2015.10.015

Rudenko, N., Golovchenko, M., Cihlárova, V., Grubhoffer, L., 2004. Tick-borne encephalitis virus-specific RT-PCR - A rapid test for detection of the pathogen without viral RNA purification. Acta Virol. 48, 167–71.

Rymaszewska, A., Adamska, M., 2011. Molecular evidence of vector-borne pathogens coinfecting dogs from Poland. Acta Vet. Hung. 59, 215–223. https://doi.org/10.1556/AVet.2011.008

Selmi, M., Tomassone, L., Ceballos, L.A., Crisci, A., Ragagli, C., Pintore, M.D., Mignone, W., Pautasso, A., Ballardini, M., Casalone, C., Mannelli, A., 2018. Analysis of the environmental and host-related factors affecting the distribution of the tick Dermacentor marginatus. Exp. Appl. Acarol. 75, 209–225. https://doi.org/10.1007/s10493-018-0257-8

Snep, R.P.H., Opdam, P.F.M., Baveco, J.M., WallisDeVries, M.F., Timmermans, W., Kwak, R.G.M., Kuypers, V., 2006. How peri-urban areas can strengthen animal populations within cities: A modeling approach. Biol. Conserv., Urbanization 127, 345–355. https://doi.org/10.1016/j.biocon.2005.06.034

Sprong, H., Azagi, T., Hoornstra, D., Nijhof, A.M., Knorr, S., Baarsma, M.E., Hovius, J.W., 2018. Control of Lyme borreliosis and other Ixodes ricinus -borne diseases. Parasit. Vectors 11, 145. https://doi.org/10.1186/s13071-018-2744-5

Süss, J., 2011. Tick-borne encephalitis 2010: Epidemiology, risk areas, and virus strains in Europe and Asia—An overview. Ticks Tick-Borne Dis. 2, 2–15. https://doi.org/10.1016/j.ttbdis.2010.10.007

Swanson, S.J., Neitzel, D., Reed, K.D., Belongia, E.A., 2006. Coinfections Acquired from Ixodes Ticks. Clin. Microbiol. Rev. 19, 708–727. https://doi.org/10.1128/CMR.00011-06

Tagliapietra, V., Rosà, R., Arnoldi, D., Cagnacci, F., Capelli, G., Montarsi, F., Hauffe, H.C., Rizzoli, A., 2011. Saturation deficit and deer density affect questing activity and local abundance of Ixodes ricinus (Acari, Ixodidae) in Italy. Vet. Parasitol. 183, 114–124. https://doi.org/10.1016/j.vetpar.2011.07.022

Torina, A., Blanda, V., Blanda, M., Auteri, M., La Russa, F., Scimeca, S., D'Agostino, R., Disclafani, R., Villari, S., Currò, V., Caracappa, S., 2018. A Geographical Information System Based Approach for Integrated Strategies of Tick Surveillance and Control in the Peri-Urban Natural Reserve of Monte Pellegrino (Palermo, Southern Italy). Int. J. Environ. Res. Public. Health 15. https://doi.org/10.3390/ijerph15030404

Figure 1. Geographical characteristics of the study area are presented along with the administrative boundaries of the parks and the urban area of Turin. Sites' location is identified by the numbers from 1 to 15, and for each one the average of I. ricinus nymphs' density is reported.

