

Review Article

Methods of Control of the *Leishmania infantum* Dog Reservoir: State of the Art

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Leishmania infantum is a protozoan parasite causing severe vector-borne visceral diseases both in humans and dogs. The latter are the most important natural reservoir and therefore should be the main target of control measures. The real efficacy of seropositive dogs culling as a direct control method is still debated, and the new sensitivity of large part of population considers ethically unacceptable this kind of approach. Treatment of infectious dogs with one of the available therapeutic protocols is recommendable as it allows to reduce parasite burdens and therefore the possibility of transmission of *Leishmania infantum* to vectors. Vaccination has been proven to be a very effective control tool, but the absence of a commonly recognized diagnostic method able to distinguish vaccinees from seropositive individuals is still an important limit. Concerning indirect control methods, a number of studies have demonstrated the efficacy of topical insecticides treatment (collars, spot-on, and sprays) in reducing incidence and prevalence of *L. infantum*. Also, the reduction of the odds of seroconversion in humans in endemic areas has been reported after the application of indirect control measures on dogs. The contemporary use of direct and indirect methods is even more effective in reducing seroprevalence in dogs.

1. Introduction

Leishmania infantum is a protozoa, obligatory intracellular parasite of mammalian macrophages. This parasite is transmitted by phlebotomine sandflies causing severe vector-borne visceral infectious diseases: zoonotic visceral leishmaniasis (ZVL) in human and canine leishmaniasis (CanL) in dogs. Infected dogs, both owned and not, can be an important threat to public health, because *L. infantum* has the domestic dog as principal reservoir. To date, ZVL is endemic to large areas of Europe, Asia, and Africa, particularly in the countries of the Mediterranean Basin [1, 2]. In Latin America ZVL is linked to *L. chagasi*, now considered as a synonymous of *L. infantum* and therefore included in the same species [3–5].

During the past years, the importance of ZVL public health concerns has increased, considering the increase in risk factors associated with environmental changes, human migration, and, mostly, the presence of *Leishmania infantum*-infected dogs in previous nonendemic areas. In fact, the emergence of cases of ZVL in new territories is usually

preceded by an increase in the prevalence of CanL. An effective control of CanL is therefore crucial for the prevention of ZVL in humans: in particular, vector control through the employment of repellent drugs seems to be an efficient tool for the reduction of seroprevalence [5–9]. Culling of infected dogs is also used in some areas, but animal welfare concerns could arise from this issue, and its real usefulness is debated: epidemiological studies reported evidences of the inefficacy of ZVL control programs that provide for the elimination of infected dogs [10–12]. Treatment of infected dogs reduces or eliminates clinical signs, but it is not usually parasitological curative [13] and treated dogs could still be capable of transmitting the parasite [14]. Recently, a canine vaccine has been licensed in some countries and has been proved to be an efficient preventive tool in order to diminish both the number of human and canine cases [1, 12]. However, the use of this vaccine is still debated in many countries, considering its possible effect on the serological condition of healthy vaccinated individuals and, therefore, on the efficacy of serological control campaigns [15, 16].

Here, after a wide description of ZVL and CanL clinical and epidemiological aspects in humans and dogs, we consider what is the state of the art about CanL control methods, particularly regarding their efficacy and feasibility for large scale-control plans.

2. *Leishmania infantum* in Humans: Zoonotic Visceral Leishmaniasis (ZVL)

Human leishmaniasis is a parasitic disease caused by intracellular protozoan of the genus *Leishmania*. These parasites infect many mammalian species, and the transmission from one species to another happens through arthropods vectors, mostly phlebotomine sandflies. Among 15 species of *Leishmania*, 13 have a zoonotic nature and cause severe diseases transmitted from mammalian reservoirs to humans. There are two main forms of zoonotic leishmaniasis: the zoonotic visceral leishmaniasis (ZVL) and the zoonotic cutaneous leishmaniasis (ZCL).

ZCL is less severe, usually not fatal and self-healing, but responsible for a large number of cases, mostly in Latin America and Middle East [17]. It is caused by many different species of the genus *Leishmania*, including dermatropic *L. infantum*, but it is mostly due to *L. major* and other species hosted by rodents and many tropical wild animals (armadillos, sloths, and procavias). Clinical forms of ZCL in humans usually consist of localized nodoulcerative lesions; rarely these forms evolve to more severe and diffuse cutaneous leishmaniasis, with multiple nodules over large areas of skins.

ZVL is much more severe than ZCL, and it is caused by the viscerotropic *L. infantum*. Viscerotropic parasites addressed to the genus *Leishmania* have been classified into four species, precisely, *L. donovani*, *L. archibaldi*, *L. infantum*, and *L. chagasi*. The last one is now considered a New World synonymous of *L. infantum* [4]. In fact, *L. infantum* is thought to have been imported into the Americas by the dogs of European settlers during the colonization [18]. In humans, all these parasites cause visceral leishmaniasis, an acute, life-threatening disease during which the agents multiply in the macrophages of the reticuloendothelial system, resulting in generalized symptoms like fever, splenomegaly, and pancytopenia. *L. donovani* and *L. archibaldi* cause anthroponotic visceral leishmaniasis (AVL) that is only transmitted from human to human via sandfly bites and affects people of all ages while ZVL from *L. infantum* affects mainly young children and is transmitted from dogs. The only domestic and primary reservoir of this zoonotic disease is the dog [19], in which *L. infantum* causes the canine leishmaniasis (CanL). ZVL is a disease re-emerging in the whole Mediterranean Basin [19]: about 200 cases are yearly reported in Italy, usually in the same areas where CanL is endemic [20]. World-wide reports show that sixty-six Old World and twenty-two New World countries are endemic for human leishmaniasis. The estimate yearly incidence of ZVL is 500,000 cases [21, 22]. About 90% of ZVL occur in rural and suburban areas of five countries (Bangladesh, India, Nepal, Sudan, and Brazil); however, this non uniform distribution is due to the evidence that official data are probably underestimated, because it was

