

SUMMARY

Detection and identification of zoonotic pathogens in ticks collected in urban parks of Rome Italy

Key words: urban parks, tick-borne diseases, Rome

Aim

The aim of this research project is the detection of zoonotic tick-borne pathogens (*Rickettsia* spp., *Coxiella burnetii*, *Borrelia burgdorferi* s.l. and *Piroplasmida*) in urban parks of Rome city (Central Italy) by molecular tests on free-living ticks and ticks removed from domestic animals. Risk of been infected by the investigated pathogens for humans was assessed. The relationship between above-mentioned pathogens and tick species has been also investigated.

Materials and Methods

We selected parks, reserves and villas inside the Rome city that met the following criteria: area of at least 100 ha, free accessible by people, characterized by the presence of a pathway network and considered important recreation areas for many inhabitants of Rome. Parks and reserves belong to a network of green areas with some of them harbouring livestock together with wild fauna; selected villas are instead surrounded by walls and do not harbour livestock.

Questing ticks were collected using a standardized dragging method. Sampling was carried out from April to September, in 2018 and 2019, every ten days. Ticks were morphologically classified into family, genus and species using taxonomic keys. After morphological identification, ticks were individually stored in vials with 70% ethanol addicted with 5% glycerol until DNA extraction and testing. Genomic DNA was extracted using an automatic DNA extractor. The presence of *Rickettsia* spp., *C. burnetii*, *Borrelia burgdorferi* s.l. and *Piroplasmida* was investigated by Polymerase Chain Reaction (PCR) detecting the following targets: citrate synthase gene (*gltA*) common to *Rickettsia* genus; *ompA* and *ompB* genes encoding rickettsial outer membrane proteins A (rOmpA) and B (rOmpB) common to Spotted Fever Group (SFG) and Typhus Group (TG) *Rickettsiae*; Hypothetical protein gene RC0338 di *Rickettsia* spp; *IS1111* gene associated to *heat shock-inducible* htpAB operon of *C. burnetii*; *16S rRNA* gene of *Borrelia burgdorferi* s.l. and *18S rRNA* of *Piroplasmida*. Amplicons of *gltA*, *ompA*, *ompB* and *18S rRNA* were purified and sequenced to identify *Rickettsia* and piroplasms species. The obtained sequences were aligned and analyzed for nucleotide sequence identity by comparing them with reference strains of *Rickettsia* and *Piroplasmida* in GenBank database using the Basic Local Alignment Search Tool (BLAST).

Results

A total of 93 ticks were collected: 65 by mean of dragging method, in five out of 11 surveyed green areas, 28 were collected on infested animals. Collected ticks belonged to five species: *Rhipicephalus bursa* (42%), *Ixodes ricinus* (27%), *Rhipicephalus turanicus* (23%), *Rhipicephalus sanguineus* (7%) and *Hyalomma marginatum marginatum* (1%). The highest number of tick species was recorded in the Parco regionale dell'Appia antica, even though with few collected individuals. Most of the questing ticks were collected in the Parco regionale urbano del Pineto, which is the

green area with the highest density of questing ticks, together with the peri-urban park Riserva naturale di Nomentum. Highest values were reported, respectively, during April-May and September for the first green area, June, July and August for the latter. The following pathogens were detected: *R. massiliae* (8,6%), *R. monacensis* (6,5%), *Theileria ovis* (2,7%), *Rickettsia* spp (2,2%) e *R. aeschlimannii* (1,1%). The total prevalence of *Rickettsia* spp. was 18,3% while *Coxiella burnetii* e *Borrelia burgdorferi* s.l. were not detected. The ticks infected by *Rickettsiae* and *Piroplasmida* were found in the following parks: Appia Antica, Parco Regionale urbano del Pineto, Tenuta di Tormarancia, Valle Dei Casali e Tenuta dei Massimi.

Discussion and Conclusions

The results obtained by this research project allow to perform an acarological and infectivological risk assessment for urban green areas of Rome city. Our results allowed us to draw up some recommendation to those parks where ticks and tick-borne pathogens were more abundant or relevant for public health. Firstly, we urged park authorities to program weed removal, grass cut along pathways and general cleaning actions even during spring and early autumn; secondly to provide appropriate signage to inform park users on the use of protective clothing and repellents, avoidance of tick habitats and employ adequate hygiene measures. Furthermore, family doctors and pediatricians should be informed in order to be able to include Spotted Fever group rickettsiae in the differential diagnosis in patients with fever of unknown origin who attended urban parks during the period of vector activity.