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**Controlling phlebotomine sand flies to prevent canine
Leishmania infantum infection: a case of knowing your enemy**

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Abstract

Leishmaniosis caused by *Leishmania infantum* is a widespread zoonotic disease that can be transmitted to animals and humans by their vectors, blood-sucking phlebotomine sand flies. To prevent canine leishmaniosis across the whole Mediterranean region, vector control is essential. Because of phlebotomine breeding sites are diverse, environmental larval controls have limited practical value. Control methods of adults are being evaluated, such as selective baits based on sugar feeding of males and females or Attractive Toxic Sugar Baits (ATSB), and the indoor use of Long-Lasting-Insecticidal Nets (LLINs) treated with permethrin to prevent sand fly bites complementing the Indoor Residual Spraying (IRS) approach suggested by WHO. Although several strategies exist, the best control measure to prevent canine *Leishmania infantum* is to treat dogs using biocidal topical formulations based on legal insecticides (PTs18) or repellents (PTs19) (as collars, spot-ons and/or sprays) during the period when the vector is active. This means we need to really know the biology and life cycle of the sand fly vector. According to available data, by mapping ambient temperatures we can already predict high risk areas where vector densities will be higher. In ongoing research, new candidates are emerging to fight against sand flies including natural plant extracts with low impacts on the environment and host animal. Other options in the future could be systemic insecticides to help reduce sand fly populations in high density areas. In parallel, health authorities and professionals involved in animal and public health (veterinarians, physicians, entomologists and epidemiologists) must work together in a One Health approach to minimize *Leishmania* infection. Veterinarians play a crucial role in liaising between key stake holders and dog owners to

ensure the latter act responsibly in using repellents as a preventive measure against sand fly bites.

Keywords: sand flies, *Leishmania infantum*, dog, canine leishmaniosis, pyrethroids, control

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Introduction

Leishmaniosis caused by *Leishmania infantum* syn. *L. chagasi* is a widespread zoonotic disease. In areas where this parasite is endemic, it may be transmitted to animals and humans by blood-sucking phlebotomine sand flies (Killick-Kendrick, 1999; Ready, 2013).

The distribution range of phlebotomine sand flies spans tropical, subtropical and temperate bioclimates zones within a belt between a northern latitude of 50° N and southern latitude of 40° S, yet they are absent from New Zealand and the Pacific islands (Maroli et al., 2013).

The dog is the main reservoir of *L. infantum* throughout the world. Prevention of canine leishmaniosis (CanL) is a challenge that requires measures focused on dogs and the environment based on the epidemiology of this disease in a given area. Preventing sand fly bites serves the dual purpose of protecting dogs from this potentially severe disease and reducing the risk of humans acquiring this infection (Otranto and Dantas-Torres, 2013).

In Europe the scenario for *L. infantum* infection has recently changed and endemic areas have expanded as numbers of infected dogs have increased in a northward direction (Espejo et al., 2014). A high proportion of infected dogs are cases of relocation or travel to and from endemic regions (Maia and Cardoso, 2015; Vrhovec et al., 2017). The introduction of infected dogs into previously CanL-free regions in absence of competent vectors has revealed other possible routes of transmission such as venereal or vertical (Boggiatto et al., 2011; Naucke and Lorentz, 2012) or even through blood transfusions (de Freitas et al., 2006) and horizontal dog to dog (Naucke et al., 2016; Petersen and Barr, 2009). Moreover, the northward expansion of the geographical distribution range of the sand fly vector has also been attributed to climate change (Koch et al., 2017; Ready, 2010). In such a situation is essential to consider an integrated epidemiological approach of what is going on in

northern European regions. Since the first report in 1999 (Naucke and Pesson, 2000), it is widely known that *P. mascittii* is the northern mostly distributed phlebotomine species in Europe and the only sand fly species found in certain other regions (Melaun et al., 2014). Several studies support the suspected vector capacity of *P. mascittii* identifying *Leishmania infantum* in wild caught sand flies by ITS1 nPCR and DNA sequencing (Obwaller et al., 2016; Vaselek et al., 2017).

Thus, on the basis of the facts stated, more than ever control strategies against CanL, besides vaccination, will essentially need to consider all aspects related to sand fly requirements (Miró et al., 2017).

Sand fly biology: knowing your enemy

The control of CanL needs to be based on an in depth knowledge and understanding of the biology of sand flies (Table 1). To obtain a good idea of the target of our measures the questions that need to be addressed are:

- Which are the vector species of CanL in a specific area?
- What are their environmental preferences?
- How long is their activity period?
- What are their climate requirements?
- How will climatic change modify the density and spread of sand flies?
- How do they behave?
- Which are their preferred blood sources?

Sand flies are ascribed to the order Diptera, suborder Nematocera of the family Psychodidae and subfamily Phlebotominae. They undergo complete, or holometabolous, metamorphosis, meaning their life cycle includes four stages: egg, larva (four instars), pupa and adult. To identify adults, the morphological features to look for are a hump-shaped thorax, hairy body, long legs and small size from 2 to 3 mm (Figure 1). Common characteristics are they cannot fold their wings when at rest and they typically hop around when they move (Killick-Kendrick, 1999; Maroli et al., 2013).

The biological rhythm of these insects is highly dependent on daily oscillations and climate variability and it also shows synchronization with host and parasite circadian rhythms (Marques, 2013). Ambient temperatures affect several factors such as the time period from a blood meal to egg maturation, egg hatching, the lengths of the different development stages and parasite development time (Reisen, 2010). Vector densities vary seasonally due to their sensitivity to climate variability (mainly temperature and rainfall) and therefore affect the probability of *Leishmania* transmission to dogs (Gálvez et al., 2010; Ready, 2013). The nocturnal activity of the sand fly species *Phlebotomus perniciosus* is crepuscular and has been described in areas of eastern Spain and southern Italy during the period of *L. infantum* transmission risk (Gaglio et al., 2014; Lucientes et al., 2005). In this last study, most sand flies were captured early at night time from sunset to midnight when the temperature was 18.7 to 21.6°C and humidity was 77.4 to 6.3% (Lucientes et al., 2005). Similarly, it has been reported that the minimum temperature at which sand flies remain active is 17°C (Killick-Kendrick, 1990). Sand fly activity patterns have been described also in *P. perfiliewi*. There was a distinct activity peak between 20.00–22.00 h for both male and female with more than 45% and 30% of the population sampled occurring between 20.00–

21.00h and 21.00–22.00h, respectively (Chaskopoulou et al., 2018). In the Greek Aegean Islands, preferences of aggregate sand fly populations was found between 21–29°C, nevertheless there were reported significant differences between species (Tsirigotakis et al., 2018). Other authors have described average annual temperatures for Mediterranean basin phlebotomine sand fly vectors ranging from 12.7°C for *P. ariasi* in France to 19.1°C for *P. tobbi* in Cyprus (Alten et al., 2016). Based on available temperature and risk data from the literature, we provide a predictive map showing the distribution of sand flies throughout endemic and non-endemic European countries for a minimum temperature of 17°C in July, as the month of maximum risk of *L. infantum* transmission (Figure 2).

The habitat of sand flies is described as a buffer area with resting places for adults and breeding sites. Adult resting sites are humid and cool. In effect, cool temperatures are optimal temperature for *Leishmania* development, digesting a blood meal, and for the maturation of egg batches (Ready, 2013). The blood meal sources of *P. perniciosus* are a wide range of mammals and birds (Bravo-Barriga et al., 2016; Gonzalez et al., 2015). Eggs are laid by adult females in warm, dark, moist soils rich in organic matter (Figure 3), which is required by the larvae for feeding and is also where they pupate. The larvae are capable of moving only very short distances from the egg laying, or oviposition site. The adult dispersion range is a few hundred meters on average and is determined by the proximity of larval breeding sites and the presence of moderate wind and lack of rainfall (Maroli et al., 2013). Regarding flight height preferences, significant differences was observed in *P. perfiliewi* numbers from sticky traps placed at 2 m compared to those from 0.5 to 1.5m indicating a preference of these sand flies species to fly below 2m (Chaskopoulou et al., 2018).

The biting behaviour of adult females depends on the species. Phlebotomines are classified as exophagic or endophagic according to whether they bite outdoors or indoors, and into exophilic and endophilic according to whether they rest outdoors or indoors after a blood meal respectively. The main *Leishmania* vectors in southern European countries, females of *P. perniciosus*, are predominantly exophagic and exophilic (they rest outdoors while their eggs mature). Thus, to apply effective control measures, behaviour knowledge of a specific vector is important (Killick-Kendrick, 1999).

Topical insecticides to ward off sand flies and avoid bites

Vector control represents a significant part of CanL prevention and consists of measures focused on both dog and environment (Travi et al., 2018). However, the use of topical products on dogs with proven insecticide and repellent efficacy is the first choice to avoid *L. infantum* infection. For their commercial use, both the insecticide and repellent effects of the active ingredients of these formulations must be tested. Among the topical compounds with proven insecticide and repellent efficacy against sand fly bites synthetic pyrethroids are the most widely used as they have shown the best tolerance and safety in dogs (Gramiccia, 2011). Today, different presentations are available: protective collars made of a polymer matrix, spot-on pipettes or sprays. The different formulations require different application regimens and show different onset of action (Miró et al., 2017; Wylie et al., 2014). The use of state-of-the-art technology in the case of collars, and the combination of generic products with other active ingredients in the case of spot-on pipettes, have promoted the commercialization of new topical formulations. Below, we provide an exhaustive review of the tested products available.

