Survey on the presence of phlebotominae sandflies in eastern Sicily and connected risk of leishmaniasis

Oscar Lisi, Valerio Vaccalluzzo & Vera D'Urso

Department of Biological, Geological and Environmental Sciences - Section of Animal Biology, University of Catania, via Androne 81 - 95124 Catania, Italy

Corresponding author, e-mail: olisi@unict.it

ABSTRACT The authors summarize the results of all the searches for phlebotomes in eastern Sicily, including the connected risk for humans and dogs to contract leishmaniasis, and point out the current situation with new risks, and the main goals for present and future research.

KEY WORDS Phlebotomus sergenti; Leishmania tropica; leishmaniasis; Eastern Sicily.

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INTRODUCTION

Sicily is a region in which sandflies are much widespread and leishmaniasis is endemic, falling into the small group of Italian regions with highest values of incidence of the disease; this is always due to Leishmania infantum Nicolle, 1908 (Trypanosomatida Trypanosomatidae), responsible for all kinds of leishmaniasis, both human and canine, its main vector is Phlebotomus perniciosus Newstead, 1911 (Diptera Psychodidae), present in all environments, followed by P. perfiliewi Parrot, 1930, whose role as vector is practically limited to rural environments with sufficient presence of farm animals. Two forms of human leishmaniasis are known in Italy; visceral leishmaniasis (VL) is a very serious illness which invariably requires hospitalization; from about 10 to more than 40 new cases/year in Sicily are reported to the Ministry of Health. Cutaneous leishmaniasis (CL) does not require hospitalization and always has benign esit, therefore it is often nondenounced or even non-recognised (Gradoni, 2013); for this reasons, though the number of new cases/year recorded is more or less similar to VL, it is believed that the real incidence is far higher.

Among the possible factors determining such a stable situation of the disease, well rooted in the territory, one can record the very high number of infected dogs, constituting the parasite reservoir, the increasing number of persons with immunodeficiency and of travellers and migrants accross the Mediterranean, and the effects of global warming, which have had a positive influence on the vectors' survaillance and distribution.

STATE OF ART

The first study on phlebotomes in Sicily was due to Adler & Theodor (1931), who carried out a very important pioneering investigation on several foci of leishmaniasis in the Mediterranean, including the town of Catania, in which they found *P. perniciosus*, *P. neglectus* Tonnoir, 1921, *P. sergenti* Parrot, 1917, *P. papatasi* Scopoli, 1786 and *Sergentomyia minuta* (Rondani, 1843). They concluded that the main vector had to be *P. perniciosus* and observed that the distribution of the disease and the vector were rather inhomogeneous in the town, anyway with more incidence in the periphery than the centre of the town.

Unfortunately, the results of their research, especially as regards the phlebotome species composition, were misrepresented by the fact that the authors based their search for phlebotomes mostly inside sick person's houses, without taking into appropriate consideration the environments of adult emerging and daytime shelter of these insects out of houses.

Biocca et al. (1977) reported the results of their collections in many sites all over Italy, with a few data also on Sicily. Among the others, they found *P. perniciosus* (30.6% of the specimens), *P. perfiliewi* (20.0%), and, only in Sicily, *P. sergenti* (0.1%). They confirmed *P. perniciosus* as the main vector of leishmaniasis in Italy and noticed its ecological plasticity, finding it in various habitats and from 0 to 1000 m a.s.l.. They reported *P. perfiliewi* in Sicily as vector for CL. They also found the very common and abundant species *S. minuta* (47.8%), which however stings mostly anphibians and reptiles and is not involved in the transmission of *Leishmania* Borovsky 1898 (Ross 1903) for humans or dogs.

After 70 years from Adler's and Theodor's investigation, finally the attention focused again on Sicily, thanks to Ruta et al., 2002, who carried out a research in the hinterlands of Catania and Siracusa; they collected more than 2000 specimens, and found collectively P. perniciosus (50.4%), P. neglectus (0.3%), P. papatasi (0.2%), P. sergenti (0.3%) and S. minuta (48.8%); however, it must be stressed that in a site they found about 90% of P. perniciosus. The authors observed a flight season from May to, in some sites, November (October in others), and reported two generations during the flight season, with just a very slight sign of a third generation in the sites with the longest flight season, immediately stopped by the incoming of the cold season.

They stated that temperature and photoperiod proved to be important to determine start and end of the flight season, while during it humidity proved to be the most important factor which allowed sandflies survival. That a parameter proved to influence the more or less presence of *Phlebotomus* Loew 1845 species with respect to *Sergentomyia* França et Parrot, 1920, the former being more linked to humidity, the latter more resistant to aridity. Last but not least, the authors remarked on the fact that the risk of transmission of leishmaniasis is not constant during the whole flight season, it becoming noticeable later than the appearance of adults (for the fact that they need first to get contact with infected hosts), and the risk becomes maximum in correspondence with the two peaks of phlebotome density during the season. The authors found more phlebotomes close to the coast then in inland, thus determining a different risk.

