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Current knowledge on the presence of *Leishmania* vectors in Eastern Sicily, and possible risks for human and canine health

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Summary

The authors point out the dispersal of Phlebotomes in Eastern Sicily, especially about Catania city, from current literature, and take into consideration the risk of contracting leishmaniasis and a possible extension of *Phlebotomus sergenti* Parrot, 1917 distribution area in the rest of Sicily and in southern Italy with a connected risk of introduction of *Leishmania tropica* (Wright, 1903) in those areas.

Key Words

Phlebotomus perniciosus, *P. sergenti*, *Leishmania tropica*, leishmaniasis, health.

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Introduction

Leishmaniasis is a group of diseases which affect primarily mammals, including dogs and humans, caused by flagellated protozoans of the family Trypanosomatidae, genus *Leishmania* Ross, 1903. The parasite protozoans have as intermediate hosts (vectors) small dipterans of the family Psychodiidae, the Phlebotomes (commonly named "sandflies"), whose females get infected, and subsequently cause the infection of a new mammal host, due to the fact that, like mosquitoes, they take a vertebrate blood meal in order to ripen their eggs.

In Italy, all types of leishmaniasis (human both cutaneous and visceral, and canine) are due to *Leishmania infantum* Nicolle, 1908 (species complex), whose main reservoir are dogs, while humans get infected occasionally. Human leishmaniasis can be, in Italy, cutaneous or visceral; the former causes skin ulcerations on the parts of the body usually exposed to stings, leaving permanent scars but normally, apart from possible secondary infections, the illness has a benign ending.

Visceral leishmaniasis is instead a very serious illness, characterized by skin darkening, fever, weight loss, anaemia and liver and spleen swelling; if not treated, the illness has fatal ending.

Canine leishmaniasis, very common in Sicily, is usually quite a serious illness (but with a large degree of variability in different individuals), and is difficult to treat because the parasite cells are not localized in defined parts of the dog body but invade all the organism; besides, in spite of great research efforts in the last decades, specific treatments, which completely eliminate the parasite without damaging the dog too, are still lacking.

It is important to underline that direct transmission dog to dog or dog to human is not possible, as the parasite becomes infective for a new mammal host ~~also~~ in the phlebotome, and also in the case of having in one's home an ill dog, it is ~~higly~~ improbable to get infected through a phlebotome which has stung the dog, because after the insect has taken the *Leishmania*, this needs time to complete part of its life cycle in the insect finally rendering it infective, but in the meantime the phlebotome will have moved away outdoors searching for an opportune site to lay its eggs.

Leishmania infantum, finding its reservoir in dogs, has a so-called zoonotic transmission, but other species of *Leishmania* may find their reservoirs in humans; this is the case of *Leishmania tropica* (Wright, 1903), that has anthroponotic transmission, which renders the control of the disease even more difficult. As it will be discussed further, there is a risk of introduction of *L. tropica* at least in Sicily.

Leishmaniasis in Sicily: state of art

Sicily is a region in which leishmaniasis, both human and canine, is endemic.

The incidence of human leishmaniasis, both cutaneous and visceral, in Sicily is an important, urgent problem that needs great attention in order to find a solution. As a matter of fact, in the '90s years about 40–50 cases/year were reported of the disease (cutaneous plus visceral) (Ascione et al., 1996; Gramiccia, 1997), and an average of 30 visceral leishmaniasis cases/year and 24.42 cutaneous leishmaniasis cases/year were reported for the region in the period 2000–2006 (data from the website of "Bollettino Epidemiologico del Ministero della Salute"), and those values are probably underestimated.

Very interesting are the data obtained in these last years by C.Re.Na.L. (Centro di Referenza Nazionale per le Leishmaniosi e laboratorio di Riferimento per l'Organizzazione Mondiale per la Sanità Animale) of the Istituto Zooprofilattico Sperimentale della Sicilia as results of direct and indirect tests made in Sicily on humans, dogs and other animals (especially cats). In particular, direct tests made in 2015 through PCR *Leishmania* on human patients with possible leishmaniasis symptoms (cutaneous or visceral), produced 50% of positive individuals. In dogs, random screening produced a good 16% of positive individuals.