Collars contain a polymer matrix system capable of continuous low-dose release of active ingredients at the site of direct contact by spreading over the entire skin surface via the lipids and hair coat of treated animals. There are two collars on the market containing synthetic type II pyrethroids: deltamethrin (DEL) and flumethrin plus imidacloprid (FLU/IMI). DEL collar has been widely validated for its protective efficacy against sand flies in dogs in both laboratory (David et al., 2001; Halbig et al., 2000; Killick-Kendrick et al., 1997; Lucientes, 1999) and field studies (Ferroglia et al., 2008; Foglia Manzillo et al., 2006; Gavgani et al., 2002; Lopes et al., 2018; Maroli et al., 2001; Reithinger et al., 2004; Reithinger et al., 2001; Silva et al., 2018). As adequate anti-feeding efficacy has been confirmed after one week, the collar should be preferably worn at least one week before risk exposure. Its prevention of blood sucking by phlebotomine sand flies is long term, lasting for a period of 5 to 6 months (Killick-Kendrick et al., 1997). DEL is not absorbed systemically and insects are exposed to this active substance only through contact. FLU/IMI collar has been proven in field studies to diminish the risk of *L. infantum* infection in both dogs and cats (Brianti et al., 2017; Brianti et al., 2014; Brianti et al., 2016), but the efficacy of the product in terms of preventing sand fly bites has not been yet tested. So far, its effects have been validated to last for 7 to 8 months against fleas, ticks and biting lice (Brianti et al., 2013; Otranto et al., 2017; Stanneck et al., 2012b). Like DEL, the FLU component is a synthetic type II pyrethroid and an active ectoparasiticide, while IMI is a systemic neonicotinoid. These low-dose release collars do not need to be replaced more than once a year in temperate areas like the Mediterranean basin, where adult sand flies are absent during the cold months.

Spot-on pipettes are a colourless to yellowish or brownish ready-to-use topical solution. The active ingredient common to all commercially available pipettes is permethrin (PER), a synthetic type I pyrethroid known to prevent sand fly bites in dogs. The protective activity of PER lasts for 2 to 4 weeks, such that higher PER concentrations show longer duration effects. Spot-ons have short-lasting effects and rapidly spread across the dog's body surface via the hair coat of treated animals. As the onset of action is usually 24-48 h after application, this must be taken into account before exposure to ensure animals are properly protected. In Europe, there are six spot-ons of proven anti-feeding and insecticide efficacy against sand flies: permethrin/indoxacarb (48/15 mg/kg) (PER/IND), permethrin/imidacloprid (50/10 mg/kg) (PER/IMI); a permethrin/fipronil 1 (50.48/6.76 mg/kg) (PER/FIP 1), a permethrin/fipronil 2 (60/6.7 mg/kg) (PER/FIP 2), permethrin (47.66 mg/kg) (PER), and permethrin/dinotefuran/pyriproxyfen (46.6/6.4/0.6 mg/kg) (PER/DIN/PYR) (Figure 4). It should be stressed that the real dose received depends on the dog's weight as spot-on dosages are prepared for a fixed weight range (Miró et al., 2017). This means that in the same range, larger dogs receive lower doses of pyrethroids; what can lead to a lower protection of those dogs.

According the scientific bibliography and regarding the anti-feeding efficacy of these formulations against *P. perniciosus* (Figure 4.A), 90% efficacy is provided up to one week by the PER spot-on (from 99.13% on day 1 to 86.80% on day 15 post-treatment) (Ferroglio et al., 2008; Molina et al., 2012); up to day 14 by the PER/IND spot-on (from 99% at 2 days to 84% at 29 days post-treatment) (Frenais et al., 2014); up to day 22 by PER/IMIa (from 97.7% on day 1 to 74% on day 29 post-treatment) (Miró et al., 2007; Otranto et al., 2010; Otranto et al., 2007; Podaliri Vulpiani et al., 2009) up to day 28 ($\geq 88.1\%$) by

PER/IMIb (Bouhsira et al., 2018); up to day 21 by PER/FIP 1 (from 94.1% at 1 day to 89.7% at day 28 post-application) (Franc et al., 2015); up to day 29 by PER/FIP 2 (from 98.2% on day 1 to 90.3% on day 29) (Dumont et al., 2015); and up to day 14 by the triple-agent PER/DIN/PYR (from 96.9% on day 1 to 87% on day 28 post-application) (Lienard et al., 2013). For other species, as *P. papatasi*, the PER/IMI spot-on performs worse than against *P. perniciosus*, with 90% anti-feeding efficacy shown up to day 8 (from 94.6% on day 1 to 55.9 % on day 29) (Mencke et al., 2003). Regarding *Lutzomyia longipalpis*, this product performs similarly efficacy than against *P. perniciosus*, with up to three weeks of over 90% repellent efficacy (Mencke et al., 2005).

In the same way, for data gathered regarding the insecticidal efficacy against *P. perniciosus* (Figure 4.B), the permethrin alone spot-on (PER) showed an insecticidal efficacy from 97.6% on day 1 to 43.18% on day 15 post-application (Molina et al., 2012), being label indications for use of one week; PER/IND spot-on provided a knock-down effect recorded after one hour of exposure in treated dogs was only significant in the first week after treatment (37%) (Frenais et al., 2014); PER/IMIa spot-on showed a rather low insecticidal efficacy from 53.2% on day 0 to 2.9% on day 29 post-treatment, and only within the first week was this activity significant (Miró et al., 2007), being the values against *P. papatasi*. from 60% on day 1 to 29.3% on day 29 post-treatment (Mencke et al., 2003); PER/IMIb revealed an insecticidal activity up to day 21 over 95% (Bouhsira et al., 2018); PER/FIP 1 spot-on provided a high performance throughout the study, from 98.9% at day 1 to 89.7% at day 28 post-treatment (Franc et al., 2015) and was attributed to the synergic action of both active principles (Dumont et al., 2015); PER/FIP 2 spot-on has the same active ingredients at almost the same concentrations and shows a similar efficacy to the PER/FIP

1 spot-on, being the knock down effect reported 4h post-exposure 98.7% on day 1 to 78.9% on day 29, yet was still over 90% up until day 21 post-application (Dumont et al., 2015); and finally, the triple-agent PER/DIN/PYR spot-on insecticidal efficacy was highest on days 1 (97.8%) and 7 (99.8%), felling on day 14 to 73.7% and to 39.6% on day 28 after treatment. A synergistic effect was observed when dinotefuran was administered in combination with permethrin, leading to more persistent insecticidal activity over time for up to one month (Lienard et al., 2013).

In summary, commercial spot-ons show a 90% anti-feeding efficacy against sand flies that lasts up to one week (PER), two weeks (PER/IND and PER/DIN/PYR), three weeks (PER/IMI and PER/FIP 1) or a maximum of four weeks (PER/FIP 2). In contrast an insecticidal efficacy against sand flies as high as 90% only lasts one day (PER), one week (PER/DIN/PYR) or at most four weeks (PER/FIP 1 and PER/FIP 2) (Figure 4).

If we look at the indications on the label that specify for how long these formulations provide anti-feeding activity against *P. perniciosus*, we get that the values are up to two weeks (PER) three weeks (PER/IND, PER/IMI and PER/FIP 2), four weeks (PER/FIP 1 and PER/DIN/PYR). In respect to *P.papatasi*, the value of the PER/IMI is of two weeks. Comparing this information with the one extracted from the available data we can conclude that in all cases, the indications give an additional week of efficacy over the 90% value, except for PER/IMI that is the same and PER/FIP 2 in which a week is reduced.

Sprays are directly applied to the hair coat so that the active ingredients penetrate the skin. It is important these products are properly applied to the whole body surface to avoid unprotected body areas on dogs (Miró et al., 2017). One commercial product was available against sand flies: Duowin[®], Virbac (permethrin/pyriproxyfen 18.8/0.2 mg/ml). The anti-

feeding efficacy of this spray for *P. perniciosus* was high on days 7 (91.5%) and 14 (92%), and slightly fell on days 21 (89.3%) and 28 (90.8%), yet showed notable efficacy for up to 4 weeks. Even so, its insecticidal activity proved fairly low from the moment of application on day 7 (29.6%) and was almost null on day 28 (0.8%) (Molina et al., 2006). There are also some sprays containing permethrin on the market that presumably show the same efficacy against sand flies but was not experimentally proven. The main benefit of these presentations is its immediate effect, and its main drawback is its low persistence.

Modes of action of active ingredients in available topical insecticide formulations

Pyrethroids have been widely validated as adequate agents for sand fly control. These synthetic chemical insecticides are analogues of pyrethrins, which are natural insecticides derived from chrysanthemum flowers (Rehman et al., 2014). Synthetic pyrethroids have several characteristic features in terms of their toxicity, efficacy and chemical properties: they show selective toxicity for a wide spectrum of arthropod species, low mammalian toxicity but extreme toxicity towards aquatic organisms; powerful repellent and rapid insecticidal activity; and they act through contact, are lipophilic, water resistant, scarcely volatile, highly stable in sunlight and rapidly break-down in the environment (Thatheyus and Gnana Selvam, 2013).

