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REVIEWS

Review

Novel Areas for Prevention and Control of Canine Leishmaniosis

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There have been multiple recent advances regarding tools for the control and prevention of canine leishmaniosis (CanL), including new preventative vaccines. In this review, these advances are evaluated based on control targets, including vector and parasite. Leishvet recommendations are provided for control practices based on the dog's risk of infection. New topical insecticide formulations have proven to be effective in preventing sand fly bites, and subsequently infection. Parasite control occurs through chemotherapeutic or immunologic means, which decrease or prevent transmission to other animals, including humans. Leishmaniosis control programs that include a combination of coordinated measures, either in individuals or for prevention across reservoir populations, are required.

CanL Epidemiology and Control

Visceral leishmaniasis is caused by *Leishmania* spp, including *L. donovani*, *L. infantum,* and, in rare instances, by visceralizing strains of *L. braziliensis* and *L. tropica*. Canine leishmaniosis (CanL), caused by infection with *Leishmania infantum,* syn. *L. chagasi*, is a vector-borne, protozoal, zoonotic disease. CanL affects approximately 2.5 million dogs in Mediterranean and peri-Mediterranean areas each year [1]. Beyond the Mediterranean basin, additional CanL endemic areas include the Americas and Asia. As dogs are the predominant reservoir host for human infection, there is a significant overlap between locations where there is high seroprevalence of CanL and human mortality attributed to this disease [2,3]. *Leishmania* parasites predominantly transmit to mammalian hosts via phlebotomine sand flies. Vector control is an important part of prevention but has limitations. Prevention and management of CanL has progressed considerably over the last two decades due to a better understanding of the canine immune response during infection. This progress has provided better assessment of treatment efficacy and growing knowledge regarding the multihost ecology of *Leishmania* infection beyond dogs [4–7]. The goal of this review is to summarize the most recent literature regarding *Leishmania* control and prevention in dogs.

Progress in CanL Vector Control

Transmission of metacyclic promastigotes to dogs occurs immediately sand fly bite. Consequently, the main way to avoid *Leishmania* infection is to use topical insecticides with proven activity for bite prevention. These formulations have the dual effect of both warding off and killing sand flies when they contact a protected dog [8–10]. This **killing effect** (see Glossary) identifies the insecticidal efficacy, while the ability to ward off sand flies is a combination of **contact repellency** and anti-feeding effect. However, these two activities do not always have the same duration of action. For example, a study that investigated the activity of a permethrin

Trends

Recent advances in canine leishmaniosis (CanL) prevention.

The main way to avoid *Leishmania* infection is to use topical insecticides with proven activity against the bite of the female sand fly. Higher permethrin concentrations had a longer duration of effect. Owner compliance is critical for correct product application.

The combined use of second-line human leishmanicidal agents (e.g., pentavalent antimonials, miltefosine) and allopurinol is currently the first-line treatment for CanL. Xenodiagnosis studies indicated that anti-*Leishmania* drugs reduced or completely abolished infectiousness of treated dogs for at least 4 months.

There is no justification for mandated euthanasia of infected and/or sick dogs.

Recent studies have proven both sexual transmission from sire to dam and vertical transmission to puppies. Any dog to be bred should be tested with an accurate quantitative serological test prior to breeding. Test-positive dogs should not be bred. Travelling, rehoming, sale, transfer of infected dogs or reproductive materials for breeding should not be imported into nonendemic areas.

Protective and effective vaccines against canine leishmaniosis constitute a newer, essential, tool for preventing CanL. Vaccination does not prevent the establishment of infection and may allow maintenance of an infected but clinically healthy status

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containing spot-on [11], found that 7 days after application and 1 h after treated dogs were exposed to sand flies, both the **insecticidal effect** and **anti-feeding efficacy** were high (99.8% and 99.6% respectively). In comparison, at 21 days postapplication, the insecticidal effect fell to 24.1% whereas bite prevention was still effective at 83.5%.

The efficacy of several topical formulations, such as collars, spot-ons or sprays, has been experimentally demonstrated to prevent sand fly bites. These formulations contain different synthetic pyrethroids proven to be effective against *Phlebotomus* species. Pyrethroid group molecules (Table 1) provide rapid and prolonged efficacy to prevent insect bites. Some pyrethroid-based compounds have been specifically shown to be effective against *Leishmania* infection in dogs living in endemic areas (Table 2), while others have been demonstrated to be effective only in controlled experiments. The main limitation of these studies is often their short-term follow-up of exposed dogs, usually one or two transmission seasons, which does not inform regarding the effect of these products on *Leishmania* incidence over longer periods.