Tests made in the same year 2015 through indirect immunofluorescence for *Leishmania* (IFAT Leishmania) gave as result that on 11 human cases examined, three were positive and eight suspected, while on the very numerous canine cases tested (about 17500), 41.9% resulted positive and 26.7% suspected. The same test made on cats, though did not produce a high proportion of positive individuals (14 on a total of 271 tested, i.e. 5.1%), gave a very high percentage (59.4%) of suspected, i.e. with an antibody titer between 1:40 and 1:80; this means that also in cats, at least in Sicily, the parasite can actively "circulate".

In spite of the attempts to contain the diffusion of the disease, the situation is still critical because of a huge number of infected dogs, that constitute the main reservoir of the parasite; while fighting against the diffusion of the disease is relatively simple regarding domestic dogs (eg. by using repellents, night shelter etc.), it is much more difficult with strays, which are very numerous in towns.

Another important factor to take into consideration in the struggle against leishmaniasis is the distribution and the bio-ecology of the vectors: the Phlebotomes, commonly named "sandflies".

The phlebotomes

Phlebotomes are small dipterans of the family Psychodidae, vaguely similar to very small mosquitoes, usually amber in colour and about 2–5 mm long.

Adults are present in summer, and partially in spring and autumn depending on the climate of the specific region; they flight at night, while at daytime they take shelter in various refuges having in common a lower temperature and a higher humidity with respect to the external environment, and relative darkness; daytime shelters can be for example wall holes or cracks, trunk or rock or soil cavities, animal nests, or indoor environments having too the above mentioned characteristics. In the same type of microenvironments, with in addition the presence of organic material in decomposition, females lay their eggs and larvae feed and grow.

As mentioned in the first paragraph, females sting vertebrates to take a blood meal and ripen their eggs; it is due to this behaviour that they act as vectors of leishmaniasis and other diseases. Though showing in some cases moderate preferences, it seems that female phlebotomes act as "opportunistic feeders", taking the necessary vertebrate blood from the host more at hand (including not only mammals but also birds).

In Italy, the proven vectors of *L. infantum* are *Phlebotomus perniciosus* Newstead, 1911 and *P. perfiliewi* Parrot, 1930, both present in Sicily, while two other species, *P. neglectus* Tonnoir, 1921 and *P. ariasi* Tonnoir, 1921 are suspected vectors, but the latter is absent in Sicily. *Phlebotomus papatasi* (Scopoli, 1786) and *P. sergenti* Parrot, 1917 are also present in Sicily, not involved, as far as is known until now, in the transmission of ~~leishmaniais~~, but they are vectors in other countries of *L. major* and *L. tropica* respectively.

As regards standardizable methods for collecting high numbers of adult sandflies, the most commonly used are sticky traps and luminous traps (CDC).

Sticky traps, without attracting substances, are

simply obtained by sheets of ordinary paper cut into squares 20x20 cm and soaked in castor oil. The traps are put out-of-doors inside favourable daytime shelters, or indoors (into houses, cellars or courtyards) positioned in order to intercept the insects during their nocturnal flight. The traps can be placed at regular intervals during the whole flight season.

Phlebotomes can also transmit other human pathogens, including *Phlebovirus* (causing sandfly fever) such as Sicilian and Toscana viruses, (see Maroli et al., 2013 for a review); Toscana (TOS) virus, involved in acute infections of the central nervous system, was isolated from sandflies collected in Italy. It turned out to be closely related to Sandfly fever Naples (SFN) virus (Verani et al., 1984). Recent investigations have indicated that virus diversity in the Mediterranean basin is higher than initially suspected, and that populations living south and east of the Mediterranean Sea are exposed to a high risk of infection during their life (Maroli et al., 2013). A recent investigation (Calamusa et al., 2012) reported the results of a test made on 271 individuals in Sicily; 90 subjects (33.2%) were positive for Toscana virus (TOSV) IgG, 25 (9.2%) were positive for Sicilian virus (SFSV) IgG and 11 (4%) were positive for both the viruses.

State of art about the presence of phlebotomes in Eastern Sicily

The first study on Sicilian sandflies was due to Adler & Theodor (1931), who carried out a pioneer research on visceral leishmaniasis (VL), also named "Kala-Azar", in the Mediterranean. Being one of the three greatest endemic foci in the Mediterranean, with 150–200 cases of VL per year out of a population of 264.000 inhabitants, Catania was included in the study.