Once sand flies contact the skin of dogs treated with synthetic pyrethroids these agents show different modes of action against phlebotomines according to the time of exposure to the insecticide (Maroli et al., 2010). Sand flies may remain on the skin for sufficient time to

absorb a lethal dose of insecticide (*killing dose*), or they may be only temporarily exposed causing their inability to move or fly away (*knockdown dose*) or causing irritation and disorientation (*repellent dose*). They are classified as sodium channel modulators (IRAC, 2017). After contact, synthetic pyrethroids quickly penetrate the nerve system of the insect, where they selectively disrupt nerve transmission increasing nerve cell membrane sodium channel permeability. This results in hyperexcitability are followed by paralysis, tremor and finally killing of the exposed ectoparasite. Permethrin is readily metabolized by humans and animals, but is toxic to arthropods because of their much slower rate of metabolism and elimination of the chemical (Thatheyus and Gnana Selvam, 2013).

Topical formulations based on synthetic pyrethroids are often combined with other chemicals (eg. imidacloprid, fipronil or dinotefuran) that sometimes act as a synergist ingredient enhancing their effectiveness. Permethrin is the most commonly used synthetic pyrethroid of all veterinary ectoparasiticide products. Presently, another synthetic pyrethroids, deltamethrin and flumethrin, are commercially available as a collar. Below we provide a comprehensive review of the modes of action of other chemicals that have become typical components of topical formulations for use in dogs.

Oxadiazines (Indoxacarb)

Indoxacarb belongs to the oxadiazine family of chemicals and it is classified as voltage-dependent sodium channel blockers (IRAC, 2017). This insecticide enters the insect mainly through ingestion although it can be absorbed to a lesser extent through contact. In the mid-gut of susceptible insect species, indoxacarb is transformed into a metabolite that blocks sodium channels in the insect's nervous system. Its effectiveness has been extensively documented against adults and immature stages of fleas in the immediate surroundings of

the treated dog (Dryden et al., 2013). According to *in vitro* data, indoxacarb is toxic for the adult blowfly (Calliphoridae) and for mosquito larvae (Frenais et al., 2014). However, because of the blood sucking, or haematophagous behaviour of the sand fly, it is not likely it will be very susceptible to the insecticidal effects of indoxacarb.

Neocotinoids (Imidacloprid and Dinotefuran)

Imidacloprid and dinotefuran are systemic insecticides belonging to the neonicotinoids group of chemicals which are classified as nicotinic acetylcholine receptor (nAChR) competitive modulators (IRAC, 2017). They are effective through contact or ingestion, and because of their high affinity for nicotinic acetylcholine receptors in the insect nervous system, they inhibit cholinergic transmission causing a block in nerve impulse propagation followed by insect death. Both dinotefuran and imidacloprid show a low affinity for mammalian acetylcholine receptors. The efficacy of imidacloprid against adult fleas and immature flea stages has been widely established (Mehlhorn et al., 2001). The combination of synthetic pyrethroids (permethrin or flumethrin) with imidacloprid has been found to significantly enhance neurotoxic activity over the added effects of each agent alone (Stanneck et al., 2012a). A synergistic effect was reported when dinotefuran was administered in combination with synthetic pyrethroids causing a faster onset of insecticidal activity (Lienard et al., 2013).

Phenylpyrazoles (Fipronil)

Fipronil is a systemic insecticide and acaricide belonging to the phenyl-pyrazole family classified as GABA-gated chloride channel blockers (IRAC, 2017). The major metabolite of fipronil is its sulphone derivative that binds to gamma-aminobutyric acid (GABA) receptors, blocking pre- and post-synaptic transfer of chloride ions across cell membranes.

This leads to the uncontrolled activity of the central nervous system and death of arthropods. The combination of fipronil and permethrin has a strong insecticidal effect against sand flies that can be attributed to their synergic action. This product combination showed a faster onset of flea adulticidal activity than fipronil alone after treatment administration (Halos et al., 2016).

Insect Growth Regulator (Pyriproxyfen)

Pyriproxyfen is an insect growth regulator (IGR) that mimics the actions of juvenile hormones (IRAC, 2017). It acts through contact and hinders moulting which affects the immature stages of arthropods (eggs, larvae and pupae). Pyriproxyfen only affects developing stages, so it is often formulated in combination with adulticides.

Oral insecticides as possible candidates for sand fly control

Some systemic insecticides, without specific therapeutic indications against sand flies, have been assessed for this purpose both in laboratory and field studies (Table 2). Results for these molecules are described below.

Fipronil

The insecticidal efficacy of fipronil against adult and larval stages of sand flies has been demonstrated in laboratory and field studies. Female sand flies are killed if they feed on treated animals and larval stages are also susceptible to this agent as fipronil is excreted in the faeces of treated animals on which larvae feed.

Laboratory studies have shown a high insecticidal efficacy of fipronil against the larval stages of sand flies after orally treating rodents and cattle (Derbali et al., 2014; Mascari et

al., 2013; Poche et al., 2013). Levels of fipronil significantly toxic to sand fly larvae have been detected following 21 days after the end of a fipronil-containing diet in hamsters (*Mesocricetus auratus*), and cows (*Bos indicus*) and 5 weeks after the treatment of desert jirds (*Meriones shawi*) with a concentration of 0.005% of fipronil (Derbali et al., 2014). Further, in blood feeding bioassays it was shown that fipronil was present in the peripheral blood of hamsters at a concentration that was significantly toxic for adult female sand flies 49, 29, and 21 days respectively after the hamsters, jirds and cows had been withdrawn from their fipronil-containing diets (Derbali et al., 2014; Mascari et al., 2013; Poche et al., 2013).

In field studies, fipronil was shown to reduce adult sand fly populations (95-97%) after treating cattle 3-6 times per year (Poche et al., 2017). In another two studies, 80% reductions in *P. papatasi* populations were detected up to 6 weeks after the application of 0.005% fipronil to rodent baits (*M. shawi*) (Derbali et al., 2017; Derbali et al., 2014).

Recently, Poche et al., (2016) developed an individual-based, stochastic, life-stage-structured model to represent a sand fly vector population and simulate the effects of vector control using orally administered fipronil-based drugs. This simulation model for a small village in India predicted an 83-95% reduction in sand flies after administering oral fipronil treatment to cattle. This reduction percentage range was found to vary depended on the timing of drug administration relative to the sand fly life cycle (Poche et al., 2016).

Imidacloprid

Imidacloprid acts on fleas via direct contact with the compound. This active substance becomes incorporated in the water-resistant lipid layer secreted by the sebaceous glands, and spreads over the entire skin and fur of the host and it is excreted in the faeces and urine (Baynes, 2009).

Laboratory studies have shown that 100% of *P. papatasi* were killed within 24h after blood feeding on *M. shawi* treated up to four weeks previously with a single imidacloprid dose (0.05%) (Derbali et al., 2013). Similar results were reported by Wasserberg et al. (2011) who observed a range of 81.3% (dose 1 mg/ml) to 89.8% (dose 5 mg/ml) mortality in adult female *P. papatasi* feeding on imidacloprid-treated rabbits (Wasserberg et al., 2011). In larvicide experiments, 90-100% of *P. papatasi* larvae that had consumed the faeces of rodents treated with imidacloprid-loaded bait did not survive up to pupation, although first stage larvae were more sensitive than older stages and *Lutzomyia longipalpis* larvae were less sensitive (Derbali et al., 2013; Mascari et al., 2012b; Wasserberg et al., 2011).

Ivermectin

The main indications of macrocyclic lactones (MLs) in dogs and cats is to prevent heartworm as they are effective against nematodes and moreover against mites, and lice (Nolan and Lok, 2012).

When the ML ivermectin was assessed as a rodent systemic and feed-through insecticide for the control of adult and immature *P. papatasi*, the agent proved fully effective against blood-feeding sand flies for up to one week after rodents were fed ivermectin-containing diets (Mascari and Foil, 2010; Mascari et al., 2012a, b). In addition, larvae fed with hamster faeces (at 0, 3, 7 and 14 days of treatment) died before pupation (Mascari and Foil, 2010).

Other MLs such as abamectin have returned inconclusive results as high doses of abamectin (>10 mg/kg) were not palatable for rodents and palatable doses (10 mg/kg) did not cause 100% mortality of blood-feeding sand flies (Mascari et al., 2012b).

The killing effect of other MLs (e.g. moxidectin) on adult and larval sand flies has not yet been tested. However, some of these MLs are available in combination with other

ectoparasiticides (e.g. imidacloprid/moxidectin and spinosad/milbemycin oxime). Thus further work is needed to assess their efficacy and safety.

Spinosad

Spinosad comprises spinosyn A and spinosyn D and is used as an insecticide against fleas in dogs and cats through oral administration. Its excretion is primarily via the bile and faeces, and to a lesser extent in urine (Baynes, 2009). In a laboratory study, 100% mortality of blood-feeding sand flies was observed for at least 1 week after rodents were fed with spinosad (5000 mg/kg) (Mascari et al., 2012b). These studies in mice models need to be repeated in canids.

Other Insect Growth Regulators (IGRs)

Other Insect Growth Regulators (IGRs) such as diflubenzuron, lufenuron and novaluron (chitin synthesis inhibitors) or methoprene and pyriproxyfen (juvenile hormone analogues), interfere with the molting process of insects development. All of them are used in the control of fleas (Baynes, 2009). Some IGRs have been incorporated in the rodent diet to study their efficacy against immature stages of sand flies. The effects observed were that diflubenzuron caused mortality from the larva to pupa moult stage; novaluron caused mortality at the larval moult stage; methoprene caused mortality at the fourth instar or pupal stage; and, pyriproxyfen caused mortality at the fourth instar stage (Mascari et al., 2011; Mascari et al., 2012a).

