Review

The prevention of canine leishmaniasis and its impact on public health

Domenico Otranto[1] and Filipe Dantas-Torres[1,2]

[1] Department of Veterinary Medicine, University of Bari, 70010 Valenzano, Bari, Italy
[2] Department of Immunology, Aggeu Magalhã es Research Institute, Oswaldo Cruz Foundation, 50670-420 Recife, Pernambuco, Brazil

Canine leishmaniasis (CanL) caused by *Leishmania infantum* is a vector-borne disease of great veterinary and medical significance. Prevention of CanL requires a combined approach including measures focused on dogs and the environment where the vectors perpetuate. Over past decades, considerable effort has been put towards developing novel and cost-effective strategies against CanL. Vaccination is considered among the most promising tools for controlling CanL, and synthetic pyrethroids are useful and cost-effective in reducing risk of *L. infantum* infection in dogs. The effectiveness of the use of vaccines plus repellents in preventing *L. infantum* infection and subsequent disease development should be assessed by means of large-scale, randomized controlled field trials because this combined strategy may become the next frontier in the control of CanL.

An emerging zoonotic threat

Canine leishmaniasis (CanL) caused by *Leishmania infantum* is among the most important vector-borne parasitic diseases of dogs, occurring on all continents, except Oceania [1]. In dogs, the infection may be asymptomatic (over 80% of cases in some areas) or may evolve to life-threatening overt disease, with a wide range of clinical signs (from localized skin alterations to severe loss of weight and generalized lymphadenomegaly). For its potential severity in dogs and its zoonotic nature, the prevention of this infection is not only desirable but also a must for both dog and human health (Figure 1). Because the infection to a receptive host occurs through the bite of sand flies of the genus *Phlebotomus* (in the Old World) and genus *Lutzomyia* (in the Americas) [2], the management of this disease is extremely complex [1,3]. In this regard, the prevention of CanL should include measures targeting animals (at individual and population level) and the environment. However, the adoption and transportation of dogs from areas of CanL endemicity has resulted in the introduction and spread of disease to regions where infections were not previously found [4–6], which may create new epidemiological scenarios, further complicating the zoonotic potential. This often occurs in combination with emerging immunosuppressive conditions in humans (e.g., HIV/AIDS) that may increase the risk of zoonotic diseases such as visceral leishmaniasis (VL) [7]. Although the risk of CanL transmission is reputed to be low in the absence of sand flies, other ways of transmission, such as venereal and transplacental transmission, should be seriously considered [8]. Here, we summarize the main difficulties in setting control plans as well as current knowledge on the prevention and control of CanL, with emphasis on currently available tools and future research needs.

Canine and human leishmaniasis

Dogs are regarded as the principal reservoirs of *L. infantum*, and for this reason they are the target of control programs in some countries, such as in Brazil, where approximately 3500 human cases of zoonotic VL are reported each year [1]. This figure is significantly different from the Mediterranean region, where approximately 875 human cases are reported annually [9]. However, although CanL is thought to be highly prevalent in several countries, mainly in South America and in the Mediterranean region (Figure 1), a precise account of its actual distribution and frequency is currently unavailable. Based on serological surveys, it has been estimated in the past that over 2.5 million *L. infantum*-infected dogs are present in southern Europe [10]; from these numbers, pervasive infection of dogs with *L. infantum* does not necessarily imply a higher incidence of the disease in humans. Importantly, stray dogs that are not treated with proper preventatives (e.g., insecticide-impregnated collars) may potentially play a role in maintaining CanL, in areas where the disease is endemic. In some European countries, the existence of municipal kennels, where dogs are kept throughout their life, may represent a major barrier to the control of CanL (Figure 1). Under these conditions, focal spots of CanL may easily occur with infection of up to 35% of dogs exposed to sand fly bites over a single season [11]. This picture highlights how pivotal it is to control CanL by different approaches according to the epidemiology of the disease in a given area and the difficulties inherent to local conditions [1].