Sandflies made their appearance in Catania towards the end of May or the beginning of June. *Phlebotomus papatasi* and *P. perniciosus* were the first species to appear, and about a week later the other three species, *P. neglectus* (then referred to as *P. major*), *P. sergenti* and *Sergentomyia minuta* (Rondani, 1843) (= *P. parroti* var. *italicus*) were found. The comparative rarity of the last three species made it difficult to determine the exact date of their appearance.

The authors observed that the distribution of the disease was not continuous within the very wide focus, and that human and canine leishmaniasis coexisted. Their study on phlebotomes consisted in capturing the insects at sight principally in sick person's houses, and, in some cases, outdoor but only on humans. They noticed that the distribution of the disease was consistent with propagation by *P. papatasi* and *P. perniciosus* but their quantitative study showed that *P. perniciosus* was the principal vector, while *P. papatasi* could be excluded as a vector of the disease in Italy. Adler & Theodor (1931) found that in some outlying districts there was the maximum incidence of VL, and in some houses in those districts *P. perniciosus* was the most common species. On the contrary, in the centre of the town both the phlebotomes and the incidence of disease were scarse. *Phlebotomus neglectus* was found in many districts of Catania in which the disease was widespread but where *P. perniciosus* was also abundant. Besides, the distribution of the disease turned out to be more extensive than that of *P. neglectus*, so this species could not be the only vector of the disease but it might contribute, together with *P. perniciosus*, to making Catania the focus of maximum intensity of the leishmaniasis. The authors observed that *P. perniciosus* was a zoophilic species which fed much more willingly on cattle and dogs than on humans, and that it could be captured both indoors and out-of-doors. The distribution of *P. sergenti* in Catania was irregular. It was common in a few streets only. More than half of the total female specimens were collected in July. Adler & Theodor (1931) collected only few specimens of *S. minuta* and did not consider them important because at that time the species was believed to sting only cold-blooded vertebrates; today it is known that the species prefers such animals but may also sting birds and mammals, man included, and some females of *S. minuta* (as well as other congeneric species) positive for mammalian *Leishmania* have been found; however, it is still unclear whether *Sergentomyia* França et Parrot, 1920 species can really play a role in the diffusion of mammalian leishmaniasis (Maia & Depaquit, 2016).

Biocca et al. (1977) carried out a search in whole Italy finding as dominant species *S. minuta* (47.8% of the specimens), *P. perniciosus* (30.6%) and *P. perfiliewi* (20.0%); only in Sicily, they found also *P. sergenti* (0.1%). The authors confirmed the role of *P. perniciosus* as main vector of visceral leishmaniasis and remarked about its ecological versatility as it was found in various environments from 0 to 1000 m a.s.l.; the authors also hypothesized a role of *P. perfiliewi* as vector of cutaneous leishmaniasis (also in Sicily).

Ruta et al. (2002) carried out a study in the hinterlands of Catania and Syracuse collecting more than 2000 specimens belonging to *P. perniciosus* (50.4%), *P. neglectus* (0.3%), *P. papatasi* (0.2%), *P. sergenti* (0.3%) and *S. minuta* (48.8%), but it must be stressed that in a site they found about 90% *P.*

perniciosus. The authors observed a flight season beginning in the middle of May with two generations and stated that temperature and photoperiod are important factors determining start and end of the flight season, while during it, it is humidity to influence adult and larvae surveillance especially as regards the genus *Phlebotomus* (while *Sergentomyia* is more resistant to aridity). Another important statement regarded the risk of contracting leishmaniasis which is not constant during the whole flight season, it being low at the beginning of the flight season because the vectors need time to get infected, and highest in correspondence to the density peaks of the phlebotomes. The authors observed at the end of the flight season in some sites a slight tendency to form a third peak (i.e. a third generation of adults) which was immediately blocked by the cold season overcoming. The authors found more phlebotomes along the coast than in inland, thus concluding for the former environment a higher risk for humans and dogs to contract the disease.