In summary, according to the results of published studies to date, a strategy to control sand fly populations could be to orally administer systemic insecticides to rodents, cattle or other mammals as bait. Systemic insecticides kill female sand flies after they feed on the blood of these animals, and also kill larval stages that feed on animal faeces. However, futures

studies are needed to assess the field efficacy of this measure testing the use of well characterized insecticides as well as new potential candidates as such as oral isoxazolines: afoxolaner (Otranto, 2014; Shoop et al., 2014), fluralaner (Gassel et al., 2014), sarolaner (McTier et al., 2016) and lotilaner (Little, 2017) whose efficacy against sand flies bites is still unknown. Indeed, recent studies have shown that afoxolaner could be a good candidate due to its insecticidal efficacy against *Aedes aegypti* on treated dogs on day 2 (98%) and day 29 (75.3%) of treatment (Liebenberg et al., 2017). In addition, new results indicate that fluralaner shows insecticidal activity against *P. papatasi* feeding on the blood of treated dogs between 60-80% for 30 days (Gómez et al., 2018).

Natural active ingredients: plant extracts

In studies *in vitro*, some authors have tested natural plant extracts as repellents and insecticides against sand flies (Dinesh et al., 2014b). Such oil extracts have been obtained from widely distributed plants including castor oil, lavender, lemon grass (citronella), *Geranium* spp., jasmine nightshade, lesser bougainvillea, caper bush, bull mallow, eucalyptus, common myrtle, *Monticalia greenmaniana*, snakeroot and *Derris* spp. (containing rotenone, a natural broad-spectrum insecticide); or regionally distributed plants like southern marigold (native to South America), *Acalypha fruticosa* (widely distributed in East and Southern Africa), camphor bush (widespread across southern Africa) and neem (found in tropical and subtropical regions) (Cardenas et al., 2012; Dinesh et al., 2015; Torres et al., 2017). Despite promising results, these candidate insecticides have not been yet tested on dogs (Miró et al., 2017). If these natural products are found to prove effective

in the future, their benefits over synthetic formulations will be a low environmental impact and lack of toxicity for the host.

Environmental vector control

Environmental management entails controlling sand fly vectors through actions designed to reduce sand fly numbers in the dogs' environment.

The environmental control of immature sand flies is difficult because they are terrestrial and thus breeding sites could be anywhere including a wide variety of microhabitats rich in organic matter favourable for both larvae and pupae (eg, tree roots and holes, animal burrows, leaf litter, manure, holes and crevices in walls, etc) (Otranto and Dantas-Torres, 2013). Searching for immature phlebotomine stages in soil samples is like looking for a needle in a haystack (Feliciangeli, 2004). A strategy described as useful to reduce larval sources is the removal of microhabitats suitable for sand flies to develop (eg, avoiding the build-up of organic matter, plant cuttings or rubbish) (Miró et al., 2017). Notwithstanding, addressing larvae and pupae control remains a challenge and sand fly control programs have mainly targeted adults. Common practices like making compost in gardens will provide a favourable habitat for sand flies to breed. Adults rest close to oviposition sites, and both resting and breeding sites are usually cool, humid and dark micro-habitats (Figure 3) (eg. cellars, stables, crevices, soil, dense vegetation, trees, burrows, etc) (Killick-Kendrick, 1999; Maroli et al., 2013). However, spraying large areas of suspected natural resting and breeding sites with insecticides can be ineffective since residual effects are short-lived in open environments (Sharma and Singh, 2008). In contrast, the use of insecticides in animal shelters can diminish sand fly populations because animals will

attract these vectors (Otranto and Dantas-Torres, 2013). An example is the disinfection measures undertaken since 2011 in the area of the leishmaniosis outbreak in south-western Madrid. This environmental control measure along with others have effectively reduced sand fly populations (Gomez-Barroso et al., 2015). In addition, *P. perfiliewi* populations can be highly controlled using Ground ultra low volume (ULV) space spray applications of a deltamethrin, being effective even in a heavily infested sand fly environment such as the kennel sites (Chaskopoulou et al., 2018).

Other measures are the use of nets in shelters and homes (with holes of 0.3 to 0.4 mm²) to avoid flies biting dogs, or keeping dogs indoors from dusk to dawn (Miró et al., 2017). Permethrin treated bednets can be a useful technology to control canine leishmaniosis. Permethrin creates an effective barrier against adults of some species of sand flies (Rowland et al., 2015). Long-Lasting Insecticidal Nets (LLINs) have been evaluated as vector control alternative to Indoor Residual Spraying (IRS) to prevent visceral leishmaniosis in the Indian subcontinent under the umbrella of the World Health Organization (WHO) and the Governments of India, Nepal and Bangladesh (Picado et al., 2015). A novel and interesting approach of sand-flies control based on adult behaviour is the use of Attractive Toxic Sugar Baits (ATSB) on green non-flowing vegetation or within bait stations (Qualls et al., 2015). The authors significantly reduced densities of both female and males of *P. papatasi* and *P. sergenti* along five-week in Morocco, with low non-target impact.

Biological control of *Leishmania* vectors

Biological control is one of the most environmentally friendly methods used to control animal parasites. Most insects are infected by different pathogens, including viruses, bacteriae, fungi, protozoa, nematodes, entomophages arthropod and insectivorous vertebrates. The biocontrol of sand flies is a research field to be developed, but some records show its potential value. In Argentina *Pintomyia fischeri* is infested by juveniles of entomoparasitic nematodes of the genus *Tylenchida* (Fernández et al., 2016). These nematodes are known as parasites of different species of sand flies from Africa, the Middle East and Central America. In India, mites have been recorded predating sand fly 2nd to 4th larval instars under laboratory conditions (Dinesh et al., 2014a). These authors also recorded a spider eating females of sand flies. The value of entomopathogenic virus, bacteriae and fungi as environmental larvicide of phlebotomids for bio-control of the visceral leishmaniosis could be limited because of the diversity of breeding sites of the sand flies in their natural habitats (Ebrahim, 2015): *Bacillus thurigiensis* var. *israelensis* infects larvae of *Phlebotomus papatasi* and *Lutzomyia longipalpis*. *B. sphaericus* has been used as biocontrol agent of *P. martini*, and can have inhibitory effect on *P. duboscqi* eggs. Further research is needed to evaluate the biological control of sand fly populations by natural enemies.

Overview of recommendations

The only way to determine which control strategy will be appropriate for a given site is to experimentally assess the possible efficacy of the different methods and options in situ. The measures implemented should be based on local epidemiological information. In endemic zones, the best control strategy will be the use of insect repellents in the whole dog

population and a feasible combination of vaccination in healthy seronegative dogs. Although both these measures are considered equally necessary in healthy dogs, vaccination does not avoid infection while repellents could indeed reduce the risk of transmission.

Abbreviations

ATBS = Attractive Toxic Sugar Baits; DEL = deltamethrin; DIN = dinotefuran; FIP = fipronil; FLU = flumethrin; GABA = gamma-aminobutyric acid; GIS = Geographic Information System; IGRs = Insect Growth Regulator; IMI = imidacloprid; IND = indoxacarb; LLINs = Long-lasting Insecticidal Nets; ML = macrocyclic lactone PER = permethrin; PYR = pyriproxyfen.

Conflict of interest

The Authors declare no competing interests.

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References

Alten, B., Maia, C., Afonso, M.O., Campino, L., Jimenez, M., Gonzalez, E., Molina, R., Banuls, A.L., Prudhomme, J., Vergnes, B., Toty, C., Cassan, C., Rahola, N., Thierry, M., Sereno, D., Bongiorno, G., Bianchi, R., Khoury, C., Tsirigotakis, N., Dokianakis, E., Antoniou, M., Christodoulou, V., Mazeris, A., Karakus, M., Ozbel, Y., Arserim, S.K., Erisoz Kasap, O., Gunay, F., Oguz, G., Kaynas, S., Tsertsvadze, N., Tskhvaradze, L.,

Giorgobiani, E., Gramiccia, M., Volf, P., Gradoni, L., 2016. Seasonal Dynamics of Phlebotomine Sand Fly Species Proven Vectors of Mediterranean Leishmaniasis Caused by *Leishmania infantum*. PLoS neglected tropical diseases 10, e0004458.

Baynes, R.E., 2009. Ectoparasiticides, in: Papich, J.R.a.M. (Ed.), Veterinary Pharmacology and Therapeutics. Willey-Blackwell.

Boggiatto, P.M., Gibson-Corley, K.N., Metz, K., Gallup, J.M., Hostetter, J.M., Mullin, K., Petersen, C.A., 2011. Transplacental transmission of *Leishmania infantum* as a means for continued disease incidence in North America. PLoS neglected tropical diseases 5, e1019.

Bouhsira, E., Deuster, K., Lienard, E., Le Sueur, C., Franc, M., 2018. Evaluation of the anti-feeding and insecticidal effects of a topically administered combination of imidacloprid and permethrin (Advantix(R)) against *Phlebotomus (Larrousius) perniciosus* (Newstead, 1911) in dogs following monthly administration. Parasites & vectors 11, 120.

Bravo-Barriga, D., Parreira, R., Maia, C., Afonso, M.O., Blanco-Ciudad, J., Serrano, F.J., Perez-Martin, J.E., Gomez-Gordo, L., Campino, L., Reina, D., Frontera, E., 2016. Detection of *Leishmania* DNA and blood meal sources in phlebotomine sand flies (Diptera: Psychodidae) in western of Spain: Update on distribution and risk factors associated. Acta tropica 164, 414-424.