Difficulties in setting control plans

The control of CanL is a difficult task because of the complex transmission cycle of *L. infantum*. Based on current knowledge and considering the tools available, the environmental control of immature sand flies is unfeasible because the microhabitats of larvae and pupae are extremely variable, including, for example, tree roots, animal burrows, decaying foliage, and tree holes [2]. Similarly,

Corresponding author: Otranto, D. (domenico.otranto@uniba.it).
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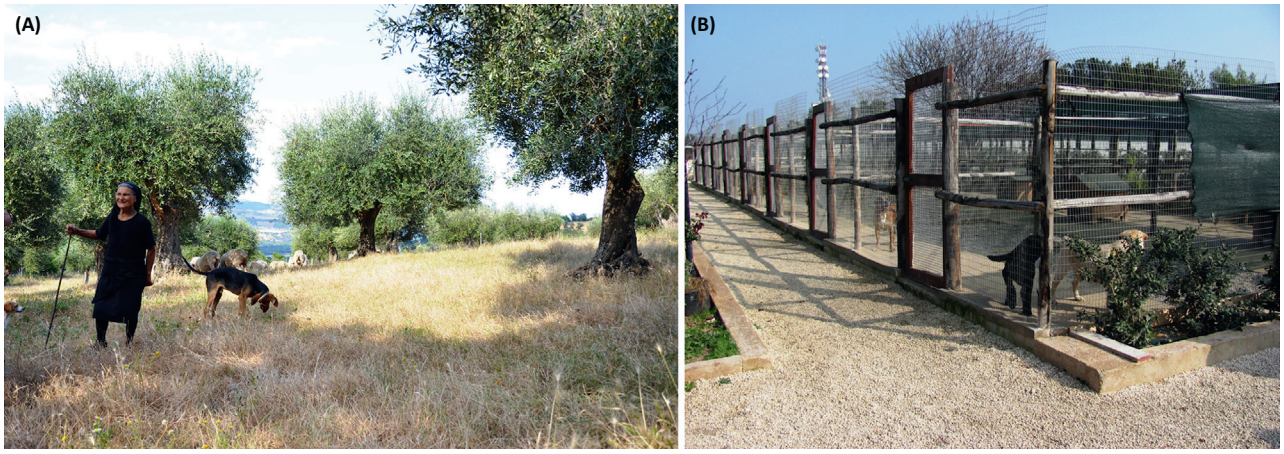

Figure 1. Mediterranean environments where canine leishmaniosis is endemic. **(A)** Rural hilly areas of southern Italy where shepherd and hunting dogs live in close contact with humans. **(B)** Typical shelter where hundreds of stray dogs live throughout their life and are exposed to sand fly bites.

evidence indicates that spatial fogging for adult sand fly control is useless and that the residual effect of house wall spraying is very short [12], making the residual spraying of houses impractical and ineffective, particularly in rural areas [13].

A proper assessment of the infection status of dogs is fundamental for a better determination of actions to be taken, to start the treatment in the initial stages of the disease, and to monitor the effectiveness of control measures. In areas where *L. infantum* is prevalent, most of the dogs and people exposed to sand flies will come into contact with the parasite, but remain asymptomatic [14,15]. Although asymptomatic dogs may potentially be infectious to sand flies [16,17], the role of asymptomatic humans in the epidemiology of VL has yet to be ascertained [15]. It is worth noting that the definitive diagnosis of *L. infantum* infection in asymptomatic dogs and people is often troublesome due to the inherent limitations of serological and parasitological methods [15,18]. For example, serology may not be a good indicator of infection due to the variable time in seroconversion. Additionally, the costs attached to the systematic serological testing of dogs may be difficult to handle by local public health authorities, especially in large countries such as Brazil where the number of dogs living in areas with a high rate of infection might be huge [1,6,14,19]. Conversely, microscopic examination of stained smears (e.g., lymph node and bone marrow) is a simple method, but has a low sensitivity, particularly in asymptomatic dogs [6], whereas molecular tools, although the most sensitive and specific, often have prohibitive costs [6,20]. Available data clearly show that there is no gold standard to detect *Leishmania* infections in asymptomatic dogs [6], indicating that improved diagnostic tests are needed.