D'Urso et al. (2002) collected in several localities of Eastern Sicily a total of more than 10000 specimens belonging to the following species: *P. perniciosus*, *P. perfiliewi*, *P. neglectus*, *P. papatasi*, *P. sergenti* and *S. minuta*. The most abundant species were *S. minuta* (63.6%) and *P. perniciosus* (34.7%), while *P. sergenti* was only 1.7% and the other species less than 1%.

For the first time, it was remarked about the presence of *P. sergenti* which is known in Italy only for Sicily, and is known in other Mediterranean countries as vector of *L. tropica*.

The authors did not find significative differences at different altitudes or distance from the coast, underlining the fact that these small insects are linked to the specific conditions of the very small area in which they live and move. The authors found significative differences, instead, between the Aetnean and the Iblean territories, the former being richer in *Phlebotomus* species (*P. perniciosus*, *P. neglectus* e *P. sergenti* the most abundant), the latter dominated by *S. minuta*, with presence of *P. perniciosus* only in the most ~~antropized~~ environments. These ~~difference was~~ explained considering that the Iblean area is more arid and uniform, and less ~~antropized~~, while the Aetnean more humid and ~~antropized~~ (thus more suitable for the genus *Phlebotomus*), and offering a larger variety of environments

As a consequence, the Aetnean territory is characterized by a higher incidence of ~~liesbmaniasis~~.

D'Urso et al. (2004) focused on *P. sergenti* in the ~~triennal~~ 1997–1999 studying a site at the foot of Etna and another in the Iblean area; they found in the former 77.7% *P. perniciosus* and about 2% *P. sergenti* (the rest were *S. minuta* and other minor species); in the Iblean site they found mostly *S. minuta*, while only 14.4% *P. perniciosus* and less than 0.02% P. sergenti. It was not possible to comment on a possible role of *P. sergenti* in the ~~transimission~~ of leishmaniasis but the authors stated this species is associated to urban and periurban domestic environments between the sea level and 750 m a.s.l.

Maroli et al. (2005) studied the ecology of sandflies as vectors of leishmaniasis in Sicily during 2004, monitoring 18 sites with various habitats suitable for phlebotomes such as farms with various livestock, chicken coop and wall cracks. Over a total of 8821 specimens collected, the most abundant were *S. minuta*, *P. perniciosus* and *P. perfiliewi*, the other species found, in very low percentages, were *P. neglectus*, *P. sergenti* and *P. papatasi*. The two proven *Leishmania* vectors, *P. perniciosus* and *P. perfiliewi*, were found with significative abundance; *Phlebotomus perniciosus*, though less abundant in farms, ~~had proven~~ to be able to colonize all types of environments studied (urban areas included), while *P. perfiliewi*, species linked to livestock, was very abundant in farms (or anyway rural environments) while rare in other environments (especially urban ones).

Maroli et al. (2006) studied the seasonality and feeding habits of *P. sergenti* in a focus of Eastern Sicily in which in previous searches this species had proven to be abundant. They collected in 2004 and 2005 843 specimens represented by *P. sergenti* (54.1%), *P. perniciosus* (35.0%), *S. minuta* (6.2%), *P. neglectus* (3.0%) and *P. papatasi* (1.7%). *Phlebotomus sergenti* turned out to have a shorter flight season than *P. perniciosus* with only one peak between the end of July and the beginning of August.

The authors also studied the blood meal of females of *P. sergenti* and *P. perniciosus* and found out that *P. sergenti* had fed on dogs (77.8%), avian (8.3%) and humans (2.8%), while *P. perniciosus* had fed only on dogs (60.0%) and humans (13.3%).

D'Urso et al. (2008a, 2008b, 2009) and Lisi et al. (2014) performed a long research lasted several years, in the town of Catania and nearby localities. After about 80 years from the studies of Adler & Theodor (1931), Catania was again studied for phlebotomes with a big monitoring in 2006 in 51 sampling sites distributed in the main urban tissue. Most sites (45) proved to be positive for phlebotomes, and a total of 4341 specimens was collected, belonging to 6 species (*S. minuta*, *P. perniciosus*, *P. papatasi*, *P. mascitti*, *P. perfiliewi*, *P. sergenti*). The results of that search induced the authors to make additional tests in the subsequent years. *Phlebotomus perniciosus* was present in all the positive sites for phlebotomes.