Brianti, E., Falsone, L., Napoli, E., Gaglio, G., Giannetto, S., Pennisi, M.G., Priolo, V., Latrofa, M.S., Tarallo, V.D., Solari Basano, F., Nazzari, R., Deuster, K., Pollmeier, M., Gulotta, L., Colella, V., Dantas-Torres, F., Capelli, G., Otranto, D., 2017. Prevention of feline leishmaniosis with an imidacloprid 10%/flumethrin 4.5% polymer matrix collar. Parasites & vectors 10, 334.

Brianti, E., Falsone, L., Napoli, E., Prudente, C., Gaglio, G., Giannetto, S., 2013. Efficacy of a combination of 10% imidacloprid and 4.5% flumethrin (Seresto(R)) in slow release

collars to control ticks and fleas in highly infested dog communities. *Parasites & vectors* 6, 210.

Brianti, E., Gaglio, G., Napoli, E., Falsone, L., Prudente, C., Solari Basano, F., Latrofa, M.S., Tarallo, V.D., Dantas-Torres, F., Capelli, G., Stanneck, D., Giannetto, S., Otranto, D., 2014. Efficacy of a slow-release imidacloprid (10%)/flumethrin (4.5%) collar for the prevention of canine leishmaniosis. *Parasites & vectors* 7, 327.

Brianti, E., Napoli, E., Gaglio, G., Falsone, L., Giannetto, S., Solari Basano, F., Nazzari, R., Latrofa, M.S., Annoscia, G., Tarallo, V.D., Stanneck, D., Dantas-Torres, F., Otranto, D., 2016. Field Evaluation of Two Different Treatment Approaches and Their Ability to Control Fleas and Prevent Canine Leishmaniosis in a Highly Endemic Area. *PLoS neglected tropical diseases* 10, e0004987.

Cardenas, J., Rojas, J., Rondon, M., Nieves, E., 2012. Adulticide effect of *Monticalia greenmaniana* (Asteraceae) against *Lutzomyia migonei* (Diptera: Psychodidae). *Parasitology research* 111, 787-794.

Chaskopoulou, A., Miaoulis, M., Kashefi, J., 2018. Ground ultra low volume (ULV) space spray applications for the control of wild sand fly populations (Psychodidae: Phlebotominae) in Europe. *Acta tropica* 182, 54-59.

David, J.R., Stamm, L.M., Bezerra, H.S., Souza, R.N., Killick-Kendrick, R., Lima, J.W., 2001. Deltamethrin-impregnated dog collars have a potent anti-feeding and insecticidal effect on *Lutzomyia longipalpis* and *Lutzomyia migonei*. *Memorias do Instituto Oswaldo Cruz* 96, 839-847.

de Freitas, E., Melo, M.N., da Costa-Val, A.P., Michalick, M.S., 2006. Transmission of *Leishmania infantum* via blood transfusion in dogs: potential for infection and importance of clinical factors. *Veterinary parasitology* 137, 159-167.

Derbali, M., Chelbi, I., Cherni, S., Barhoumi, W., Boujaama, A., Raban, R., Poche, R., Zhioua, E., 2013. [Laboratory and field evaluation of an imidacloprid treated rodent oral bait for a systemic control of *Phlebotomus papatasi* Scopoli, 1786 (Diptera: Psychodidae)]. *Bull Soc Pathol Exot* 106, 54-58.

Derbali, M., Dachraoui, K., Barhoumi, W., Chelbi, I., Safedine, C., Poche, R., Zhioua, E., 2017. Field evaluation of rodent bait treated with fipronil for feed through and systemic control of *Phlebotomus papatasi*. , 6th World Congress on Leishmaniosis, Toledo. Spain.

Derbali, M., Polyakova, L., Boujaama, A., Burruss, D., Cherni, S., Barhoumi, W., Chelbi, I., Poche, R., Zhioua, E., 2014. Laboratory and field evaluation of rodent bait treated with fipronil for feed through and systemic control of *Phlebotomus papatasi*. *Acta tropica* 135, 27-32.

Dinesh, D.S., Kumar, V., Kesari, S., Das, P., 2014a. Mites and spiders act as biological control agent to sand flies. *Asian Pacific Journal of Tropical Disease* 4, S463–S466.

Dinesh, D.S., Kumari, S., Kumar, V., Das, P., 2014b. The potentiality of botanicals and their products as an alternative to chemical insecticides to sandflies (Diptera: Psychodidae): a review. *Journal of vector borne diseases* 51, 1-7.

Dinesh, D.S., Kumari, S., Pandit, V., Kumar, J., Kumari, N., Kumar, P., Hassan, F., Kumar, V., Das, P., 2015. Insecticidal effect of plant extracts on *Phlebotomus argentipes* (Diptera: Psychodidae) in Bihar, India. *The Indian journal of medical research* 142 Suppl, S95-S100.

Dryden, M.W., Payne, P.A., Smith, V., Heaney, K., Sun, F., 2013. Efficacy of indoxacarb applied to cats against the adult cat flea, *Ctenocephalides felis*, flea eggs and adult flea emergence. *Parasites & vectors* 6, 126.

Ebrahim, A.Y., 2015. Biological control of vector borne protozoan parasites of veterinary importance. *Applied Scientific Reports* 10, 122–127.

Espejo, L.A., Costard, S., Zangmutt, F.J., 2014. Modelling canine leishmaniasis spread to non-endemic areas of Europe. *Epidemiology and infection*, 1-14.

Feliciangeli, M.D., 2004. Natural breeding places of phlebotomine sandflies. *Medical and veterinary entomology* 18, 71-80.

Fernández, M., Santini, M., Diaz, J., Villarquide, L., Lestani, E., Solomón, O., Achinelly, M., 2016. Parasitism by tylenchid nematodes in natural populations of *Pintomyia fischeri* (Diptera: Psychodidae: Phlebotominae) in Argentina. *SM Tropical Medicine Journal* 1, 1001.

Ferroglio, E., Poggi, M., Trisciuglio, A., 2008. Evaluation of 65% permethrin spot-on and deltamethrin-impregnated collars for canine *Leishmania infantum* infection prevention. *Zoonoses and public health* 55, 145-148.

Foglia Manzillo, V., Oliva, G., Pagano, A., Manna, L., Maroli, M., Gradoni, L., 2006. Deltamethrin-impregnated collars for the control of canine leishmaniasis: evaluation of the protective effect and influence on the clinical outcome of *Leishmania* infection in kennelled stray dogs. *Veterinary parasitology* 142, 142-145.

Frenais, R., Flochlay-Sigognault, A., Milon-Harnois, G., 2014. Anti-feeding efficacy of Activyl(R) Tick Plus topical treatment of dogs against *Phlebotomus perniciosus*. *Parasites & vectors* 7, 217.

Gaglio, G., Brianti, E., Napoli, E., Falsone, L., Dantas-Torres, F., Tarallo, V.D., Otranto, D., Giannetto, S., 2014. Effect of night time-intervals, height of traps and lunar phases on sand fly collection in a highly endemic area for canine leishmaniasis. *Acta tropica* 133, 73-77.

Gálvez, R., Descalzo, M.A., Miró, G., Jiménez, M.I., Martín, O., Dos Santos-Brandao, F., Guerrero, I., Cubero, E., Molina, R., 2010. Seasonal trends and spatial relations between

environmental/meteorological factors and leishmaniosis sand fly vector abundances in Central Spain. *Acta tropica* 115, 95-102.

Gassel, M., Wolf, C., Noack, S., Williams, H., Ilg, T., 2014. The novel isoxazoline ectoparasiticide fluralaner: selective inhibition of arthropod gamma-aminobutyric acid- and L-glutamate-gated chloride channels and insecticidal/acaricidal activity. *Insect Biochem Mol Biol* 45, 111-124.

Gavgani, A.S., Hodjati, M.H., Mohite, H., Davies, C.R., 2002. Effect of insecticide-impregnated dog collars on incidence of zoonotic visceral leishmaniasis in Iranian children: a matched-cluster randomised trial. *Lancet* 360, 374-379.

Gomez-Barroso, D., Herrador, Z., San Martin, J.V., Gherasim, A., Aguado, M., Romero-Mate, A., Molina, L., Aparicio, P., Benito, A., 2015. Spatial distribution and cluster analysis of a leishmaniasis outbreak in the south-western Madrid region, Spain, September 2009 to April 2013. *Euro Surveill* 20, 11-20.

Gomez, S.A., Curdi, J.L., Hernandez, J.A.C., Peris, P.P., Gil, A.E., Velasquez, R.V.O., Hernandez, P.O., Picado, A., 2018. Phlebotomine mortality effect of systemic insecticides administered to dogs. *Parasites & vectors* 11, 230.

Gonzalez, E., Gallego, M., Molina, R., Abras, A., Alcover, M.M., Ballart, C., Fernandez, A., Jimenez, M., 2015. Identification of blood meals in field captured sand flies by a PCR-RFLP approach based on cytochrome b gene. *Acta tropica*.

Gramiccia, M., 2011. Recent advances in leishmaniosis in pet animals: epidemiology, diagnostics and anti-vectorial prophylaxis. *Veterinary parasitology* 181, 23-30.