obtained through incomplete, passive case detection [17]. The clinic evolution of ZVL can greatly vary according to the immune status of the patient: in fact, it usually has a chronic course, but in people with reduced immune response (e.g., HIV-affected people) fulminant disease has been described [23]. In the classic, chronic evolution, early symptoms include abdominal pain, weight loss, weakness, and typically remittent febrile episodes, with two spikes per day. Later, lymphnodes swelling and liver and/or spleen enlargement may occur. Typically, there is hypergammaglobulinemia with circulating immune complexes and nephritis. Patients in the final stages of the disease show thrombocytopenia, bleeding, haematuria, and consequent anemia. Death may occur following hemorrhage and complications due to reduction of the immune response.

3. Canine Leishmaniasis (CanL): Epidemiology, Pathogenesis, and Clinical Signs

Canine leishmaniasis (CanL) is a chronic visceral-cutaneous syndrome, which clinical signs could noticeably change from a patient to another: it may be manifested as a subclinical infection, often self-limiting [24] or can be a nonself-limiting and severe illness [25]. The transmission of *L. infantum* from domestic dogs to humans by the bite of previously infected sandflies was first demonstrated in 1930 [26, 27]. Since then, many studies confirmed the role of the domestic dog as the main reservoir of ZVL [17]. In fact, dogs have a high prevalence of both infection and infectiousness, have long-lasting infections, and are common in the peridomestic environment where usually the transmission to human occurs [5]. During the past years the scientific interest in CanL has grown considering the progressive increasing of the global incidence of ZVL and its occurrence in areas previously not involved, possibly as a consequence of global climate changes [17, 28]. Moreover, it is possible that the zoonotic potential of CanL has been enhanced, considering the more and more abundant presence of immunocompromised humans, particularly in developing countries [29–32]. However, even if dogs are recognized as the most important peridomestic reservoir of *L. infantum* infection to humans, only one study has shown that the owner of an infected dog could be at risk of transmission of the infection from his pet [33]. In fact, the presence of an infected dog in a household does not seem to increase the risk of infection for the members of the family in endemic areas [25], as the infected sandfly can transmit *L. infantum* in a range of 2.5 km [34]. Pathogenesis of CanL is common to others forms of leishmaniasis. In fact, all protozoan of the genus *Leishmania* are parasites that need two hosts to complete its life cycle: a phlebotomine sandfly vector and a mammal. The arthropod harbors the flagellated extracellular promastigote while the nonflagellated, intracellular amastigote develops in the mammal. The metacyclic promastigote (e.g., the final promastigote form) is transmitted to a new mammal host, that can belong to the same species of the previous extraphlebotomine host, or to another receptive species. The transmission occurs when

an infected female sandfly feed on the mammal, inoculating promastigotes via the proboscis. Male sandflies are not effective vectors as they are not hematophagous [35]. In dogs, the subsequent course of infection is linked to the host immune response and to the degree of persistence and multiplication of the parasite [36]. As it has already been shown in humans [37], the presence or absence of T-cell response is important for the developing of clinical signs: analyses of circulating lymphocytes in symptomatic dogs have shown low levels of CD4⁺ and CD8⁺ T-cells and a decrease in CD21⁺ B-cells and CD14⁺ monocytes, and changes related to bone marrow parasite density [38]. The absence of an adequate T-cell response leads to insufficient control of the parasite and consequent appearance of clinical signs, high *Leishmania*-specific antibody levels, and high parasite burdens in skin, bone marrow, spleen, liver, and lymphnodes [14, 39]. Asymptomatic dogs, in which *Leishmania* infection is manifested as a subclinical, self-limiting disease, are characterized by the presence of protective immunity that is CD4⁺ T-cells-mediated [11, 40, 41]. On the contrary, symptomatic dogs are characterized by disease susceptibility associated to the production of a marked humoral non-protective immune response and a reduced cell-mediated immunity [36, 42]. Therefore, CanL can range from a mild dermatitis associated with specific cellular immunity and low humoral response to a severe disease characterized by immune complex deposition due to a massive humoral, non-protective response and renal damage consequent to the frequent development of glomerulonephritis and tubulointerstitial nephritis [43, 44].

Clinical signs of CanL show after a period of incubation from two to eight months [45], and up to fifteen months [46]. One author reported the development of clinical signs in dogs living in a nonendemic country after 5–7 years they had visited an endemic area [47]. Clinical signs vary widely from a patient to another due to the diversity of immune responses and the variety of organs affected, resulting in several clinical findings [36, 42, 48]. Any organ could be potentially involved; however, some signs are more frequent than others. The main groups of clinical signs of CanL are described below.