Collars

Two collars proven effective are currently available with different active ingredients. Deltamethrin-impregnated collars release insecticide that reaches maximum efficacy 2 weeks after application [12,13]. The repellent effect of deltamethrin-impregnated collars can last 6–8 months [12,14]. The active ingredients in these collars act against several phlebotomine species present in Europe, Asia, and South America. Widespread use in dogs as a control measure significantly reduced canine and human seroprevalence of leishmaniosis. Estimated population protection rates ranged from 50% to 86% [15–17]. Flumethrin- and imidacloprid-containing collars (flumethrin 4.5%, imidacloprid 10%), were an effective preventive measure against *L. infantum* infection in puppies under 6 months of age, as tested in a kennel at a hyperendemic area in southern Italy [18,19]. At present, there is no information on the experimental contact **repellency** of this collar against sand fly bites.

Spot-ons

Topical preparations applied as spot-ons can provide adequate repellent levels with shorter lasting effects than collars [20]. These products provide relatively rapid spread of insecticide on the body surface [21]. Permethrin 50% (insecticide-acaricide) and imidacloprid 10% acted synergistically to lengthen the repellent effect against sand flies [22,23]. Otranto *et al.* [24] reported that population-based application of permethrin plus imidacloprid spot-ons on dogs was able to reduce *Leishmania* infection incidence over a 2-year period. A combination, including dinotefuran (5.0%) (neonicotinoid insecticide), permethrin (36.1%), and pyriproxyfen (0.4%), had repellency and insecticidal efficacies of 96% and 88% at 14 and 21 days, respectively [11]. More recently, a combination of permethrin (48%) and indoxacarb (15%; oxadiazine insecticide) had antifeeding effects lasting 4 weeks against *Phlebotomus perniciosus* [25]. New combinations of permethrin and fipronil (pheylpyrazole insecticide-acaricide) confirmed a significant repellent effect against *P. perniciosus.* A fipronil (6.7%) and permethrin (50.5%) treatment maintained repellency above 90% until day 29 [26]; another combination (permethrin [44.9%] and fipronil [6%]) had repellency above 87% up to 28 days after application [27]. Different efficacy studies are hard to compare, as protocols and repellent effect calculations can be slightly different. Moreover, due to the difficulty of maintaining a sand fly colony, the number of sand flies used in studies was generally limited and reproducibility was questionable. In general, a higher permethrin concentration had a longer duration of effect. The real dose (mg/kg) received by dogs varied depending on the dog's weight as spot-on dosages are fixed for compartmentalized weights.

Sprays and Natural Compounds

Insecticide lotions marketed as sprays based on 65% permethrin, or combinations with pyriproxyfen, offer good repellent and insecticidal properties [8,20,28]. Their efficacy is

in some dogs. The decision to vaccinate should be based upon the following: individual benefit/risk to the dog, age, breed, life-style or use, habitat, reproductive status, and owner compliance.

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Table 1. Insecticide Molecules with Efficacy to Prevent Sand Fly Bite[a]

Active ingredient	Pyrethroid (range)	Type	Onset	Duration	Brand name (Company)	Refs
Permethrin Indoxacarb	48 mg/kg (48–96)	Spot-on	24–48 h	3–4 weeks	Activyl® Plus (MSD)	[25]
Permethrin Imidacloprid	50 mg/kg (50–125)	Spot-on	24–48 h	3–4 weeks	Advantix® (BAYER)	[23]
Permethrin	–	Spray	Instant	2–3 days	Duowin® (VIRBAC)	[8]
Permethrin Fipronil	60 mg/kg (60–160)	Spot-on	24–48 h	4 weeks	Effitix® (VIRBAC)	[27]
Permethrin	47.6 mg/kg	Spot-on	24–48 h	2 weeks	Ex-spot ® (MSD)	[28]
Permethrin Fipronil	50.48 mg/kg (50.5–101)	Spot-on	24–48 h	4 weeks	Frontline Tri-Act® (MERIAL)	[26]
Deltamethrin	40 mg/g	Collar	7 days	6 months	Scalibor® (MSD)	[12]
Flumethrin Imidacloprid	56 mg/g	Collar	–		Seresto® (BAYER)	
Permethrin Dinotefuran Piriproxyfen	46.6 mg/kg (46.6–158.8)	Spot-on	24–48 h	4 weeks	Vectra 3D® (CEVA)	[11]

[a]The focus of this table are products licenced in Europe; however, certain products are also available on other continents.

immediate after application, but correct manual administration is critical to avoid creating unprotected zones on the dog and therefore loss of protection. Their residual effect is less than that of other products, and applications separated by 1 to 2 weeks are required to obtain protection [8]. Dogs tolerate these sprays well and without reported adverse reactions to the active ingredients. The safety margin after following the manufacturer's recommendations was high with these applications. The manufacturer's recommendations need to be repeated by the recommending veterinarian to dog owners to optimize repellent and insecticidal effects. Insecticide spray use can be of particular importance in cases of short exposure, or to immediately protect a dog in case of transient loss of protection from other products (during and immediately following grooming).