Halbig, P., Hodjati, M.H., Mazloumi-Gavgani, A.S., Mohite, H., Davies, C.R., 2000. Further evidence that deltamethrin-impregnated collars protect domestic dogs from sandfly bites. *Medical and veterinary entomology* 14, 223-226.

- Halos, L., Fourie, J.J., Fankhauser, B., Beugnet, F., 2016. Knock-down and speed of kill of a combination of fipronil and permethrin for the prevention of *Ctenocephalides felis* flea infestation in dogs. *Parasites & vectors* 9, 57.
- IRAC, 2017. Mode of Action Classification Scheme. Version 8.3. <http://www.irac-online.org/documents/moa-classification/>, in: Committee, I.R.A. (Ed.).
- Killick-Kendrick, R., 1990. Phlebotomine vectors of the leishmaniasis: a review. *Medical and veterinary entomology* 4, 1-24.
- Killick-Kendrick, R., 1999. The biology and control of phlebotomine sand flies. *Clinics in dermatology* 17, 279-289.
- Killick-Kendrick, R., Killick-Kendrick, M., Focheux, C., Dereure, J., Puech, M.P., Cadiergues, M.C., 1997. Protection of dogs from bites of phlebotomine sandflies by deltamethrin collars for control of canine leishmaniasis. *Medical and veterinary entomology* 11, 105-111.
- Koch, L.K., Kochmann, J., Klimpel, S., Cunze, S., 2017. Modeling the climatic suitability of leishmaniasis vector species in Europe. *Sci Rep* 7, 13325.
- Liebenberg, J., Fourie, J., Lebon, W., Larsen, D., Halos, L., Beugnet, F., 2017. Assessment of the insecticidal activity of afoxolaner against *Aedes aegypti* in dogs treated with NexGard((R)). *Parasite* 24, 39.
- Lienard, E., Bouhsira, E., Jacquet, P., Warin, S., Kaltsatos, V., Franc, M., 2013. Efficacy of dinotefuran, permethrin and pyriproxyfen combination spot-on on dogs against *Phlebotomus perniciosus* and *Ctenocephalides canis*. *Parasitology research* 112, 3799-3805.
- Little, S.E., 2017. Lotilaner - a novel systemic tick and flea control product for dogs. *Parasites & vectors* 10, 539.

Lopes, E.G., Seva, A.P., Ferreira, F., Nunes, C.M., Keid, L.B., Hiramoto, R.M., Ferreira, H.L., Oliveira, T., Ovallos, F.G., Galati, E.A.B., Villegas, T.J., Bortoletto, D.V., Valadas, S., Soares, R.M., 2018. Vaccine effectiveness and use of collar impregnated with insecticide for reducing incidence of *Leishmania* infection in dogs in an endemic region for visceral leishmaniasis, in Brazil. *Epidemiology and infection*, 1-6.

Lucientes, J., 1999. Laboratory observations on the protection of dogs from the bites of *Phlebotomus perniciosus* with Scalibor® protector bands: preliminary results., in: Killick-Kendrick (Ed.), *Canine leishmaniasis: an update*. Proceedings of the International Canine Leishmaniasis Forum, Barcelona, pp. 92-94.

Lucientes, J., Palmero, J., Guarga, J.L., Gracia, M.J., Peribañez, M.A., Zarate, J., Castillo, J.A., 2005. Risk of transmission of canine leishmaniasis in eastern Spain. *The Veterinary record* 156, 743-744.

Maia, C., Cardoso, L., 2015. Spread of *Leishmania infantum* in Europe with dog travelling. *Veterinary parasitology*.

Maroli, M., Feliciangeli, M.D., Bichaud, L., Charrel, R.N., Gradoni, L., 2013. Phlebotomine sandflies and the spreading of leishmaniasis and other diseases of public health concern. *Medical and veterinary entomology* 27, 123-147.

Maroli, M., Gradoni, L., Oliva, G., Castagnaro, M., Crotti, A., Lubas, G., Paltrinieri, S., Roura, X., Zini, E., Zatelli, A., 2010. Guidelines for prevention of leishmaniasis in dogs. *Journal of the American Veterinary Medical Association* 236, 1200-1206.

Maroli, M., Mizzon, V., Siragusa, C., D'Oorazi, A., Gradoni, L., 2001. Evidence for an impact on the incidence of canine leishmaniasis by the mass use of deltamethrin-impregnated dog collars in southern Italy. *Medical and veterinary entomology* 15, 358-363.

Marques, M.D., 2013. Biological rhythms and vector insects. *Memorias do Instituto Oswaldo Cruz* 108 Suppl 1, 59-62.

Mascari, T.M., Clark, J., Gordon, S., Mitchell, M.A., Rowton, E.D., Stout, R., Foil, L.D., 2011. Oral treatment of rodents with insecticides for control of sand flies (Diptera: Psychodidae) and the fluorescent tracer technique (FTT) as a tool to evaluate potential sand fly control methods. *J Vector Ecol* 36 Suppl 1, S132-137.

Mascari, T.M., Foil, L.D., 2010. Oral treatment of rodents with ivermectin for the control of *Phlebotomus papatasi* (Diptera: Psychodidae) under laboratory conditions. *Veterinary parasitology* 171, 130-135.

Mascari, T.M., Stout, R.W., Foil, L.D., 2012a. Evaluation of three feed-through insecticides using two rodent and two sand fly species as models. *J Am Mosq Control Assoc* 28, 260-262.

Mascari, T.M., Stout, R.W., Foil, L.D., 2012b. Laboratory evaluation of oral treatment of rodents with systemic insecticides for control of bloodfeeding sand flies (Diptera: Psychodidae). *Vector borne and zoonotic diseases* (Larchmont, N.Y 12, 699-704.

Mascari, T.M., Stout, R.W., Foil, L.D., 2013. Oral treatment of rodents with fipronil for feed-through and systemic control of sand flies (Diptera: Psychodidae). *Journal of medical entomology* 50, 122-125.

McTier, T.L., Chubb, N., Curtis, M.P., Hedges, L., Inskeep, G.A., Knauer, C.S., Menon, S., Mills, B., Pullins, A., Zinser, E., Woods, D.J., Meeus, P., 2016. Discovery of sarolaner: A novel, orally administered, broad-spectrum, isoxazoline ectoparasiticide for dogs. *Veterinary parasitology* 222, 3-11.

Mehlhorn, H., Hansen, O., Mencke, N., 2001. Comparative study on the effects of three insecticides (fipronil, imidacloprid, selamectin) on developmental stages of the cat flea

(*Ctenocephalides felis* Bouche 1835): a light and electron microscopic analysis of in vivo and in vitro experiments. *Parasitology research* 87, 198-207.

Melaun, C., Kruger, A., Werblow, A., Klimpel, S., 2014. New record of the suspected leishmaniasis vector *Phlebotomus* (*Transphlebotomus*) *mascittii* Grassi, 1908 (Diptera: Psychodidae: Phlebotominae)--the northernmost phlebotomine sandfly occurrence in the Palearctic region. *Parasitology research* 113, 2295-2301.

Miró, G., Petersen, C., Cardoso, L., Bourdeau, P., Baneth, G., Solano-Gallego, L., Pennisi, M.G., Ferrer, L., Oliva, G., 2017. Novel Areas for Prevention and Control of Canine Leishmaniosis. *Trends in parasitology*.

Molina, R., Miró, G., Gálvez, R., Nieto, J., Descalzo, M.A., 2006. Evaluation of a spray of permethrin and pyriproxyfen for the protection of dogs against *Phlebotomus perniciosus*. *The Veterinary record* 159, 206-209.

Naucke, T.J., Amelung, S., Lorentz, S., 2016. First report of transmission of canine leishmaniosis through bite wounds from a naturally infected dog in Germany. *Parasites & vectors* 9, 256.

Naucke, T.J., Lorentz, S., 2012. First report of venereal and vertical transmission of canine leishmaniosis from naturally infected dogs in Germany. *Parasites & vectors* 5, 67.

Naucke, T.J., Pesson, B., 2000. Presence of *Phlebotomus* (*Transphlebotomus*) *mascittii* Grassi, 1908 (Diptera : Psychodidae) in Germany. *Parasitology research* 86, 335-336.

Nolan, T.J., Lok, J.B., 2012. Macrocyclic lactones in the treatment and control of parasitism in small companion animals. *Curr Pharm Biotechnol* 13, 1078-1094.

Obwaller, A.G., Karakus, M., Poepl, W., Toz, S., Ozbek, Y., Aspöck, H., Walochnik, J., 2016. Could *Phlebotomus mascittii* play a role as a natural vector for *Leishmania infantum*? New data. *Parasites & vectors* 9, 458.