Environmental and on-host vector control

In spite of the major concerns related to the complex ecology of vectors, control measures aimed at reducing vector populations in the environment have also been employed. The application of insecticides may eventually have a transitory effect but is typically unsustainable in the long term for several technical and economic reasons. For example, a variety of sand fly species may be potentially involved in the transmission of *L. infantum* [2], and the ecology and behavior of each species may vary widely. Similarly, the size of the area to be treated in countries such as Brazil where leishmaniasis is endemic may be vast and make environmental control economically unaffordable.

The application of insecticides on the walls and roofs of human habitations (indoor residual spraying; IRS) and in animal shelters (e.g., chicken pens and corrals) was shown to be effective in reducing the population of sand flies [13,21]. Environmental and human health hazard concerns around the employment of organochlorides and other chemical groups (e.g., organophosphates and carbamates) have progressively led to their substitution by synthetic pyrethroids (e.g., α-cypermethrin, cypermethrin, deltamethrin, and λ-cyhalothrin), which are currently used by public health authorities in several countries.

Another major constraint in employing insecticides in the environment for sand fly control relies on the fact that their natural resting and breeding sites are difficult to find [13]. However, IRS may be useful in particular situations, such as when a high density of sand flies is found near or in human habitations. Similarly, chicken coops, pigsties, corrals, and dog shelters may represent a 'natural lure' for the vectors [22,23] and should be targeted by control activities. In any case, information on sand fly population dynamics is fundamental to optimize timing and modalities of insecticide applications. Microhabitats favorable to their development, for example, in crevices and cracks on the walls and humid soil in shaded areas, should also be removed [13]. Furthermore, the destruction of these microhabitats has been considered as one of the few examples of effective noninsecticidal control of sand flies [13], but such a measure is difficult to apply; there is no convincing scientific evidence showing that cleaning microhabitats may have an impact on the incidence of VL in humans and dogs.

The use of repellents such as synthetic pyrethroids on dogs has become the most effective tool for prevention of *L. infantum* infection in these animals. Their mode of action, a toxic and irritating effect on sand flies, causes insect disorientation and sudden abandonment of the host

Table 1. Efficacy of pyrethroids to prevent CanL under field conditions

Pharmaceutical compound	Formulation	Study site	Study duration	Treated dogs	Untreated animals (incidence)	Efficacy (%)	Refs
Imidacloprid 10% and permethrin 50%	Spot-on	Italy	12 months	209[a]; 204[b]	218 (9.8%)	89%[a]; 90.3%[b]	[37]
Permethrin 65%	Spot-on	Brazil	4 months	230	160 (7.4%)	50%	[71]
Permethrin 65%	Spot-on	Italy	2 seasons	120	188 (15%)	84%	[72]
Deltamethrin 4%	Collars	Italy	2 seasons	119	188 (15%)	84%	[72]
Imidacloprid 10% and permethrin 50%	Spot-on	Italy	24 months	71	56 (47.6%)	100%	[3]
Deltamethrin 4%	Collars	Italy	2 seasons	354	371 (25.8%)	50–86%	[34]
Deltamethrin 4%	Collars	Italy	2 seasons	60	60 (41.2%)	51%	[73]
Deltamethrin 4%	Collars	Tunisia	2 seasons	42	38 (15.8%)	100%	[74]
Deltamethrin 4%	Collars	Iran	6 months	354	466 (6.6%)	54%[c]	[71]
Deltamethrin 4%	Collars	Brazil	12 months	136	97 (17.6%)	50%	[33]
Imidacloprid 10% and flumethrin 4.5%	Collars	Italy	24 months	63	61 (35.3%)	100%	[11]

[a]Dogs treated once a month.

[b]Dogs treated twice a month.

[c]This is an estimation of reduction in dog seroconversion during a transmission season.

followed by death soon after the landing of an insect on the coat of a treated animal. Hence, bloodfeeding usually does not occur and infection is usually prevented (Table 1). The effect of synthetic pyrethroids in spot-on formulations or collars (Figure 2) may last from 1 to approximately 8 months, respectively [3,11,24].