3.1. Cutaneous Lesions. Cutaneous lesions are the most common clinical manifestations of CanL, they are reported in more than 50% of cases [36], even 90% according to several authors [36, 47], and an extensive range of dermatological entities have been described and classified in groups (reported percentages are from a study of Ferrer on 43 dogs): (1) alopecia and peeling (nonpruritic exfoliative dermatitis) generalized or localized over face, ears, and limbs (60%); (2) ulcerative dermatitis with ulcers in the skin of the limbs and joints (23%); (3) focal or multifocal nodular dermatitis (11%); (4) pustular dermatitis and general exanthema (6%). Papular dermatitis has also been described [44] and therefore considered as the fifth group of main dermatological entities associated to CanL [25]. Other less frequent cutaneous manifestations are depigmentation, digital, and nasal hyperkeratosis, while pyodermitis by *Staphylococcus* spp. is a frequent complication [25, 49]. Onychogryphosis

has been reported as present in 75% of cases in a study by Semiao-Santos et al. [50]. Perionyxis and onychorrhhexis have also been reported [47, 50].

3.2. Lymphadenopathy and Splenomegaly. Lymphadenopathy (increasing of size and consistency of lymph nodes) is a very common clinical sign, usually appearing relatively early during the course of the disease. The presence of this clinical sign has been reported in the 93.5% of cases [36, 50]. The general increase of lymph nodes size facilitate palpating of superficial ones, in particular, popliteal, prescapular, and submaxillar [36]. Considerable splenomegaly have also been described [25, 47].

3.3. Ocular Manifestations. Ocular manifestations are quite frequent during CanL, being reported from 16 to 80% of cases [25]. The most frequent eye lesions are conjunctivitis, with mucous to mucopurulent conjunctivitis reported in 18.5% of cases [50]. These are characterized by a yellowish secretion that adhere to the lacrimal margins while the mucosa is usually pale, due to anemia [36]. Similarly, blepharitis (exfoliative, ulcerative, or nodular), anterior uveitis and keratoconjunctivitis have also been reported [51]. Keratoconjunctivitis can also progress to ulceration and blindness [52]. Uncommon are ocular consequences of systemic hypertension (e.g., retinal detachment), reported in 5.7% of hypertensive dogs with CanL [25, 53].

3.4. Renal Disease. Renal disease may be the only clinical manifestation of CanL [25]. Glomerulonephritis and tubulointerstitial nephritis due to the deposition of immune complex are the most common pathological findings in leishmaniotic dogs that develop chronic renal failure [43], which is the principal cause of death in CanL [25, 36]. However, renal disease associated to this pathology does not always reach these severe developments: membranoproliferative glomerulonephritis is usually associated with chronic renal failure while dogs without clinical evidence of renal disease more frequently reveal mesangioproliferative lesions [54].

3.5. Other Clinical Signs. CanL is a systemic disease that could involve many organs, so a variety of other clinical signs are possible, often making difficult differential diagnosis to other diseases. Weight loss, locomotor abnormalities, apathy and anorexia are still quite frequent, being reported in 15–30% of cases; less frequent are polydipsia, polyphagia, polyuria, epistaxis, vomiting, and diarrhea [36, 50]. In particular, cachexia and muscular atrophy are common findings in dogs at the final stages of the disease, when locomotor abnormalities are also frequent. These could be associated to arthritis, osteomyelitis, and arthrosynovitis in uncommon clinical forms of leishmaniosis [47, 55]. Other uncommon clinical forms include neurological disease and polymyositis [56], as well as various autoimmune and cardiovascular disorders (pericarditis, vasculitis) [25].

4. Canine Leishmaniasis (CanL): Laboratory Diagnosis

Accurate and early diagnosis of CanL is crucial to identify infectious dogs and organize control strategies. In order to diagnosis CanL, clinical, anatomopathological, and laboratory findings should be evaluated together. However, control programs are usually based mostly on mass screening, therefore laboratory techniques will be primarily taken into consideration: a large part of the CanL reservoir is composed by infected clinically healthy dogs. As concerns clinical signs and anatomopathological findings in CanL patients, also useful for the diagnosis in infected dogs with clinically evident disease, we recommend to refer to the previous chapter.

Different methods of laboratory diagnosis are currently available, and they can be divided between (1) *parasite isolation* (direct and indirect) and (2) *immunodiagnostic techniques* for the detection of cellular and humoral responses [36]. The detection of the parasite is the main conclusive diagnosis and it is usually carried out directly through the demonstration of amastigotes in smears or after culture of samples (aspirates) obtained from cutaneous lesions, bone marrow, lymphnodes, and spleen [25]. The most important indirect method of detection of the parasite is the polymerase chain reaction (PCR), characterized by high sensitivity and specificity. It can be carried out from samples of blood, skin, lymphnodes, conjunctiva, and bone marrow [36]. Obtaining blood could be the most simple and noninvasive procedure in order to perform PCR, but the parasite load is often lower than in other tissues. Therefore, the application of parasite detection techniques is often difficult when a high number of animals have to be sampled for screening purposes. In this cases, immunodiagnostic techniques are of great utility, in order to detect anti-*Leishmania* circulating antibodies (mainly IgG): immunofluorescent antibody test (IFAT), enzyme linked immunosorbent assay (ELISA), and direct agglutination test (DAT) are the most used methods. To date the IFAT test, the most specific and sensitive among the serological techniques mentioned above, is reasonably considered the “gold standard” for mass screening of dogs [57, 58]. In fact, infectiousness is positively associated with high antiparasite antibody response and low CD4⁺ T-cell count [11, 40, 41], therefore high antibody levels are considered conclusive of a diagnosis of CanL [25, 38]. However, false-positive results due to serological cross-reactivity with other pathogens (e.g., *Trypanosoma cruzi*) have been described [59] and low sensitivity in detecting asymptomatic dogs has also been reported [60]. The cutoff titre to distinguish positive and negative results ranges from 1:40 to 1:160 according to different laboratories [61].