D'Urso et al. (2002) performed a research in Catania, Siracusa and Ragusa provinces during the triennial 1997–99, collecting more than 10,000 specimens. They found, collectively: *S. minuta* (63.6%), *P. perniciosus* (34.7%), *P. sergenti* (1.7%) and lastly, *P. neglectus*, *P. papatasi* and *P. perfiliewi* (<1%). Though not very high percentage, they found more frequently *P. sergenti* than in the previous searches, and the authors remarked on the fact that this species, though not in Italy, transmits *L. tropica* in other Mediterranean countries.

The authors did not find noticeable differences in sandflies abundance and species composition linked to different altitudes and distance from the coast, pointing out the role of the specific kind of environment (e.g. more or less anthropized, with different humidity and vegetation, with a different presence of animals). They find on average a difference between the Aetnean area and the Hyblean one, the former being more anthropized and humid with a higher sandfly biodiversity and an important presence of *P. perniciosus*, *P. sergenti* and *P. neglectus*, the latter more rural and dry, dominated essentially by *S. minuta*, therefore with lower risk of leishmaniasis.

The presence of *P. sergenti* in Sicily, induced D'Urso et al. (2004) to focus on this species, analyzing collections in the triennial 1997–99 in one collecting site at the foot of Etna, and another in the Hyblean area. In the former site the authors found 77.7% of P. perniciosus and 2.0% of P. sergenti, while in the latter, apart from a great deal of specimens of S. minuta, P. perniciosus was only 14.4% and P. sergenti less than 0.02%. The authors pointed out that P. sergenti is associated with domestic environments in urban and periurban areas between 0 and 750 m a.s.l. but were not able to comment on a possible role in transmission of leishmaniasis due to the low density found in the studied sites. In any case it is worth to mention that gradually, a scenario was coming out, in which this species was not always so rare as had resulted from the oldest searches.

Another research focusing on P. sergenti was due to Maroli et al. (2006), and was practically the continuation of the previous mentioned (D'Urso et al., 2004), which allowed the authors to find an Etnean site in which P. sergenti was the dominant species (about 54%). Maroli et al. (2006) sampled in the flight seasons 2004 and 2005 finding that this species had a shorter flight season than P. perniciosus, with only one main density peak (i.e. one main generation of adults). They also tested females with blood meal, finding that *P. sergenti* fed mostly on dogs (77.8%), far less on avians (8.3%) and only little on humans (2.8%), while P. perniciosus only on dogs (60.0%) and humans (13.3%); however, it must be stressed that in spite of possible preferences, phlebotomes are oportunistic feeders which take their blood meal on the animals more at hand.

Maroli et al. (2005) carried out a research on phlebotome ecology, sampling in 18 sites in various parts of Sicily during 2004 flight season. Among the various environments chosen for putting the traps, they can be recorded: farms with various livestock, chicken pens and wall crevices. They collected a total of 8821 specimens mostly belonging to S. minuta (69.9%), while among the Phlebotomus species the proportions were: P. perniciosus (52.9%), P. perfiliewi Parrot, 1930 (46.5%), P. neglectus (0.5%), P. sergenti (0.1%) and P. papatasi (0.03%). The authors pointed out that the two proven vectors of Leishmania infantum, P. perniciosus and P. perfiliewi, were abundant, the former more present in domestic environments, the latter more linked to farm animals (chickens excluded).

Finally, our research group decided to go on with the studies and see what had happened to the phlebotomes in the town of Catania after more than 70 years of urbanistic and sanitary progress, with a series of searches, started with a big monitoring in 51 sampling sites distributed in the urban tissue, in 2006 flight season. A good 45 sites resulted positive for phlebotomes, collecting a total of 4341 specimens, belonging to six species, one of which, *P. mascittii* Grassi, 1908, was new for Sicily. With respect to Adler & Theodor (1931) investigation, the presence of phlebotomes in the town had not only kept more than sufficient for the illnes' maintenance and propagation, but, differently from Adler's and Theodor's results, the sandflies, and in

particular *P. perniciosus*, turned out to be abundant also in the centre of the town. Besides, with respect to recent investigations in Sicily (e.g. Ruta et al., 2002), it was observed in some sites a very long flight season: from May to December, with a clear, though lower, third peak in phlebotome density, which means a third generation (D'Urso et al., 2008a, 2009).

In some collecting sites, also *P. sergenti* was abundant: it was found to be up to 45.5% of the specimens, which was the highest percent ratio ever recorded in Sicily (D'Urso et al., 2008b).

Those results induced our research group, in collaboration with a group of colleagues from the "Istituto Superiore di Sanità" (Rome), to make additional investigations in 2008, 2012 and 2013, both in the most interesting sites of Catania, and in several Aetnean sites, integrating the research also with immunological, molecular and cultural techniques in order to analyze females of Phlebotomus searching for viruses and Leishmania, and about the latter we found that 11% of the investigated females of P. perniciosus were positive for genomic Leishmania DNA (Lisi et al., 2014); unfortunately it was not possible to determine the species; according to the current knowledge it should be L. infantum, but the aim of the investigation was also to check if some exotic Leishmania species had been able to reach Sicily and set itself up there, idea justified for several reasons, as it is discussed in the following paragraph.