When a spot-on formulation is applied, it usually takes 24 h for the insecticide to spread throughout the *stratum corneum*. It is recommended to apply the first treatment 1 month before the sand fly season occurs in order to achieve the highest level of protection [3]. On the contrary, powders and sprays have an immediate effect but a short residual activity. Moreover, the use of a slow-release collar matrix system with a combination of 10% imidacloprid and 4.5% flumethrin (Seresto®, Bayer Animal Health) has resulted in an increased period of efficacy against ticks and fleas [25,26].

The efficacy of several repellents against sand flies has been evaluated under laboratory and field conditions with encouraging results. For example, the insecticidal effect of deltamethrin and permethrin, alone or in combination with other insecticides (e.g., imidacloprid), was experimentally tested against different sand fly vectors by evaluating the number of unfed female sand flies collected soon after contact with a treated animal and their mortality within 24 h after exposure to treated dogs [27–32]. The results of laboratory studies were generally positive, with an antifeeding effect ranging from 84% to 96% of sand flies and an insecticidal activity of near 100% in the few fed females. These results fostered field investigations in the Old and the New Worlds with the ultimate goal of finding an effective product to protect dogs. The use of pyrethroids with repellent properties in impregnated collars [33] and spot-on formulations [3] was demonstrated to be a suitable approach to reduce the risk of *L. infantum* infection in dogs. Specifically, collars containing 4% deltamethrin (Scalibor®, Intervet) [34,35] or 10% imidacloprid and 4.5% flumethrin [11], and a spot-on formulation containing 10% imidacloprid and 50% permethrin (Advantix®, Bayer) [3] have been used for reducing the biting rate of sand fly vectors, exhibiting protection rates ranging from 50% to 100% over two consecutive transmission seasons (Table 1). Based on current knowledge, topical insecticides used on dogs represent a promising tool for reducing the transmission of infection to dogs [11,34–37]. Large-scale studies in Brazil would be needed to assess whether the massive use of collars in dogs living in a given community would impact on the incidence of the disease in humans.

Figure 2. Dog with a pyrethroid-impregnated collar. Synthetic pyrethroid-impregnated collars were shown to be effective in protecting dogs form *Leishmania infantum* infection under field conditions.

Vaccination

Over the past decades, considerable effort has been made towards selecting potential *Leishmania* antigens as vaccine candidates as well as the best adjuvant for such vaccines [38]. Because initial unsuccessful attempts using inactivated vaccines prepared with disrupted promastigotes of *Leishmania braziliensis* or alum-precipitated, autoclaved *Leishmania major* with *Bacillus* Calmette–Guerin as an adjuvant [39–41], second-generation vaccines composed of whole cultured parasites or their excretory–secretory (ES)

products have been tested under field conditions and made available in recent years. For example, a vaccine prepared with a glycoprotein known as the fucose mannose ligand of *Leishmania donovani* (Leishmune®, Fort Dodge Animal Health) has been licensed for use in Brazil [42–44], and it showed a field efficacy of 76% and protection of 92% [42]. The use of this vaccine in the immunotherapy of CanL was also suggested [45], and, in the field, it was correlated with a decreased incidence of infection in dogs and humans [46]. Nonetheless, the Brazilian Ministry of Health has not adopted it as a control measure thus far, even if veterinary practitioners have recommended this vaccine at the individual level. Another vaccine using ES antigen purified from culture supernatant of *L. infantum* promastigotes and *Quillaja saponaria* (QA21) as an adjuvant (CaniLeish®, Virbac Animal Health) has been licensed recently in Europe, and a prototype of this vaccine (with a different adjuvant) displayed 92% efficacy in protecting animals against the appearance of clinical signs, under field conditions, in France [47]. However, further large-scale field studies are necessary to assess whether this vaccine will contribute to the control of CanL in Europe. Analogously, the efficacy of other vaccines such as a vaccine using a recombinant A2-antigen of *Leishmania* amastigotes with saponin as adjuvant (Leish-Tec®, Hertape Calier) needs to be further evaluated under field conditions [48].