5. *Leishmania infantum* Vectors: Sandfly and Non-Sandfly Transmission Routes

Leishmania infantum is transmitted from the canine reservoir to other dogs or humans via sandflies bites. There is an exclusive association between sandfly species and *Leishmania* species due to specific enzyme and ligands present in the

gut of these insects: only specific *Leishmania* spp. are allowed to attach to gut's wall and replicate without being excreted [62]. Therefore, the transmission of each *Leishmania* species occurs only in that areas where the specific vectors species are present. In particular, female sandflies of the genus *Phlebotomus* (Europe, Asia, and Africa) and *Lutzomyia* (Latin America) are the vectors of *L. infantum* [19, 34, 35]. In the countries of the Mediterranean Basin the most important vector species is *Phlebotomus perniciosus* [63–65]. Other proven sandfly vectors present in the Mediterranean Basin and in the Middle East are *P. ariasi*, *P. neglectus*, *P. perfiliewi*, *P. langeroni*, and *P. tobbi* [5, 19, 35]. In northern Africa also *P. sergenti* and *P. arabicus* have been reported as proven vectors of *L. infantum* [25]. These arthropods are present both in tropical and temperate areas, but in the last ones they are usually active only during warm months, from spring to autumn, at temperatures between 15° and 28°C [25, 35]. The importance of sandfly transmission routes in the epidemiology of ZVL and CanL is demonstrated by the spatial and temporal overlapping of clinical cases and the presence of incriminated vector species [5]. For example, new cases of autochthonous ZVL in humans have been reported in areas of Northern Italy previously considered free [20], in consequence to new CanL outbreaks [66–68] and the proven northerly expansion of two specific vector species *P. perniciosus* and *P. neglectus* [28]. This situation has been attributed, just as new northward cases of CanL, to climate changes [17]. A recent European Union-funded project “Climate Change and Adaptation Strategies for Human Health in Europe” (cCASHh) concluded that so far there are no evidences that sandfly distributions in Europe have altered after recent climate changes, but it also stated that a considerable potential for climate-driven changes could be in Leishmaniasis distribution in the future [69, 70]. Moreover, the EDEN project (Emerging Diseases in a Changing European Environment) has highlighted the potential of leishmaniasis to expand to new territories with its vector as environments change, and various researches carried out in the framework of this project have come to the conclusion that significant changes have occurred in the geographic distribution of CanL and VZL cases and of flebotomine vectors, involving areas previously considered as nonendemic [71–74].

Non-sandfly transmission routes should be also considered. In fact, symptomatic CanL is characterized by wide-spread dissemination not only in blood but also in other body fluids, like semen or saliva, therefore biting [5], blood transfusions [75], and used needles [76, 77] have been suggested to be non-sandfly transmission routes, as well as sexual contact [78], congenital transmission [79, 80] and mechanical dog-to-dog transmission via ticks [81]. However, non-sandfly transmission seems to play only a marginal role in the epidemiology of leishmaniasis [25].

6. *Leishmania infantum* Dog Reservoir: Methods of Control

The primary objective of the control of CanL is to reduce the cases of ZVL in humans through the reduction of the

prevalence in dogs. Different methods of control are available for the control of the *L. infantum* canine reservoir. Control methods could be divided into two different groups: (1) methods aimed at infectious dogs (direct control methods) and (2) methods aimed at vectors (indirect control methods). As concern the first group, a big issue is to identify what dogs should be subjected to control methods. Infectious dogs should be recognized, and this is not an easy work. According to the guidelines by Solano-Gallego et al. [25], dogs in CanL endemic regions could be divided into sick (animals with clinical leishmaniosis) and healthy. Moreover, healthy dogs encompasses both noninfected and infected clinically healthy animals. Only part of infected clinically healthy dogs could develop disease (potentially “presymptomatic” dogs): in fact, there are CanL-infected dogs that could be considered “resistant” to clinical leishmaniosis that will never develop clinical signs. There is a debate about the degree of infectiousness of infected clinically healthy and infected sick dogs, also linked to the usefulness or not of therapeutic treatment on dogs for the control of ZVL in humans. Moreover, it is debated if only sick dogs are worth subjecting to direct control methods (e.g., clinical treatment or culling) in order to limit the transmission of *L. infantum*. In fact, infectiousness would increase with clinical severity, but the infectiousness of infected clinically healthy dogs would also be sufficiently high that direct control needs to be targeted to all serologically positive dogs [5]. However, according to a longitudinal study by Courtenay et al. [11], dogs infected but “resistant” to clinical leishmaniosis would contribute very little to transmission. So, direct control methods targeted to infectious dogs (presymptomatic and symptomatic dogs) could be effective if a reliable test were available. Although of undoubted utility in mass screening, IFAT is not a sensitive and specific test to detect infectiousness, neither are skin biopsies, in fact, studies comparing infectiousness and parasite loads in the skin or lymphnodes have not shown the presence of a correlation [82]. The use of quantitative PCR for the assessment of skin parasite load could be more informative [5], but to date the detection of anti-*Leishmania* circulating antibodies with a serological titre $\geq 1:160$ is the preferred screening method to identify dogs with a very high chance to be infectious and, therefore, worth to be subjected to direct control methods [19]. It is obvious that all dogs (infectious and noninfectious) should be subjected to indirect control methods. Hereinafter, direct and indirect control methods are described, including their efficacy, advantages, and disadvantages.