- Otranto, D., 2014. NEXGARD(R). Afoxolaner, a new oral insecticide-acaricide to control fleas and ticks in dogs. Editorial. *Veterinary parasitology* 201, 177-178.
- Otranto, D., Dantas-Torres, F., 2013. The prevention of canine leishmaniasis and its impact on public health. *Trends in parasitology* 29, 339-345.
- Otranto, D., Dantas-Torres, F., Napoli, E., Solari Basano, F., Deuster, K., Pollmeier, M., Capelli, G., Brianti, E., 2017. Season-long control of flea and tick infestations in a population of cats in the Aeolian archipelago using a collar containing 10% imidacloprid and 4.5% flumethrin. *Veterinary parasitology* 248, 80-83.
- Petersen, C.A., Barr, S.C., 2009. Canine leishmaniasis in North America: emerging or newly recognized? *Vet Clin North Am Small Anim Pract* 39, 1065-1074, vi.
- Picado, A., Ostin, B., Rijal, S., Sundar, S., Singh, S.P., Chappuis, F., Das, M.L., Khanal, B., Gidwani, K., Hasker, E., Dujardin, J.C., Vanlerberghe, V., Menten, J., Coosemans, M., Boelaert, M., 2015. Long-lasting insecticidal nets to prevent visceral leishmaniasis in the Indian subcontinent; methodological lessons learned from a cluster randomised controlled trial. *PLoS neglected tropical diseases* 9, e0003597.
- Poche, D., Garlapati, R., Torres, Z., Grant, W., Wang, H.H., Mukherjee, S., Clarke, T., Norris, A., Briley, L., Somers, D., Franckowiak, G., Chaubey, R., Rehman, T., Kumar, V., Hasker, E., Poch, R., 2017. The importance of system ecology: implications of vector-based field studies and quantitative analysis of vector control programs., 6th World Congress on Leishmaniosis, Toledo. Spain.
- Poche, D.M., Grant, W.E., Wang, H.H., 2016. Visceral Leishmaniasis on the Indian Subcontinent: Modelling the Dynamic Relationship between Vector Control Schemes and Vector Life Cycles. *PLoS neglected tropical diseases* 10, e0004868.

- Poche, R.M., Garlapati, R., Singh, M.I., Poche, D.M., 2013. Evaluation of fipronil oral dosing to cattle for control of adult and larval sand flies under controlled conditions. *Journal of medical entomology* 50, 833-837.
- Qualls, W.A., Muller, G.C., Khallaayoune, K., Revay, E.E., Zhioua, E., Kravchenko, V.D., Arheart, K.L., Xue, R.D., Schlein, Y., Hausmann, A., Kline, D.L., Beier, J.C., 2015. Control of sand flies with attractive toxic sugar baits (ATSB) and potential impact on non-target organisms in Morocco. *Parasites & vectors* 8, 87.
- Ready, P.D., 2010. Leishmaniasis emergence in Europe. *Euro Surveill* 15, 19505.
- Ready, P.D., 2013. Biology of Phlebotomine Sand Flies as Vectors of Disease Agents. *Annual Review of Entomology* 58, 227-250.
- Rehman, H., Aziz, A.T., Saggu, S., Abbas, Z.K., Mohan, A., Ansar, A.A., 2014. Systematic review on pyrethroid toxicity with special reference to deltamethrin. *Journal of Entomology and Zoology Studies* 2, 60-70.
- Reisen, W.K., 2010. Landscape epidemiology of vector-borne diseases. *Annu Rev Entomol* 55, 461-483.
- Reithinger, R., Coleman, P.G., Alexander, B., Vieira, E.P., Assis, G., Davies, C.R., 2004. Are insecticide-impregnated dog collars a feasible alternative to dog culling as a strategy for controlling canine visceral leishmaniasis in Brazil? *International journal for parasitology* 34, 55-62.
- Reithinger, R., Teodoro, U., Davies, C.R., 2001. Topical insecticide treatments to protect dogs from sand fly vectors of leishmaniasis. *Emerging infectious diseases* 7, 872-876.
- Rowland, T., Davidson, S., Kobylinski, K., Menses, C., Rowton, E., 2015. Efficacy of Permethrin Treated Bed Nets Against *Leishmania major* Infected Sand Flies. *US Army Med Dep Jul-Sep*, 10-15.

Sharma, U., Singh, S., 2008. Insect vectors of Leishmania: distribution, physiology and their control. *Journal of vector borne diseases* 45, 255-272.

Shoop, W.L., Hartline, E.J., Gould, B.R., Waddell, M.E., McDowell, R.G., Kinney, J.B., Lahm, G.P., Long, J.K., Xu, M., Wagerle, T., Jones, G.S., Dietrich, R.F., Cordova, D., Schroeder, M.E., Rhoades, D.F., Benner, E.A., Confalone, P.N., 2014. Discovery and mode of action of afoxolaner, a new isoxazoline parasiticide for dogs. *Veterinary parasitology* 201, 179-189.

Silva, R.A.E., Andrade, A.J., Quint, B.B., Raffoul, G.E.S., Werneck, G.L., Rangel, E.F., Romero, G.A.S., 2018. Effectiveness of dog collars impregnated with 4% deltamethrin in controlling visceral leishmaniasis in *Lutzomyia longipalpis* (Diptera: Psychodidae: Phlebotominae) populations. *Memorias do Instituto Oswaldo Cruz* 113, e170377.

Stanneck, D., Ebbinghaus-Kintscher, U., Schoenhense, E., Kruedewagen, E.M., Turberg, A., Leisewitz, A., Jiritschka, W., Krieger, K.J., 2012a. The synergistic action of imidacloprid and flumethrin and their release kinetics from collars applied for ectoparasite control in dogs and cats. *Parasites & vectors* 5, 73.

Stanneck, D., Rass, J., Radeloff, I., Kruedewagen, E., Le Sueur, C., Hellmann, K., Krieger, K., 2012b. Evaluation of the long-term efficacy and safety of an imidacloprid 10%/flumethrin 4.5% polymer matrix collar (Seresto(R)) in dogs and cats naturally infested with fleas and/or ticks in multicentre clinical field studies in Europe. *Parasites & vectors* 5, 66.

Thatheyus, A.J., Gnana Selvam, A.D., 2013. Synthetic Pyrethroids: Toxicity and Biodegradation. *Applied Ecology and Environmental Sciences* 1, 33-36.

- Torres, L., Rojas, J., Rondon, M., Morales, A., Nieves, E., 2017. Insecticide Activity of *Ageratina jahnii* and *Ageratina pichinchensis* (Asteraceae) against *Lutzomyia migonei* (Diptera: Psychodidae). *Adv Biomed Res* 6, 53.
- Travi, B.L., Cordeiro-da-Silva, A., Dantas-Torres, F., Miro, G., 2018. Canine visceral leishmaniasis: Diagnosis and management of the reservoir living among us. *PLoS neglected tropical diseases* 12, e0006082.
- Tsirigotakis, N., Pavlou, C., Christodoulou, V., Dokianakis, E., Kourouniotis, C., Alten, B., Antoniou, M., 2018. Phlebotomine sand flies (Diptera: Psychodidae) in the Greek Aegean Islands: ecological approaches. *Parasites & vectors* 11, 97.
- Vaselek, S., Ayhan, N., Oguz, G., Erisoz Kasap, O., Savic, S., Di Muccio, T., Gradoni, L., Ozbek, Y., Alten, B., Petric, D., 2017. Sand fly and *Leishmania* spp. survey in Vojvodina (Serbia): first detection of *Leishmania infantum* DNA in sand flies and the first record of *Phlebotomus* (*Transphlebotomus*) *mascittii* Grassi, 1908. *Parasites & vectors* 10, 444.
- Vrhovec, M.G., Pantchev, N., Failing, K., Bauer, C., Travers-Martin, N., Zahner, H., 2017. Retrospective Analysis of Canine Vector-borne Diseases (CVBD) in Germany with Emphasis on the Endemicity and Risk Factors of Leishmaniosis. *Parasitology research*.
- Wasserberg, G., Poche, R., Miller, D., Chenault, M., Zollner, G., Rowton, E.D., 2011. Imidacloprid as a potential agent for the systemic control of sand flies. *J Vector Ecol* 36 Suppl 1, S148-156.
- Wylie, C.E., Carbonell-Antonanzas, M., Aiassa, E., Dhollander, S., Zagmutt, F.J., Brodbelt, D.C., Solano-Gallego, L., 2014. A systematic review of the efficacy of prophylactic control measures for naturally occurring canine leishmaniosis. Part II: topically applied insecticide treatments and prophylactic medications. *Prev Vet Med* 117, 19-27.

Figure 1. Fed female of *Phlebotomus perniciosus* resting

Figure 2. Predictive map showing the distribution of sand flies throughout endemic and non-endemic European countries in July as a period of maximum risk of *L. infantum* transmission. Red = areas inhabited by sand flies according to their minimum temperature requirement of 17°C. Light grey = sand fly free areas. Average monthly minimum temperature data layers for 1970-2000 (1 km² spatial resolution) were obtained from WorldClim version2 (<http://www.worldclim.org>). Map drawn using Arc-GIS 10.4 software and projected on the WGCS 1984 coordinate reference system.

Figure 3. Sand fly habitat. (A) Resting sites for adults, holes and crevices in walls. (B) Resting sites for adults, woodshed and (C) breeding sites for larvae rich in organic matter compost in gardens.

Figure 4. (A) Anti-feeding efficacy (%) and (B) insecticidal efficacy (%) of the six described spot-on pipettes with proven repellent and insecticidal efficacy against *P. perniciosus*: PER/IND (Frenais et al., 2014), PER/IMIa (Miró et al., 2007), PER/IMI b (Bouhsira et al., 2018), PER/FIP 1 (Franc et al., 2015), PER/FIP 2 (Dumont et al., 2015), PER (Molina et al., 2012) and PER/DIN/PYR (Lienard et al., 2013).

Table 1. Sand flies requirements: crucial questions that need to be addressed for an effectively control.