Several other vaccine candidates have been tested with less promising results. These include multisubunit recombinant *Leishmania* vaccines, based on expression of polyprotein (Leish-111) [49] or *Leishmania* analog of the receptors of activated C kinase (LACK) antigen [50]. Furthermore, fourth-generation vaccines were prepared with *L. infantum* cysteine proteinases (types I and II) and protected ten dogs (90% protection) against experimental *L. infantum* infection, after 12 months of challenge [51], whereas a multiantigenic plasmid DNA encoding four proteins did not [52]. The vaccines prepared for *L. infantum* and the efficacy in experimental and field trials are summarized in Table 2. Although vaccines represent the next frontier in the control of CanL, several concerns linked with their use (e.g., prohibitive costs) still need to be addressed.

Treatment of infected dogs

The treatment of dogs with CanL is not only aimed at increasing their life expectancy and improving quality of life but also to diminish the parasite load, thereby reducing their infectiousness to sand flies. Indeed, the treatment of CanL has evolved considerably in the past decades, and available protocols may promote clinical cure of infected dogs, reduce parasite load considerably, and decrease the risk of *L. infantum* transmission [1,53]. For example, a reduction in the *Phlebotomus perniciosus* infection rate after treatment with meglumine antimoniate, alone or associated with allopurinol, was recorded in *L. infantum*-infected dogs [54,55]. Indeed, the use of allopurinol in association with meglumine antimoniate treatment could contribute to keeping dogs noninfectious, especially during the disease transmission season (from late May to early October in southern Europe). The reduced capacity of infectiousness to sand flies might be the effect of clinical improvement and reduction of parasite load on the skin, as demonstrated in Brazil where an innovative liposomal formulation of meglumine antimoniate was used in combination with allopurinol to treat dogs suffering from CanL [56]. Altogether, these studies clearly indicate the benefits of treating infected dogs in areas where *L. infantum* infection is endemic.

Dog culling: an unethical and useless practice

The elimination of dogs to control human leishmaniasis was first conducted in Palestine, China, and the Central Asian republics of the then Soviet Union [57]. Specifically, in China these initiatives were supported by the centralized state as part of the socialist revolutionary doctrine with a tremendous effort to simultaneously combine a mass treatment of human patients, vector control in the environment with dichlorodiphenyltrichloroethane (DDT), and elimination of dogs [57]. Importantly, the disease in humans was caused by both *L. donovani* (predominantly in the eastern plains) and *L. infantum* (concentrated in the mountain areas of Beijing and Gansu), responsible for anthroponotic and zoonotic VL, respectively [58]. Expectedly, human treatment and vector control resulted in the interruption of transmission in areas of anthroponotic transmission, and by the 1970s leishmaniasis was a relatively rare disease in China [57]. Even in areas of zoonotic transmission, the successful control of the disease was attributed to the mass treatment of human patients and to the use of insecticides for vector control, making the actual effect of dog elimination difficult to assess [57]. However, numerous dogs were eliminated, with no definite criteria, as any dog found in areas where infection was endemic was indiscriminately killed. In 2008, an outbreak of human VL was detected in the western part of China with the incidence rate of disease increased more than 20-fold compared with the mean annual incidence rate [59]. This suggests that the disease has never been eradicated and that the effect of dog culling, if any at all, was momentary.

Table 2. Efficacy and protection of vaccines currently licensed to prevent canine leishmaniasis

Vaccine	Antigenic composition	Efficacy/protection	Refs
Leishmune®	*L. donovani* FML antigen and QS21 and deacylated saponins of *Quillaja saponaria*	76%/92%	[75,76]
Canileish®[a]	Excreted–secreted proteins of *L. infantum* (LiESP), plus a highly purified fraction (QA-21) of the *Q. saponaria* saponin	68.4%/92.7%	(-)[b]
Leish-Tec®[a]	A2 recombinant protein plus saponin	(-)[c]	[48]

[a]Phase III trials have not been published in the international scientific literature yet.