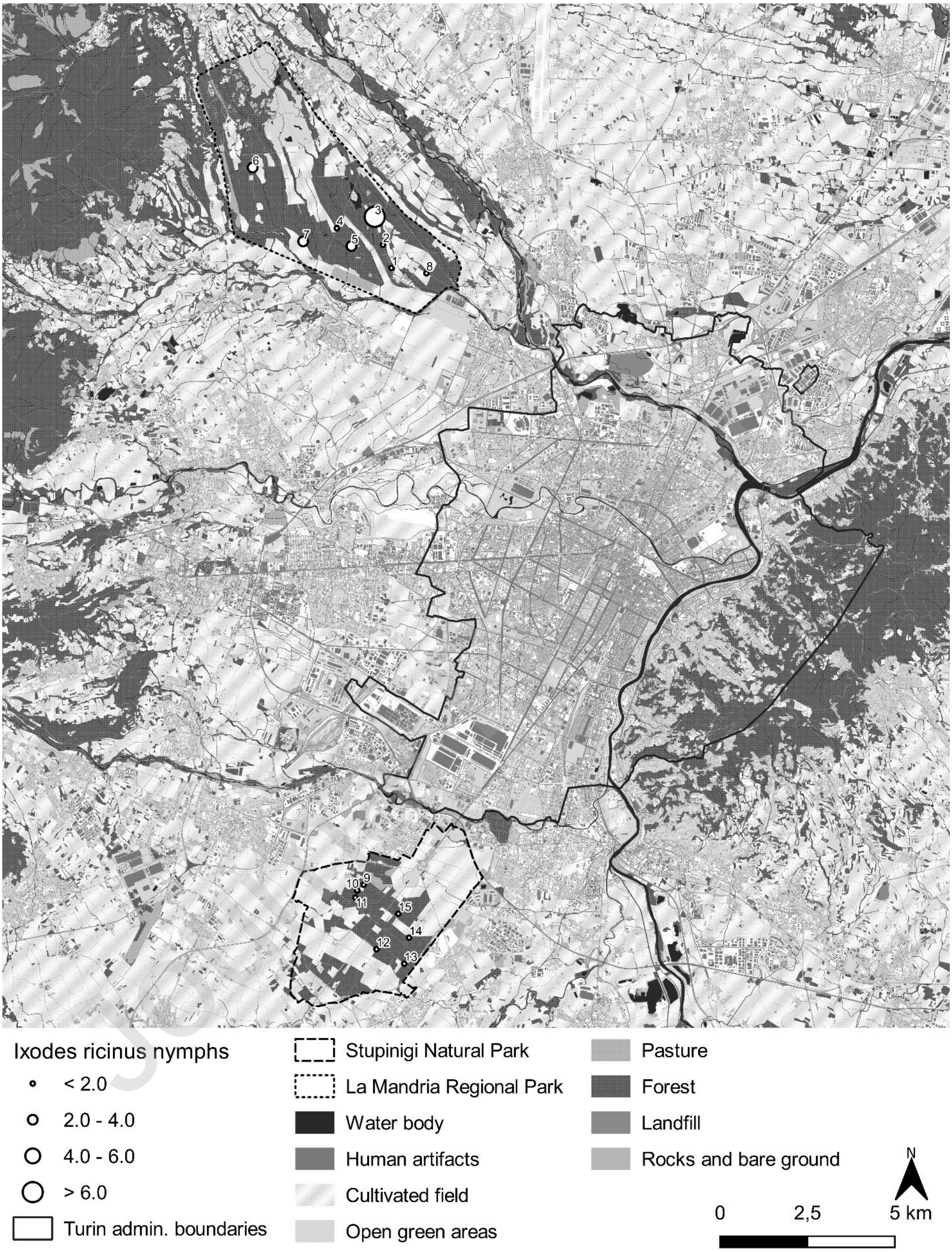
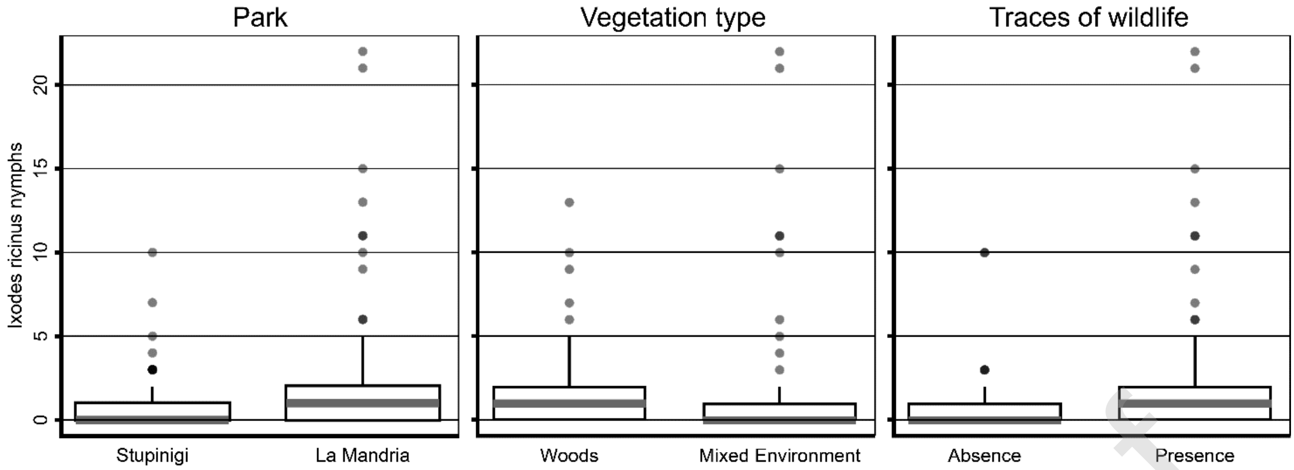