The species had a very long flight season (from May to November/December with three density peaks) and proved to be very widespread everywhere in the town, thus fully justifying the cases of human (both visceral and cutaneous) and canine leishmaniasis reported for the territory. A search for *Leishmania* DNA in 72 females of *P. perniciosus* revealed 11% infection prevalence (Lisi et al., 2014). *Phlebotomus perfiliewi* was a new record in the town, because not found by Adler & Theodor (1931), while *P. mascittii* was a new record for the whole Sicily. *Phlebotomus sergenti* turned out to be the second species of the genus *Phlebotomus* for abundance and density in Catania, but it did not reach values which allow considering it to be as a species involved in the epidemiology of leishmaniasis in Sicily. In spite of this, its presence in Sicily represents a potential risk for the introduction of *L. tropica*, which is the agent of a form of anthroponotic cutaneous leishmaniasis in other Mediterranean countries.

In conclusion, those searches revealed that the abundant presence of phlebotomes in the town of Catania not only had maintained in almost 80 years of urbanistic and sanitary progress, but indeed, phlebotomes, and in particular the main vector *P. perniciosus*, resulted abundant also in the centre of the town.

Problems about parasite reservoirs and vectors

As already stated, domestic dog is the main reservoir of human infection and sandflies are the vectors of this protozoal disease. According to Moreno & Alvar (2002) it has been estimated that at least 2.5 million dogs are infected in southwestern Europe. In the last decades, the diffusion of pets, especially dogs, has everywhere increased; one of the consequences, has been also an increase of dog abandon, thus reinforcing the already serious problem represented by stray dogs, which is unfortunately still far away from being solved. Therefore, in a right perspective of leishmaniasis containment, it is important to concentrate on the fight against vectors, the sandflies, hopefully setting up more efficient, simpler and faster fight strategies. Since it is quite difficult to detect the larvae breeding-sites, fight methods have to focus on adults. The use of repellents for phlebotomes is essential not only to preserve sound man and dog health, but also to be adopted on ill individuals in order to prevent from the parasite diffusion. Specific control measures against sandflies have been shown in Alexander & Maroli (2003).

As regards other possible reservoirs of the disease, it has been demonstrated that foxes [*Vulpes vulpes* (L., 1758)] and above all black rats [*Rattus rattus* (L., 1758)] can be infected by *Leishmania*, and that *P. perniciosus* and *P. perfiliewi* were readily attracted to, and fed on, black rat in nature; in particular, *P. perniciosus* in laboratory has transmitted *L. infantum* to the black rat (Bettini et al., 1980; Gradoni et al., 1983; Pozio et al., 1985). Thus, these animals can be considered wild reservoirs of *L. infantum*, though it is still unclear their role in the propagation of the disease to dogs and humans.

As regards the cat, though it had never been considered a habitual host for *Leishmania*, and only rare cases of feline leishmaniasis had been reported in regions in which the illness is endemic, more recent studies have demonstrated that domestic cat can get leishmaniasis also showing evident clinical symptoms. More in particular, Pennisi (2002) reported that after the occurrence in Sicily in 1997 of the first clinical case of FL (Feline Leishmaniasis) in a FIV+ (Feline Immunodeficiency Virus) cat, a study was performed on 93 cats living in the Eastern part of the island; later on, three more clinical cases were registered, two of them in FIV infected cats; besides, eighty-nine cats from the same area were recruited in 1999. For a review of the case reports on feline leishmaniasis in Sicily, but also in the world, and for its literature, see Pennisi (2002); see also the above reported results from the C.Re.Na.L. In this viewpoint, cat might represent another additional reservoir, though its real potential is still to be evaluated.

Beyond the problems about reservoirs, other studies have reported that the disease is spreading at North into the foothills of the Alps (Maroli et al., 2008) and into the Pyrenees (Ferroglio et al., 2005). This phenomenon is also to be related to phlebotome areal extension at North, especially due to global warming.