[b]Oliva, G. *et al.* (2012) Evidence for protection against active infection and disease progression in naïve dogs vaccinated with LiESP/QA-21 (CaniLeish®) exposed to two consecutive *Leishmania infantum* transmission seasons. The World Small Animal Veterinary Association (WSAVA)/Federation of European Companion Animal Veterinary Associations (FECAVA)/British Small Animal Veterinary Association (BSAVA) Congress, Birmingham, UK.

[c]Unavailable.

Abbreviation: FML, fucose mannose ligand.

In Brazil, CanL control has long been based on the culling of seropositive dogs [1]. However, there is no scientific evidence that this strategy could reduce the incidence of zoonotic VL [12]. Moreover, Brazil remains among the six countries responsible for 90% of the global cases of zoonotic VL reported worldwide [9]. Not least, the dog culling strategy has not been accepted in Brazil for ethical reasons [57], but public health authorities still insist on recommending this measure. The reasons for the failure of the dog culling strategy used in Brazil have been extensively discussed in recent years [1,12,60] and these include: (i) other animals (e.g., marsupials, rodents, and humans themselves) may act as reservoirs of *L. infantum* [60]; (ii) dog population screenings for *L. infantum* may be very inaccurate when based on serological tests [e.g., enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescence antibody test (IFAT)] [61,62]; and (iii) the rapid replacement of culled dogs with young animals increases the proportion of susceptible animals in the population [63]. The ineffectiveness of such a strategy is indicated by the rising trend in the number of human cases of zoonotic VL observed between the years 1990 and 2010 [1], in spite of the incalculable number of dogs killed during the past decades.

Concluding remarks and future perspectives

From an epidemiological point of view, CanL is a multifaceted disease and for this reason its control is not easy to achieve. In fact, the control of such a complex disease requires a well-rounded approach, based on current knowledge of the biology of the parasite and its vector, while at the same time also considering interaction with the host at individual and population levels. At present, different strategies are available for the prevention and control of CanL, including vaccination, use of repellents, and the treatment of infected dogs (Figure 3). Importantly, the effectiveness of the combined use of repellents plus vaccination in the prevention of CanL should be assessed in large-scale randomized controlled field trials.

Box 1. Control of CanL: the way forward

- **Vaccination**
  - Vaccine against infection (not only disease).
  - Vaccines based on concealed antigens of sand flies to prevent infection.
- **Diagnosis and treatment**
  - Rapid, high sensitivity, point-of-care tests for CanL.
  - Drug resistance of *Leishmania infantum* and impact on public health.
- **Transmission of the infection**
  - Role of animals other than dogs as reservoirs or amplifying hosts of *L. infantum*.
  - Alternative ways of *L. infantum* transmission in the absence of sand fly vectors.
  - Hazard for introduction of other *Leishmania* species and/or vectors.
- **Prevention and control**
  - Usefulness of repellents from natural extracts to prevention of sand fly bites.
  - Impact of vector control on the risk of *L. infantum* transmission to humans.

A high percentage of asymptomatic infections may occur in dogs [14] and humans [15], and evidence indicates that both may potentially serve as a source of infection to sand fly vectors [15–17,64]. Relevantly, the definitive diagnosis of *L. infantum* infection in asymptomatic dogs is troublesome due to the inherent limitations of serological and parasitological methods [18].

In spite of the advancements in terms of research and development in this field, several issues require more attention from researchers in the near future (Box 1). Of particular interest are studies on host immune response to leishmanial and sand fly antigens. For example, immunogenic as well as immunosuppressive molecules released by sand flies while taking a blood meal greatly interfere with the individual hemostasis and host immune response [65]. The IgG response to sand fly saliva measured in a population of dogs in southern Italy was negatively correlated with risk of *L. infantum* transmission [66]. Possibly, this approach could help to evaluate the effectiveness of vector