Figure 2. Box plot of the density of I. ricinus nymphs/100m^2 by park, vegetation type and traces of large wildlife.



Park	Site	Alt. (m)	Predominant tree species	Phytotypes	Prev. % (obs./N)	Mean ± std.dev.	Median	Min – Max
La Mandria Regional Park	01	340	Ash	Plain oak forest	52.9 (9/17)	1.3 ± 1.6	1	0-5
	02	333	Red oak	Monospecific artificial implant	41.2 (7/17)	0.8 ± 1.1	0	0-3
	03	350	White pine	Monospecific artificial implant	82.4 (14/17)	6.7 ± 7.2	4	0-22
	04	363	Poplar	Monospecific artificial implant	5.9 (1/17)	0.1 ± 0.5	0	0-2
	05	355	Swamp Spanish oak	Two-species artificial implant	58.8 (10/17)	1.4 ± 1.4	1	0-4
	06	396	Hornbeam	Plain oak forest	82.4 (14/17)	2.9 ± 3.4	2	0-13
	07	338	Hornbeam	Plain oak forest	58.8 (10/17)	1.2 ± 1.4	1	0-5
	08	382	Hazel	Plain oak forest	64.7 (11/17)	1.5 ± 1.7	1	0-6
TOT.					55.9 (76/136)	2.0 ± 3.5	1	0-22
Stupinigi Natural Park	09	257	Hazel	Plain oak forest	13.3 (2/15)	0.7 ± 2.6	0	0-10
	10	259	Hazel	Plain oak forest	26.7 (4/15)	0.3 ± 0.6	0	0-2
	11	262	Ash	Plain oak forest	50.0 (6/12)	0.7 ± 0.8	0.5	0-2
	12	256	Hornbeam	Plain oak forest	71.4 (10/14)	1.4 ± 1.3	1	0-4
	13	254	Oak	Plain oak forest	21.4 (3/14)	0.3 ± 0.6	0	0-2
	14	257	Red oak	Plain oak forest	21.4 (3/14)	0.3 ± 0.6	0	0-2
	15	261	Hazel	Plain oak forest	71.4 (10/14)	2.0 ± 2.1	1.5	0-7
TOT.					38.8 (38/98)	0.8 ± 1.5	0	0-10

Table 1. Environmental characteristics of dragging sites, prevalence of I. ricinus nymphs, and mean, median, min. and max. density of I. ricinus nymphs / 100 m^2. [Alt.: Altitude in metres above sea level; Prev.: Prevalence of transects positive for the occurrence of I. ricinus nymphs; std. dev.: standard deviation].