As regards Eastern Sicily, as already reported, competent phlebotomes, in particular *P. perniciosus*, are very widespread in different types of environments, urban included, as testified by the searches made in the town of Catania (Lisi et al., 2014); besides, the adult flight season, again thanks to global warming, has prolonged (also tending to produce a third generation of adults), thus increasing the risk for human and dogs to be infected by the parasite. This shows that today the risk is not only persistent, but even higher than in the past, thus requiring great attention to set up effective fight measures especially against *P. perniciosus*. That need is also reinforced by the fact that in other Mediterranean regions another form of leishmaniasis is present (cutaneous leishmaniasis by *L. tropica*), to which man is so vulnerable to constitute itself the main reservoir of the parasite (anthroponotic transmission); though

today that illness is not present in Sicily and the rest of Italy, it is highly at the risk of introduction in Sicily due to the presence on the island of the species of vector phlebotome *P. sergenti*.

Risk of exotic *Leishmania* introduction

In the last decades, as a consequence of the obvious phenomena of globalization and immigration, introduction of allochthonous pathogens and their vectors is more and more easy, with clear negative results on human health, and, consequently, on the socio-economic branch.

Cases already occurred in Italy of importation of human leishmaniasis by allochthonous *Leishmania* species, such as the already mentioned *L. tropica*, but also *L. major* ....., both from Africa and the Middle-East, and *L. braziliensis* .....e *L. panamenisis* .....from South America (Antinori et al., 2004).

Unfortunately, a simple clinic exam on the patient, and the morphological observation of the parasite under a microscope, do not allow sure specific diagnosis, therefore an exotic *Leishmania* might not be recognised and have all the time to install in the territory spreading itself among the population if it finds a compatible phlebotome species (competent or not for "our" *L. infantum*) which transmits the parasite.

Sicily can be considered as the interface between Southern Europe, Northern Africa and the Middle-East, since it lies in the middle of the Mediterranean, where many populations, especially coming from North Africa, settle or migrate further (these days it is actually a modern massive migration); in addition, close to Catania there is the NATO base of Sigonella, where every year a lot of soldiers from all over the word pass through. For these reasons, Sicily might thus easily represent an arrival point for new diseases which may not only install in the territory, but also propagate in the rest of Italy. Such risk is particularly concrete as regards the presence in Sicily of *P. sergenti*, the main proven vector of *L. tropica*, which is responsible for a serious form of cutaneous leishmaniasis, in Saudi Arabia and Morocco; it is believed that *P. sergenti* is the vector also in all the others foci of cutaneous leishmaniasis by *L. tropica*.

Phlebotomus sergenti is a very widespread species, in particular it is present in many Mediterranean countries; in Italy its presence seems to date confined to Eastern Sicily, but while until recently it was known only for few Aetnean sites (Adler & Theodor, 1931; Biocca et al., 1977), in the last years it turned out to be present in many habitats of Eastern Sicily, from the Aetnean to the Iblean areas (D'Urso et al., 2002, 2004; Maroli et al., 2006; Lisi et al., 2014) and it is not possible to exclude that further studies may reveal a more widespread presence of this species at least on the island; besides, it cannot be excluded the possibility for this species to colonize new territories, if really confined to that part of the island, also with the help of global warming, which can surely have a positive effect on that species, whose geographic distribution and seasonal dynamic clearly indicate that it is linked to hot climate.

In addition, Rioux (2001) demonstrated that *P. sergenti* in Morocco is highly subject to infection by *L. tropica*, and Depaquit et al. (2002), while studying the intraspecific variations of different populations of *P. sergenti*, discovered that the Sicilian and the Moroccan populations are so similar that they must be considered as sister groups, which may indicate that Sicilian *P. sergenti* may take infection by *L. tropica* as easily as the Moroccan.

For all the above mentioned reasons, there is in Sicily a high risk of introduction of *L. tropica* which requires constant attention, primarily by monitoring the presence and diffusion of *P. sergenti*, as well as general monitoring and further investigations on all *Phlebotomus* species in Sicily, in order to acquire a more complete scenario of their distribution and bioecology; such investigations should be associated to careful analyses on patients suspected to be affected by leishmaniasis in order to detect precociously eventual infection by exotic *Leishmania*. Much helpful would be to acquire a sufficient control of stray dogs and immigrants, which are goals, unfortunately, still far away from being reached.

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