6.1. Direct Control Methods

6.1.1. Culling. The removal of infectious, serologically positive dogs through euthanasia could seem the most obvious direct control method of the *Leishmania infantum* reservoir. In fact, this is a debated issue, as different studies tried to demonstrate the efficacy of this tool, without reaching definitive evidences [83, 84]. The new sensitivity of a large part of population, particularly in most developed countries, considers this kind of approach ethically unacceptable [7, 85]. For this reason, more and more countries are choosing

to follow only no-kill strategies for the control of free-roaming dogs and, therefore, for the control of the canine reservoir of ZVL. However, the main evidence against the dog culling approach to control leishmaniosis is the failure of large programs of systematic elimination of seropositive dogs carried on during past years that did not result in any reduction in the number of human cases [86]. A high proportion of dogs should be euthanized to achieve effective reduction in disease transmission [87], and this is often a too ambitious objective, mostly in undeveloped countries with limited economic resources. Moreover, the diagnosis of infectious dogs can be very difficult: sometimes serological diagnosis produces false negatives and often there is a long interval before the effective elimination of infectious dogs [88]. Finally, the removal of dogs only subjected to serological diagnosis can have counterproductive effects: infected dogs, when removed, would be replaced by susceptible dog that could become infectious in a short time [11].

6.1.2. Treatment. Treatment of symptomatic or asymptomatic dogs with high levels of anti-*Leishmania* circulating antibodies is the only way to reduce the infectiousness of these subjects in countries where culling is not allowed. Infectious dogs should always be treated, not only for ethical reasons, but also for public health reasons. In fact, there is a correlation between infectiousness of dogs and parasite burden: the reduction of parasite loads after treatment would reduce the possibility of transmission of *Leishmania infantum* to vectors and, therefore, to other dogs or humans [89, 90]. Response to treatment vary from a patient to another and according to the protocol used, but particularly for the first choice drugs it is normally quick: in few days weight increases, cutaneous lesions reduce, blood parameters return to normality, and there is general improvement of the dog's condition [36].

To date, there are different protocols of treatment for leishmaniosis in dogs, using drugs from different categories. The “classic” and still considered “first choice” treatment is based on the use of pentavalent antimonial compounds. In particular, meglumine antimoniate (Glucantime), is the most used commercial formulation, usually combined with allopurinol, according to several different protocols. Considering the possibility of significant side effects, many different protocols have been suggested, varying considerably regarding the dosage, dose interval, and duration of treatment. Possible adverse effects may be arthralgias, myalgias, gastrointestinal problems, alterations in hepatic function and nephrotoxicity [36]. The last one is the most severe effect, and occurs mostly in dogs with reduced glomerular filtration rate [25]. The preferred route for the administration of antimonial compounds is subcutaneous [25]. Intramuscular or intravenous administration has also been suggested [36], even though they could be cause of muscular fibrosis, abscess formation [13], and thrombophlebitis [47]. Allopurinol is always administered orally. Allopurinol has synergic effect in CanL therapy, therefore it is used in many different protocols. It is an inhibitor of xanthine oxidase, that results in the inhibition of the synthesis of parasite proteins [91]. It has scarce side effects (xanthine urolithiasis) and therefore can

be used for long periods [92, 93]. The protocol based on the combined use of antimonials and allopurinol considered as “first line treatment” by the guidelines from Solano-Gallego et al. [25] and Oliva et al. [94] is: meglumine antimoniate (MA) 75–100 mg/kg/SID S.C. for 4–8 weeks + allopurinol (A) 10 mg/kg/BID P.O. for six months or more [95–98]. Another effective protocol based on the use of the same drugs is: MA 50–75 mg/kg/BID S.C. for 1–2 months + A 10–20 mg/kg/die P.O. for 1–8 months [19, 99]. During treatment clinicopathological parameters should be monitored, including complete CBC, biochemical profile, serum protein electrophoresis, and urinalysis, after the first month of analysis and then at least every 3–4 months [25]. In order to limit side effects, modulation of doses according to kidney and liver functions has also been used [9]: MA 75 mg/kg/BID S.C. + A 20 mg/kg/SID P.O. were used in dogs with GOT, GPT, BUN and creatinine inside normal range; MA 50 mg/kg/SID S.C. and A 20 mg/kg/SID P.O. were used in dogs with liver or renal failure. In the same study, the moment of stopping antimonial treatment was decided on the basis of results from serum protein electrophoresis, enduring the treatment until the albumin/globulin was ≥ 0.80 .