Month	Occurrence of *I. ricinus* nymphs (%)	OR (95% CI)	*I. ricinus* nymphs' density/100m^2	IDR (95% CI)
April	65.5 ± 48.4	0.5 (0.2-1.4)	1.8 ± 2.3	0.6 (0.4-0.8)
May	77.5 ± 42.3	1 (ref.)	2.9 ± 4.4	1 (ref.)
June	60.9 ± 49.9	0.5 (0.1-1.4)	2.0 ± 3.0	0.7 (0.5-1.0)
July	58.3 ± 49.7	0.4 (0.2-1.0)	1.9 ± 3.3	0.7 (0.5-0.9)
August	23.7 ± 43.1	0.1 (0.0-0.3)	0.5 ± 0.9	0.2 (0.1-0.3)
September	11.1 ± 31.9	0.0 (0.0-0.1)	0.1 ± 0.3	0.0 (0.0-0.1)
October*	25.0 ± 46.3	0.1 (0.0-0.6)	0.3 ± 0.5	0.1 (0.0-0.3)

Table 2. For occurrence and density of I. ricinus nymphs, monthly means are reported with standard deviations. Odds Ratios and Incidence Density Ratios for monthly measures compared to May are reported along with their Confidence Interval [: only La Mandria was sampled in October 2014; OR = Odds Ratio; IDR = Incidence Density Ratio; CI = Confidence Intervals].*

Saturation Deficit	La Mandria	Stupinigi	OR	95% CI	IDR	95% CI
Minimum	0.9 ± 0.6	1.2 ± 0.9	0.4	0.2-0.8	0.5	0.3-0.6
Average	4.3 ± 1.5	4.8 ± 1.3	4.0	1.8-8.6	2.9	2.1-3.9
Maximum	6.0 ± 1.5	6.7 ± 1.4	0.2	0.2-0.5	0.4	0.3-0.5

Table 3. The average, minimum and maximum saturation deficit (SD) by parks are reported as mean and standard deviation. Odds Ratios and Incidence Density Ratios estimated by univariate analysis on occurrence and density, respectively. The OR and the IDR show the effect of a 1 mmHg increase in SD mean over a 15-

days period before the sampling date [OR = Odds Ratio; IDR = Incidence Density Ratio; CI = Confidence Intervals].

Variable	**Occurrence of *I. ricinus* nymphs**		**Density of *I. ricinus* nymphs/100m^2**	
	OR	**95% CI**	**IDR**	**95% CI**
Late-spring NDVI	0.02	0.00-0.58	0.22	0.05-0.99
Avg. daily SD (15DbS)	0.52	0.31-0.87	0.77	0.61-0.98
Traces of wildlife	1.66	0.66-4.19	2.19	1.34-3.58

Table 4. Results of the final binomial random-effects GLM for the occurrence of I. ricinus nymphs (left) and negative binomial GLM for density of I. ricinus nymphs (right) [OR = Odds Ratio; IDR = Incidence Density Ratio; CI. = Confidence Intervals].

.

	B. burgdorferi s.l.		*Rickettsia* spp.		*Anaplasma* spp.		Coinfected *I. ricinus*	
Park	**Prev.**	**95% CI**	**Prev.**	**95% CI**	**Prev.**	**95% CI**	**Prev.**	**95% CI**
La Mandria	28.5	23.3-33.7	37.2	31.6-42.7	2.9	1.2-4.6	13.9	9.9-17.9
Stupinigi	9.9	3.8-16.0	9.9	3.8-16.0	0.0	-	3.3	0.0-7.0

Table 5. Prevalence (%) of infected I. ricinus ticks in the two parks. Aggregated infection prevalence by Borrelia burgdorferi s.l., *Rickettsia spp., Anaplasma spp. and of concurrent infection by at least two different pathogens are reported [Prev. = Prevalence; CI = Confidence Interval].*

	B. burgdorferi s.l.		*Rickettsia* spp.		*Anaplasma* spp.		Coinfected *I. ricinus*	
Park	**AR**	**95% CI**	**AR**	**95% CI**	**AR**	**95% CI**	**AR**	**95% CI**
La Mandria	59.1	40.7-78.3	71.3	50.0-89.5	12.0	5.7-24.2	36.9	23.9-54.0
Stupinigi	15.2	8.1-27.3	17.4	10.6-27.9	0.0	-	6.8	2.5-18.1

Table 6. Estimated acarological risk (%) by park, i.e. the cumulative risk of finding at least one I. ricinus nymph which is infected by B. burgdorferi s.l., Rickettsia spp., Anaplasma spp. or by two or more of them, in 100m^2 [AR = Acarological Risk; CI = Confidence Interval].