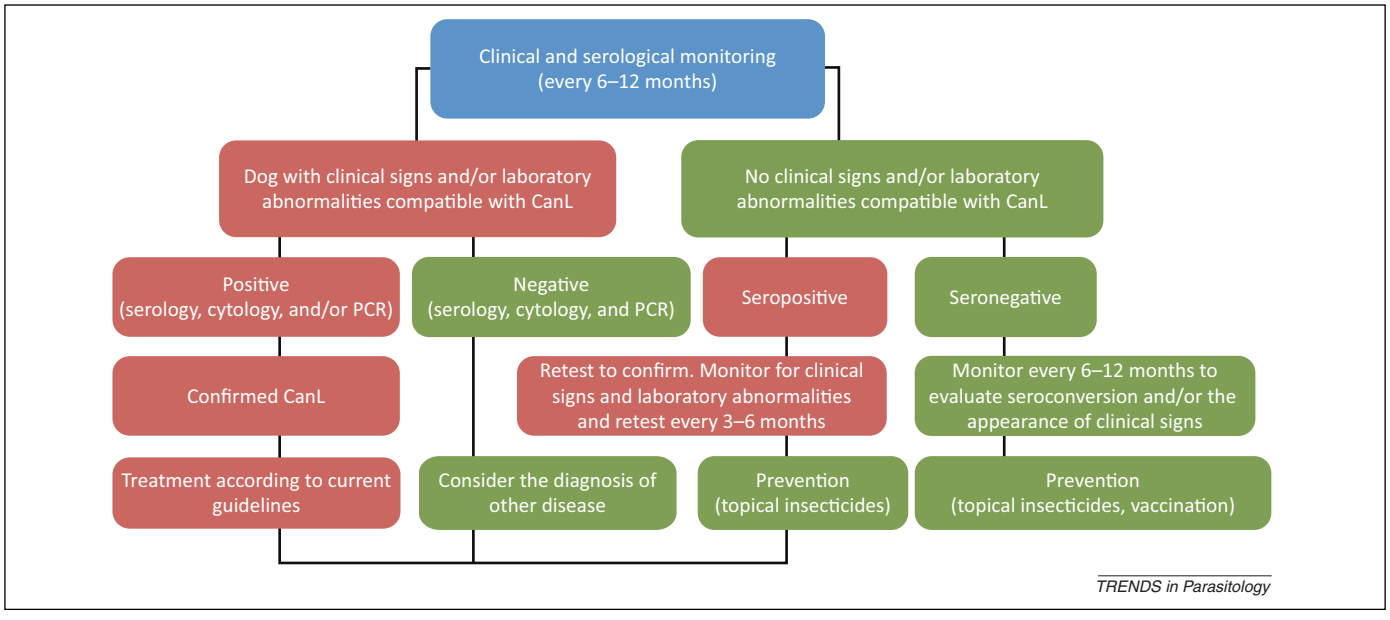

Figure 3. Suggested management of canine leishmaniosis. Abbreviation: CanL, canine leishmaniasis.

control campaigns [67,68] and to estimate the risk of infection for humans and individual dogs living nearby. In addition, further studies on the interactions between immunomodulatory molecules present in sand fly saliva and the host immune response would contribute to the development of vaccines using sand fly concealed antigen.

Currently, the use of repellents in different formulations may induce a high degree of protection in dogs at individual and population levels. Governmental authorities should settle affordable surveillance systems to optimize economic resources and to achieve the best outputs possible. Nonetheless, the costs of control campaigns at the population level are often not affordable for the local governmental authorities in developing as well as in developed countries. Therefore, in a time of global economic crisis, stray and sheltered dogs maintained in municipal kennels may represent a risk factor for zoonotic VL transmission in areas with a high rate of infection and high vector densities. Indeed, the contribution of the community wide use of dog collars on the incidence of VL in humans should be confirmed in large-scale surveys. In Iran, the use of deltamethrin-impregnated collars in dogs reduced the risk of infection in dogs by 54% and in children by 43%, as assessed by serology; however, no effect was observed when humans were assessed by skin test [69]. By contrast, in Brazil where dog culling is a common practice, cases of human VL are increasing and expanding in terms of geographical distribution in some areas [70], indicating that the use of such unethical and above-all inefficacious strategies should be discontinued.

Finally, synergism between medical physicians, veterinary practitioners, researchers, public health authorities, and politicians is central to find a one-base platform for planning sustainable control strategies against CanL, and also from a one-health perspective.

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