The second choice “classic” treatment for the therapy of CanL is amphotericin B: it is a very powerful anti-*Leishmania* drug [100], but it is preferably administered by slow intravenous infusion [25, 101], although also subcutaneous administration has been suggested [102]. The speed of intravenous infusion is closely related to the occurrence of side effects like trembling, fever, nausea, and vomiting [103]. Another important disadvantage of amphotericin B is its severe nephrotoxicity, resulting in decreasing of the rate of glomerular filtration with consequent increasing in levels of serum creatinine and urea nitrogen [36]. The recommended protocol foresees the administration of doses from 0.1 to 1 mg/kg/SID or twice per week (lower doses when administration is daily) I.V. for two months [101, 102]. The development of formulations of amphotericin B in lipid carriers has been performed to diminish toxicity [104], but these products have high costs. Moreover, these are the first line drugs for the therapy of human leishmaniasis, so their use in dogs is not recommended to avoid drug parasite resistance [25]. Experimentally, liposomal amphotericin B has been used in dogs at the dose of 3 mg/kg/SID I.V. for five consecutive days, intravenously: results were good, but clinical signs usually reappeared after 4–6 months [105].

Recently, miltefosine has been successfully used in association with allopurinol for the treatment of CanL [6, 106]. It is an antineoplastic agent that has proved to be efficient against *Leishmania* [107], now registered for the use in dogs as Milteforan. The main advantages of this drug are the oral administration and the low toxicity, even if some gastrointestinal side effects (vomiting, diarrhea) have been reported, mostly at high doses, so that administration with food is recommended [108, 109]. Another possible side effect could be the early development of drug resistance due to the long half-life of this agent [110]. The protocol successfully used [6] foresees the administration of miltefosine 2 mg/kg/SID

P.O. for four weeks and allopurinol 10 mg/kg/BID P.O. for six months.

6.1.3. Vaccine and Immunotherapy. The use of an anti-*Leishmania* dog vaccine could be the most practical and efficient tool for the control and, possibly, the eradication of the disease in endemic areas [3, 87]. In the last years, many studies have been carried on several immunoprophylactic vaccine candidates, in order to find an efficient tool able to directly prevent leishmanian infections [111–117]. To date, only two vaccines have been licensed, both in Brazil, precisely the Leishmune (Fort Dodge Animal Health) and the Leish-Tec (Hertape Calier Saúde Animal). The second one consists of adenovirus expressing a *Leishmania donovani* A2 antigen, but only partial results on field trials testing its efficacy have been published so far [14, 118, 119]. Leishmune vaccine has already shown its efficacy in various field studies [15, 113, 120, 121]. It is a FML-saponin vaccine that protect 98% of vaccinated dogs [15], blocking the transmission of the disease to sandfly vectors. This tool acts on a crucial moment of the life cycle of *Leishmania infantum*, with important epidemiological effects: in fact, it was demonstrated that vaccinated dogs do not present the parasite in blood, skin and lymph nodes [120], not exposing the parasite to sandflies. In some areas of Brazil, the anti-*Leishmania* vaccination of healthy dogs has been used as a preventive tool systematically used in association to regular culling of serologically positive dogs, with encouraging effects: from 2004 to 2006 the vaccination of 5.7% of the healthy dogs led to a decrease of 25% of cases of CanL in dogs and of 61% of cases of ZVL in humans [12]. Anti-*Leishmania* vaccination is debated because of the possible effects on the serological response of vaccinated, healthy dogs: after vaccination they could present seroconversion, therefore being indistinguishable from infectious dogs [15, 16]. So, serological control campaigns, employing either ELISA or IFAT serological screening tests, would be no more useful, because more than 70% of vaccinated dogs would result positive [12, 16]. Recently, it has been reported that the use of a specific anti-*Leishmania* ELISA test, suggested by the Brazilian Ministry of Health, would be able to detect only 1.3% of positivity among uninfected vaccinees [12], therefore not interfering with regular serological control campaigns.

The anti-*Leishmania* vaccine has also demonstrated its efficacy as immunotherapeutic agent, used alone or in association with allopurinol and amphotericin B (chemoimmunotherapy) for the treatment of symptomatic dogs [1]. For these purposes, a vaccine formulation, different from the one registered for prophylactic purposes, was used, with positive effects both considering immunotherapy (reduction of symptomatic cases from 100% to 38%) and chemoimmunotherapy (reduction of symptomatic cases from 100% to 12%). In particular, the combination chemotherapy-vaccine (chemoimmunotherapy) resulted very effective in eliminating also latent infections, given that 80% of patients showed PCR negative lymphnodes after eight months of treatment, versus only 33% of patients with immunotherapy alone.