DISCUSSION AND CONCLUSIONS

As it can be seen, the searches of the last decade have drawn a scenario about the presence of phlebotomes in eastern Sicily, that fully justifies the presence of the illness as endemic, maintained by these insects as vectors, and by the huge number of infected dogs, as reservoir, with the problem of the stray, very numerous and practically out of control.

About the vectors, by comparing the results of the researches of the last ten years, with those of the older ones, it seems that phlebotome flight season has prolonged, and perhaps the species composition of several environments, especially urban, changed, in favour of a more efficient maintainance and propagation of the disease; all this is very probably due to global warming, which seems to have determ-

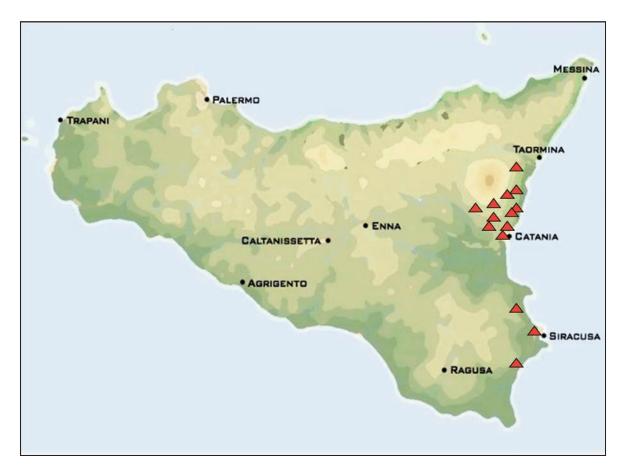


Figure 1. Distribution of *Phlebotomus sergenti* in Sicily according to the literature.

ined the prolongation of the flight season, and, supposedly (the avalaible data are still insufficient to state this with certainty), changes in species composition of some environments, especially the urban, and the general abundance of these insects. Global warming is a process still in act, thus rendering the situation dynamic, and therefore in need to be monitored.

Another very important aspect to which to pay careful attention, is the possibility for some exotic *Leishmania* to reach Sicily and set itself up there finding a species of phlebotome (competent or not for "our" *L. infantum*) suitable as a vector. The island lies in the main immigration route of the "Mediterranean boat people", migrants who fled African and Middle East countries, most of which are endemic for *L. tropica*, because of civil conflicts and/or poverty. Moreover, Catania is located close to the Sigonella NATO military base, where every year soldiers from all over the world (including *L. tropica* Wright, 1903 - endemic Middle East countries) pass through. It is therefore not unlikely that individual infected with L. tropica may reach Sicily (this, actually, has already happened), where the presence of P. sergenti, the proven vector of L. tropica in the countries in which the parasite is endemic, constitutes a high potential risk for introduction of the exotic parasite with the illness it causes. Rioux (2001) demonstrated that populations of P. sergenti from Morocco are highly subjected to get infected by L. tropica, and Depaquit et al. (2002), while studying the intraspecific variability of different populations of P. sergenti, found out that Sicilian and Moroccan populations are "sister groups", thus arising the suspect that Sicilian P. sergenti might be as easily infected by L. tropica as the Moroccan.

In Italy the distribution of *P. sergenti* seems to be limited to the East coast of Sicily (Fig. 1), but while in the close past it was known only from few Aetnean sites (Adler & Theodor, 1931; Biocca et al., 1977), it then proved to be more spread along the coast not only in the Aetnean territory, but also in the Hyblean area (D'Urso et al., 2002, 2004; Maroli et al., 2006); therefore, with more investigations, it is possible that it will be found in other areas, at least on the island; on the other hand, even if the species were really today present only in eastern Sicily, it is not possible to exclude a colonization of the rest of the island, and of southern Italy.

Besides, though less probable, it cannot be excluded that also other species of Leishmania may reach Italy finding a phlebotome species capable to establish a local cycle spreading the parasite. Today, we "only" know that cases of foreigners affected by exotic Leishmania species have already been reported: not only the above mentioned L. tropica but also L. major from Africa and Middle East, and L. braziliensis e L. panamenisis from South America. Unfortunately, neither the clinical observation of the patient nor the morphological observation of the parasite under a microscope allow to identify the Leishmania species, thus increasing the above mentioned risks since an exotic Leishmania might be at first mistaken with the local L. infantum and a correct identification might take place much later, when the exotic parasite has already spread.

To conclude, it is necessary to keep on monitoring and investigating to complete the picture of the distribution and bio-ecology of the *Phlebotomus* species, especially *P. sergenti*, and ensure that *L. tropica*, as well as any other exotic *Leishmania*, has not already started to set itself up in Sicily; in the meantime, it would be much helpful if a better sanitary control of stray dogs and immigrants might be achieved.

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