RESEARCH ACTIVITY ON TICK-BORNE DISEASES IN LATIUM REGION

Luciano Toma

Department of Infectious, Parasitic and Immune-mediated Diseases, Istituto Superiore di Sanità, Rome

Introduction

Ticks (Ixodida: *Ixodidae*) are arthropods belonging to Chelicerata Subphylum, Aracnida Class, as spiders, scorpions and mites. About 900 species of ticks are known in the world and all of them are ectoparasites. Moreover such Arthropods are obligatory aematophagous as they stay on the host for a certain period of time for the bloodmeal essential for growth and reproduction. Italian Fauna ranges 2 tick families, *Ixodidae* or "hard ticks" (ranged in 6 genera: *Ixodes, Boophilus, Hyalomma, Rhipicephalus, Dermacentor, Haemaphysalis*), and Argasidae or "soft ticks" (ranged in 2 genera *Argas* and *Ornithodoros*) to which 36 species belong (Checklist of Italian Fauna). In the last decades, Tick-Borne Diseases (TBD) are dramatically spreading.

The growth of global relations and global warming are a health threat to the introduction and spread of vector-borne diseases in Europe, such as TBD. Among the arthropods, ticks are highly efficient vectors of many pathogens of viral, bacterial and protozoan nature that cause diseases now considered emerging basing on the increased reported cases in animals and humans.

Recently, also in Italy as elsewhere, the chances for humans to contract TBD increased and in particular the high number of dogs as pet taken in public parks, the rearing livestock activities, provide new opportunities for encounter between man and ticks. The low host specificity and the ability to transmit pathogens (both veterinary and human interest) since the immature stages of larva and nymph explains their high efficiency as vectors of infectious diseases for which the possibility of transmission is directly proportional to the length of stay of the tick on the host.

The main infectious bacterial diseases transmitted by ticks, with epidemiological importance in our country are the Rickettsiosis, Lyme disease and the relapsing fever. In southern Europe, including Italy the human Rickettsiosis are caused mainly by *Rickettsia conorii* and *Coxiella burnetii*. Among TBD virus Crimean-Congo Hemorrhagic Fever (CCHF) is responsible for a disease that causes high death rates among people, transmitted by many tick species mostly belonging to the genus Hyalomma. Fortunately, this disease rarely occurs in humans, although in animals may be more common. Sporadic outbreaks of CCHF have been reported in 30 countries in Africa, the Middle East, Asia and Europe.

Recently the research activity on ticks (temporary hung up in the last years) has been started again in the Department of Infectious, Parasitic and Immune-mediated Diseases of the Istituto Superiore di Sanità. Since 2011 until now, we carry out 4 topics 3 out of them completely "money free", voluntarily, and 1 only supported by a small fund by the Fondazione Cassa di Risparmio di Civitavecchia (CARICIV Foundation) useful for mission repayment. Such research lines are here reported and described in their preliminary results, some of them already published on peer reviews or presented at national and international congresses.

Occasional record of Ornithodoros coniceps in Latium

The first record of *Ornithodoros (Alectorobius) coniceps* (Canestrini) was reported for Italy in 1877, inside the interstices of the ancient mosaics at S. Marco Basilica in Venice. Afterwards only few discoveries of the species are reported for Italy; the last record is dated back to 1984, in L'Aquila town (Abruzzo Region). The present study shows the data of a survey carried out as a result of a massive infestation by *O. coniceps* in an ancient villa in Anzio town (Latium region) recently restored. In the past decades the villa has been fallen into disrepair, becoming an occasional shelter for wild animals mainly pigeons, that colonized the whole building for generations. This case appears worthy of note because it is the first record of this species after more than 25 years in Italy. A total of 136 specimens were collected by three methods: manual, mechanical aspirators and Wilson traps. Wilson trapping indicates positive O. coniceps tropism for CO₂. It is important to point out that ticks belonging to Ornithodoros genus could be vectors and reservoirs of different species of Borrelia (*B. hermsi*, *B. perkeri* e *B. turicata*).

This study was published on the peer-review *Experimental and Applied Acarology* (Khoury *et al.*, 2011).

Bionomics of ticks in Insugherata natural reserve in Rome and detection of pathogens potentially transmitted

Since 1997 the Insugherata Natural Reserve has been an important protected area of Rome (about 740 ha), characterized by different biotopes and a significant biodiversity. This park is also an important area for human recreational activity, archaeological remains and pastures for sheep breeding. After a preliminary tick survey in 2010 followed by a bacteriological analysis, in order to investigate the tick composition and distribution and the possible presence of pathogens potentially transmitted, an annual acarological and bacteriological research was implemented during 2011. Three collection sites (woodlands and pasture) were selected and biweekly monitored. Free living ticks were sampled by dragging a white flag (about 1 m²) for 15 minutes/operator, morphologically identified and stored at -80°C.