6.2. Indirect Control Methods

6.2.1. Repellent Tools. During the last decade, a number of studies have been carried out demonstrating the efficacy of topical insecticides treatment of dogs exposed to sandfly bites in order to control the canine reservoir of *Leishmania infantum* [5, 6, 25]. It is now widely accepted that the prevention of sandfly bites on dogs is efficient in reducing not only the incidence of CanL [7, 122], but also the risk of human infections [33, 123]. There are three different types of topical preventive tools, namely, collars, spot-on and spray formulations, all based on synthetic pyrethroids at various concentrations: permethrin 50%-imidacloprid 10% (spot-on), permethrin 65% (spot-on), permethrin 1.9%-pyriproxyfen 0.02% (spray), and deltamethrin 4% (collar). These tools are efficient through two actions. (1) antifeeding effect: the presence of the insecticide on the skin has a strong repellent effect, so the sandfly has only a fleeting contact with the dog, that is sufficient to disorientate and irritate the insect, resulting in absence or reduction of the blood feeding; (2) insecticide effect: usually the sandfly, even if disorientated and incapable of feeding, remains on the skin for quite long time, absorbing a dose sufficient to achieve toxic effects [35, 124]. Therefore, these topical insecticides not only manage to prevent sandfly bites and, consequently, *L. infantum* transmission, but also can reduce the number of infectious phlebotomus by direct elimination of them. Deltamethrin-impregnated collars have antifeeding and lethality effects for six months [6], while spot-on and spray formulations have shorter effects, so they have to be applied at least once a month [7]. On the contrary, because of the slow release of the insecticide, collars achieve full protective activity only 1 week after application while spot-on formulations need 24–48 hours, and sprays are immediately active [123]. Collars have demonstrated to have a protective effect from 72% to more than 90% according to different studies [6, 36, 122]; moreover, they have shown to have a mortality effect ranging from 30% to 67% [36]. Spot-on formulations have demonstrated to be more effective, with a protection rate ranging from 88.9% to 97.7% and even to 100%, according to the intervals between one application and another (once or twice a month) [7, 124, 125]. On the contrary, the insecticide effect of spot-on formulations seems to not differ very much from the one attributed to collars, ranging from 23% to 67% [123]. The only available spray formulation has shown a repellent efficacy of 71.4% but an insecticide effect just of 7.2%, after 21 days [126, 127]. Field studies have been performed for collars and spot-on formulations, in order to evaluate the impact of mass use on the incidence of leishmaniosis, reporting reductions from 50% to 53% in tropical or subtropical countries [128, 129] and from 86% (collars) to over 90% (spot-on) in temperate countries [7, 9, 122, 130]. Moreover, in an Iranian study based on the application of deltamethrin impregnated collars a reduction of 43% of the odds of seroconversion in children was reported [33], highlighting the indirect effects of these tools on human visceral leishmaniosis. These are important achievements, but even more important results could be obtained in the future through these tools. In fact, so far

these control methods have been used only in reduced and controlled areas: the application of topical insecticides in wider areas, involving entire communities and a consistent percentage of the dog population (free-roaming and not) could have a greater effect on the incidence of both CanL and ZVL.

6.2.2. Environmental Control. The efficacy of environmental insecticides in order to control the sandfly population is debated, considering high costs, difficulties in application, pollution concerns, and the possibility of resistances. There is a great variety of species of vectors, and in some cases the presence of resistance was reported, in particular for *Phlebotomus papatasi* (resistance to DDT, permethrin, and lambda-cyhalothrin) and *Phlebotomus argentipes* (permethrin and deltamethrin) [131]. However, other studies have reported that sandfly control by insecticides should be a very effective control method: the increase of sandfly mortality reduces the number of vectors, directly influencing the disease transmission [87]. Synthetic pyrethroids are at present the most used anti-sandfly insecticides, even if their short-lived residual activity compared to DDT could be a limitation and a reason of the low efficacy sometimes reported [132]. The effectiveness of control campaigns based on insecticide spraying for the reduction of ZVL cases was reported in Italy [133], India, Greece, Israel [36] and in China [84]. In particular, the efficacy of insecticide spraying was reported to be more evident in the intra- or peridomestic environment, rather than reduce the overall sandfly population [134], as this kind of control is effective on adult insects only, because it is not known were immature forms live [133]. Moreover, spraying in areas like forests, burrows, caves, and other places where sandfly larvae and pupae are suspected to live is technically very difficult [36], and could have effects on other insects species, with consequent environmental damages. It has been suggested to concentrate spraying in the peridomestic environment to the inside walls of houses, doors, windows, kennels, hen houses, and drains [36] in order to maximize its effect against indoor-biting vectors. Recently, the use of insecticide-treated nets has been suggested as a preventive tool for ZVL in humans [135, 136]. The reduction of microhabitats favorable to sandflies has also been reported as a step to be taken in order to achieve effective prevention [25]. However, the use of repellent tools on dogs remains the most effective antivectorial method to control the *L. infantum* canine reservoir, and the use of environmental control in association to spot-on formulation should be particularly efficient in areas with large and concentrate dog populations (i.e., dog shelters).

6.3. Contemporary Use of Direct and Indirect Methods. A number of studies have shown the efficacy of single control methods of the *Leishmania infantum* dog reservoir, inducing the ministry, health organizations, and groups of scientists to suggest the contemporary use of different tools, particularly preventive and therapeutic tools, in order to enhance their effects [19, 35, 36, 133]. However, these guidelines reported the absence of field studies or mathematically predictive studies on the effect of this “holistic” approach on the

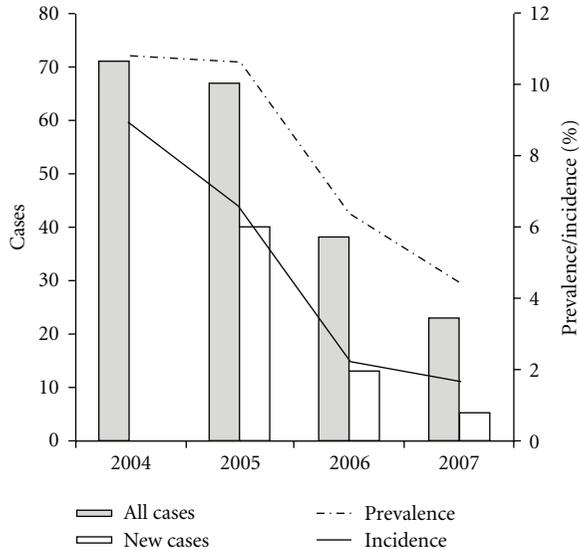


FIGURE 1: Evolution of prevalence and incidence of serological reactivity versus *Leishmania infantum* in two dog shelters during a 4-year period of contemporary use of direct (treatment with meglumine antimoniate and allopurinol) and indirect (spot-on repellent formulation imidacloprid 10%-permethrin 50%) control methods [9].