Question	Reason	Answer
Which are the vector species of CanL in a specific area?	The biting behaviour and environmental requirements depends on the species	The incriminated vectors of <i>L. infantum</i> are members of the subgenus <i>Larroussius</i> : <i>P. perniciosus</i> (W. and C. Mediterranean) and <i>P. ariasi</i> (W. Mediterranean)
What are their environmental preferences?	Identify breeding sites and/or plants that act as their sugar source	Warm, dark, moist soils rich in organic matter: plant cuttings, rubbish, compost in gardens, manure, leaf litter, tree holes and roots, holes and crevices in walls, cellars, stables, dense vegetation, animal burrows, etc
How long is their activity period?	The length of the activity period depends on the latitude. Vector densities vary seasonally due to their sensitivity to climate variability (mainly temperature and rainfall)	In temperate regions, the activity period extends from May to November, but it can be shorter or longer depending on the latitude
What are their climate requirements?	Ambient temperatures and relative humidity are related to sand flies density	It has been reported that the minimum temperature at which sand flies remain active is 17°C. High or medium ambient humidity is also desirable.
How will climatic change modify the density and spread of sand flies?	In Europe, the northward expansion of the distribution range of phlebotomines has been attributed to climate change	<i>P. mascittii</i> is the northern mostly distributed phlebotomine species in Europe. GIS models predict the northward expansion of sand flies
How do they behave?	It is important to know if they bite host indoors or are exophagic, what is their flight range and when is their preferred feeding time from dusk to dawn	<i>P. perniciosus</i> , are predominantly exophagic and exophilic. The adult dispersion range is a few hundred meters on average and is determined by the presence of moderate wind and lack of rainfall. The nocturnal activity of the sand fly species <i>P. perniciosus</i> is crepuscular or nocturnal.
Which are their preferred blood sources?	Identify potential reservoir hosts	The blood meal sources of <i>P. perniciosus</i> are a wide range of mammals and birds

Table 2. Oral insecticides tested with insecticide efficacy against sand flies

Active principle	Mechanism of action	Study type	Host	Sand fly spp	Efficacy	Sand fly stage	Time post-treatment	Ref
Fipronil	Agonist of GABA-gated chloride channels	Field	<i>B. taurus</i>	<i>P. argentipes</i>	95-97%	Adult	-	Poch
		Field	<i>M. shawi</i>	<i>P. papatasi</i>	80%	Adult	6 w	Derb
		Laboratory	Hamster	<i>P. papatasi</i>	100%	Adult	28 d	Mas
					96%	Larvae	21 d	
		Laboratory	<i>Bos taurus</i>	<i>P. papatasi</i>	100%	Adult and larvae	21 d	Poch
		Laboratory	<i>M shawi</i>	<i>P. papatasi</i>	90%	Adult	29 d	Derb
Laboratory	<i>M shawi</i>	<i>P. papatasi</i>		Larvae	5 w	Derb		
Imidacloprid	Agonist of nicotinic acetylcholine receptors	Laboratory	<i>M. shawi</i>	<i>P. papatasi</i>	100%	Adult and larvae	4 w	Derb
		Field	<i>M. shawi</i>	<i>P. papatasi</i>	90%	Adult	4 w	Derb
		Laboratory	Rabbits	<i>P. papatasi</i>	81.3-89.8%	Adult	-	Was
		Laboratory	Rodents	<i>P. papatasi</i> <i>Lu. longipalpis</i>	90-100%	Larvae	2-4 d	Was
Ivermectin	Agonist of glutamate-gated chloride channels and agonist of GABA	Laboratory	<i>M. auratus</i>	<i>P. papatasi</i>	100%	Adult	1 w	Mas 2012
Abamectin	Agonist of glutamate-gated chloride channels and agonist of GABA	Laboratory	<i>M. auratus</i>	<i>P. papatasi</i>	78%	Adult	24 h	Mas
Spinosad	Agonist of nicotinic acetylcholine receptors	Laboratory	<i>M. auratus</i>	<i>P. papatasi</i>	100%	Adult	1 w	Mas
IDIs	Inhibits chitin synthesis or deposition pathways	Laboratory	Rodents	<i>P. duboscqui</i> <i>P. papatasi</i>	90-100%	Larval	-	Mas
Fluralaner	Inhibits (GABA)-gated chloride channels and L-glutamate-gated chloride channels	Laboratory	Dogs	<i>P. papatasi</i>	60-80%	Adult	30 d	Góm

d: days; h: hours; w: weeks, *Doses non palatable for rodents; IDIs: insect development inhibitors; GABA: gamma-aminobutyric acid

Highlights

The main points highlighted by this review of sand fly control are:

- Environmental control measures against sand flies should be inexpensive, sustainable over time and should avoid environmental impacts.
- Improving the awareness of pet owners of the need for the topical application of repellents on a regular basis as preventive measures against sand fly bite minimizing *Leishmania* infection is an excellent way to control the infection in dogs.
- Because of the introduction of infected dogs, the appearance of potential vectors might be a real risk factor of *Leishmania infantum* dispersion in non-endemic areas; though detecting this new potential vectors must be major concern in these areas.
- -The application of the known natural enemies of sand flies as larvicides is limited because of their diversity of breeding places. Further research is needed to evaluate the biological control of sand fly populations by natural enemies.
- Long-lasting Insecticidal Nets (LLINs) could be an important personal protection measure to avoid bites of phlebotomines, and a valuable control measures in large areas where Indoor Residual Spraying (IRS) has a limited success.
- Attractive Toxic Sugar Baits (ATBS) spraying non-flowing green vegetation and within bait stations have been successfully tested for selective control of phlebotomine adults (male and females).
- Promoting health education focused on phlebotomine requirements could help arm the general public with useful information about local vector breeding sites.

- Predicting vector occurrence through GIS and Remote Sensing in specific regions will provide useful information on which to base the design of appropriate and focused control interventions and when they should be applied.

ACCEPTED MANUSCRIPT



Figure 1

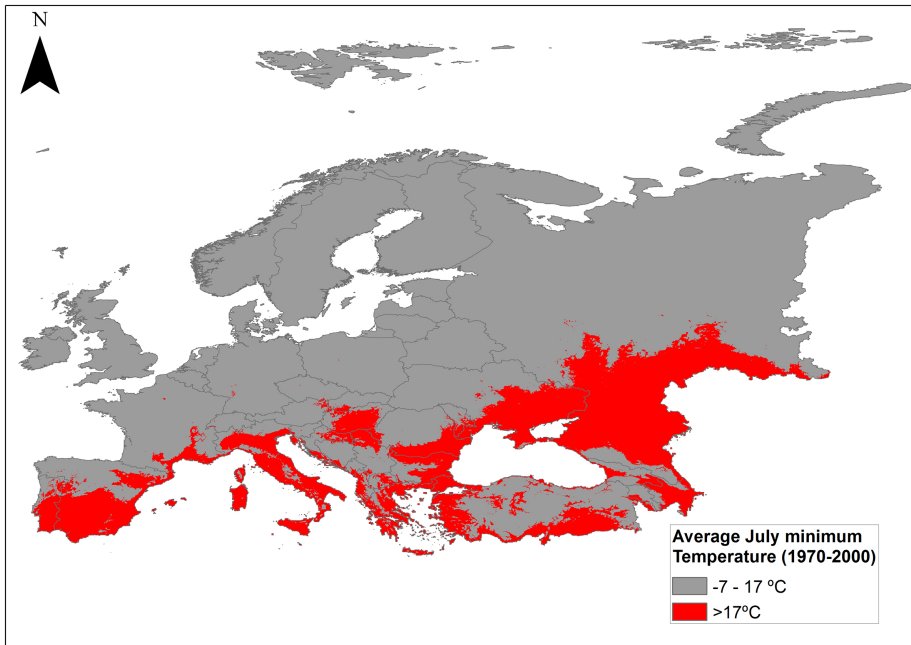
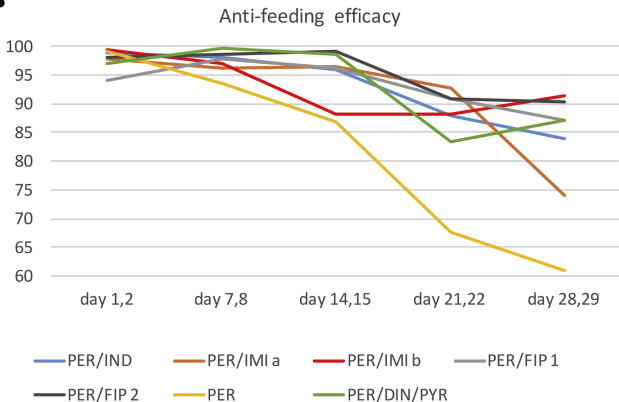
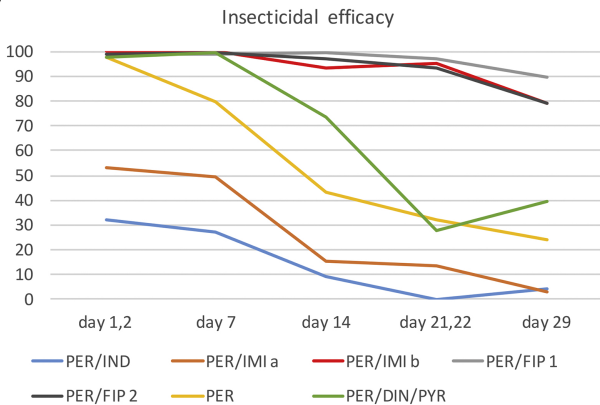


Figure 2



Figure 3

A**B****Figure 4**