For bacteriological analysis, a first Real-time PCR was performed using gltA gene to distinguish Rickettsiae species belonging to both Spotted Fever Group (SFG) and to Typhus Group (TG). A following Real-time PCR using ompB gene allowed discriminating the species within SFG. In order to identify among 15 rickettsiae of the SFG, the ompA gene was amplified and sequenced. For Borreliae and Ehrlichiae genera were used ospA and 16S rRNA genes, respectively. A total of 189 specimens were collected: Rhipicephalus turanicus (77.2%), Ixodes ricinus (13.2%), Dermacentor marginatus (8.5%) and Haemaphysalis punctata (1%). R. turanicus was widespread in all sites, especially in pasture and occurred since the end of March, reaching then high densities in the following two months. I. ricinus and H. punctata were collected only in woodlands: the first species was always present from January to April with slight abundances, whereas the second one appeared only in the half of January with two specimens. During the first four mounths, D. marginatus was present in the ecotonal area between woodland and the adjacent grassland with decreasing densities. In samples collected in 2010, 1 out of 8 R. turanicus females resulted positive for Rickettsia massiliae (100% of identity with sequences in GenBank). Until now 40 ticks of the 2011 sample were analyzed for pathogen detection: 7 I. ricinus and 8 R. turanicus specimens were found positive for Rickettsiae belonging to the SFG and 1 *I. ricinus* resulted positive for *Ehrlichia sp.* The pathogen identification at species level is ongoing. This research represents a significant contribution to the knowledge of the tick fauna and of the potential risk of introduction and spread of tick-borne diseases in a natural area of Rome. As *Rickettsia conorii,* the main agent of Mediterranean spotted fever, *Rickettsia massiliae* belongs to the SFG and represents an important etiological agent of tick-borne human spotted fever rickettsiosis. In Italy this pathogen was at first isolated in Sicily in 1985 and then identified only in 2005 in a blood human sample. In 2008 this rickettsia was identified in *R. turanicus* collected in Sardinia. In conclusion these preliminary findings seem very encouraging and this kind of approach could be extended to other areas.

The results about acarological aspects of this study have been presented as posters at *XXIII Congresso Nazionale di Entomologia*, held in Genoa on 13-16 June 2011 (Toma *et al.*, 2011a; Khoury et al., 2011). The whole study ranging also molecular data on bacteria has been presented in the same year at the *VII Ticks and Tick-borne Pathogens Conference* in Zaragoza (Toma *et al.*, 2011b; Di Luca, 2011).

Preliminary data on tick-borne disease pathogens in migratory birds in Italy

Ticks are vectors of viral, bacteric and protozoarian pathogens responsible of emerging diseases worldwide. Apart from the economic foreign trade and transport of things and people, the passive transport by migratory birds allows the spread of various tick species worlwide. In April 2010 in Latium region (Italy) we started collecting ticks on birds caught during seasonal bird ringing activities, and our data were compared with acarological records collected on birds from Sardinia region (Capoterra, nearby Cagliari) as interesting crossroads for vectors and pathogens circulation. In Latium region such activity was carried out in Castel di Guido and Paliano, nearby Rome and in Ventotene and Ponza Islands (Central Tyrrhenian Sea).

Ringing activities were carried out on Ventotene and Ponza in April and May, when huge numbers of migrating passerines stage on the small islands during their spring migration. Several migrants land at these stopover sites directly from North African coast, after non-stop flights of up to 14-16 h across the sea. In Paliano the ringing activity was in October, while in Castel di Guido in the earlier September during the post-breeding period which coincides with beginning of autumn migration for migrants. Ticks were collected directly from the birds and they were identified as morphology, then stored at -80°C in order to search for bacteria using molecular tools. For bacteriological analysis, a first Real-time PCR was performed using gltA gene as molecular marker to distinguish *Rickettsia* species belonging to both Spotted Fever Group (SFG) and to Typhus Group (TG). A following Real-time PCR using ompB gene allowed to discriminate the species within SFG. To identify Rickettsie of the SFG, the ompA gene was amplified and sequenced. For Borrelia and Ehrlichia were used molecular markers ospA gene and 16S rRNA gene. In Latium sites 126 ticks were collected on 39 birds belonging to 14 species: 3 partial migrants, European, and 11 longdistance migrants. All collected ticks were at early stages, for the main part: 122 nynphs belonging to genus Hyalomma; 2 nymphs and 2 larvae belonging to Ixodes genus. In Sardinia 2 females of Ixodes festai were collected on Common Blackbird. The molecular analysis showed positivity for *Rickettsia sp.* in 4 ticks belonging to genus Hyalomma: one was found on a Common Blackbird and three on Nightingale, trapped in Castel di Guido. Data reported showed that the prevailing ticks on partial and long-distance migratory birds are the nymphs of Hyalomma, that represents the genus comprising the most competent species for Crimean-Congo Haemorragic Fever virus (CCHF). The occurrence of 4 ticks positive for *Rickettsia sp.* confirms that bird migration could be a way of spread for tick-borne diseases. Therefore is noteworthy that the occurrence of *Ixodes festai*, unofficially reported for Sardinia has been here confirmed and observed on Common Blackbird as a new host species.

Field inquire on ticks in Tarquinia outskirts

Within the series of studies aimed to deepen the ixodidic Fauna knowledge in Latium Region, in 2011 we started a research in the surrounding of Tarquinia (Viterbo Province), in collaboration with the University of Tuscia in Viterbo, the Università Agraria of Tarquinia, the Istituto Zooprofilattico di Lazio e Toscana, supported by CARICIV Foundation. We selected 4 sites carachterized by woodlands, pastures and ecotonal situations that were monitored every 15 days for ticks collections by dragging.

Such just started study pointed out the occurrence of a various ixodidic Fauna, as we found 4 specie out of 18 specimens: *Dermacentor marginatus* 1 male, *Hyalomma marginatum marginatum* 2 females, *Ixodes ricinus* 7 nymphs, *Rhipicephalus sp.* 4 males and 4 females. The still ongoing research will be carried out in order also to complete it with molecular data. Such studies demonstrate how collaboration among ISS Departments, ISS and other Research Institutes and associations can be useful and effective; in spite of this positive aspect is impossibile to ignore that voluntary studies will be very limited and so the participation to international projects is desirable in order to achieve higher targets.

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