prevalence and the incidence of leishmaniosis [19]. It has been hypothesized that reinfection in endemic areas is likely [25], due to the persisting action of sandflies also on subjects treated with therapeutic protocols [137]. Recently, a field study of the authors [9] reported long term effects (4-year period) of therapeutic treatment associated with the application of spot-on preventive tools. The retrospective study was carried on in a dog shelter sited in an endemic area in Central Italy, reporting a significant, progressive reduction of prevalence and incidence of serologic reactivity to *L. infantum* with a very low rate of relapses (only 8% after 4 years in a pool of 67 subjects that were already seropositive at the beginning of the study). A total of 656 dogs sheltered in a municipal dog kennel were included in the study. Antimonial treatment was administered to animals assigned to one of the following three classes: (1) serological titre $\geq 1:160$, (2) serological titre = 1:80, albumins/globulins ratio < 0.80 , and (3) serological titre = 1:80 titre, albumins/globulins ratio ≥ 0.80 , and presence of clinical signs of CanL. The therapeutic protocol was based on the administration of meglumine antimoniate and allopurinol. Doses were individually adjusted according to kidney and liver functions (meglumine antimoniate 75 mg/kg/BID + allopurinol 20 mg/kg/SID in dogs with normal functions, meglumine antimoniate 50 mg/kg/SID + allopurinol 20 mg/kg/SID in dog with kidney or liver failure). Antimonial treatment endured until the serum protein electrophoresis gave an albumins/globulins ratio ≥ 0.80 ; however, it was never longer than 12 months or shorter than 2 months. Allopurinol administration continued until complete serum protein electrophoresis normalization. Since the second year of the study a spot-on preventive treatment was applied once a month to all dogs that were in the shelter,

using imidacloprid 10%-permethrin 50% (Advantix; Bayer; Germany) at a dose of 1 mL/10 kg. A progressive reduction of prevalence and incidence of seroreactivity to *L. infantum* was observed. In particular, during the four-year period seroprevalence was lowered from 10.8% to 4% ($\chi^2 = 22.02$; $P = 0.000$, significant); while incidence was reduced from 8.92% to 1.63% ($\chi^2 = 52.226$; $P = 0.000$, significant Figure 1). It should be highlighted that the introduction of the association of preventive and curative treatment was followed by a significant reduction in prevalence from the second to the third year of study (reduction of prevalence from 10.63 to 6.41; $\chi^2 = 6.90$; $P = 0.009$), which contrasts with what had been observed from the first to the second year, probably as a consequence of the absence of preventive treatment (reduction of prevalence from 10.79 to 10.63; $\chi^2 = 0.08$; $P = 0.928$, not significant). The very low rate of relapses could be considered as the key of this success. In fact, previous studies using antimonial treatment without preventing tools had reported seroreversion rates of 32% [138], 74.3% [99], 39.6% [139], and 86% [140]; these are values much more higher than the 8% reported in the research above described [9]. However, more studies are needed in order to confirm the role of preventive tools in reducing the rate of relapses and reinfections in endemic areas.

7. Conclusions

The domestic dog is the most important natural reservoir of *Leishmania infantum* and, therefore, it is crucial in the transmission of both canine leishmaniosis and zoonotic visceral leishmaniosis. A number of control methods of the *L. infantum* canine reservoir have been experimented, but at present the use of preventive tools directly applied on dogs seems to be the most successful one. Culling of infectious animals is now widely considered unacceptable from an ethical point of view: more and more countries are producing new “no-kill” laws with which control programs must necessarily deal. Moreover, it has been reported that this kind of approach often fails in achieving good results concerning its effects on reducing both the number of human and canine cases, particularly if applied without the contemporary use of repellent tools [86]. Treatment of symptomatic dogs could seem too costly to afford for many countries but, according to animal welfare laws and the new “humane” sensitivity of a large part of the population, the absence of therapies could be intended as maltreatment and therefore unacceptable [141]. The absence of treatments can also be a risk for public health, considering the correlation between infectiousness of dogs and parasite burden [89, 90]. The contemporary use of more than one control method has been repeatedly suggested in the past in order to enforce their efficacy [19, 25] and, in particular, the use of repellent tools can integrate the use of therapeutic treatments improving their effects and, therefore, their usefulness and affordability. Moreover, the community-wide use of repellent tools (spot-on or impregnated collars) could be a valid alternative or support to control programs based on dog euthanasia, both considered for their proven efficacy and limited costs. The

anti-*Leishmania* vaccination has been successfully used [12] and it could be a valuable tool in order to implement efficient surveillance programs to control the *L. infantum* canine reservoir [3]. However, until a certain and commonly recognized diagnostic method will not be available to distinguish vaccinated from seropositive individuals, the use of this tool on large-scale-control programs should not be suggested yet. At present, repellent tools associated with the treatment of infectious dogs seems to be the most desirable and widely accepted approach in order to control the *L. infantum* canine reservoir.

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