Some natural compounds are known to be insect repellents. However, their activity against sand flies is generally unknown. These products have not been tested on dogs, and the duration of repellence is thought to be very limited [29]. Neem oil or lavender lotions tested against vectors of human kala-azar showed a protection for only 7 h. Citronella (lemongrass) extract and geraniol were also tested, with very poor repellent efficacy against sand fly bites [30].

Table 2. Pyrethroid Compounds That Reduce *Leishmania infantum* Infection in Dogs.

Principle active ingredient	Type	Length of follow-up	Diagnostic tools	Power of study (*n*)	Refs
Deltamethrin	Collar	Two consecutive sand fly seasons	Serology (IFAT)	Individually collared dogs. Follow up 1st year (224) 2nd year 50–86% protection (114)	[15]
Permethrin + imidacloprid	Spot-on	One sand fly season One year	Serology (IFAT) PCR on skin and bone marrow	Treated dogs grouped in kennels 89–100% protection (63)	[24]
Flumethrin	Collar	One sand fly season Ten months	Serology (IFAT) Cytology PCR on skin and bone marrow	Individually collared dogs 93.4% protection (219)	[19]

Glossary

The activity of insecticides or repellent products can be qualified by the following terms:

Antifeeding efficacy: the primary purpose of an insecticide is to prevent insect bites. This can be grossly measured by the percentage of unfed insects placed near treated animals, as compared to control, untreated animals.

Contact repellency: the effect of modern insecticides, including pyrethroids, measured by the number of unfed sand flies alive after a certain period of time from application.

Delayed mortality (4h and 24h): the insecticidal effect on repelled insects after contact with a high concentration of insecticide.

Insecticidal efficacy: evaluates the killing effect obtained. The regulatory definition changes with time and is not necessarily similar between US and EU regulatory bodies. In Europe, insecticidal efficacy is established to be 95% efficacy up to 24 h for fleas and up to 48 h for ticks post-treatment or challenge. This time is too long for sand flies; thus, 1-hour efficacy is generally tested. This rapid efficacy is also frequently termed 'knock-down'.

Percent efficacy: established by comparing on nontreated negative control animals compared to treated animals.

Repellency: characterized by the ability of a drug to repel an insect away from a potential host. Initially developed for flying insects (flies, mosquitoes), it describes a distance effect when the insect is in contact with a vapour phase containing the insecticide. Such efficacy is linked to volatile products which consequently have a short residual effect. In practice, there is an overlap between antifeeding efficacy and the repellent effect in scientific publications. These two succinct concepts are difficult to differentiate in the case of insects that bite rapidly. It is calculated based on total unfed sand flies: alive (repelled with or without true antifeeding effect) or dead. For sand flies, the required efficacy does not have to reach 95% but only 80%, which may have consequences in terms of prevention of transmission during periods of high sand fly activity.

Environmental Vector Control

Additional measures exist beyond topical treatment to control and reduce sand fly numbers in the dogs' environment. These include protecting windows and doors of homes, shelters, or dog kennels using mesh with size ranging between 0.3 and 0.4 mm^2 [8]. In addition, removal of sand fly breeding locations (e.g., compost, pruning scraps, bins, and woodpiles), and favorable areas close to inhabited zones, is important to decrease the risk of sand fly bite [29]. Use of residual insecticides or permethrin-treated screens in dwellings and surrounding areas in hyperendemic regions can also serve to reduce the number of infectious bites [9,31]. Lastly, keeping dogs indoors from dusk to dawn, when female sand flies are questing for blood meals during high-risk seasons, is also important to prevent infection [32–35].

New Areas in *L. infantum* Parasite Control

Significant advances in the control of *L. infantum* infection in dogs have been made in three major areas:

- Chemotherapy. Drugs with leishmanicidal or leishmanistatic effects are able to reduce the parasite load in sick dogs, and a high percentage of treated animals reach a state of remission from clinical disease over long periods of time [36–38]. There is no treatment available that will achieve a 100% sterile cure in dogs.
- Immunotherapy. Given that *Leishmania* dramatically and negatively modulates the canine immune system, the main objective of immunotherapy is to modulate the host immune system so that it can restore an immune response that can control infection. This approach is an expanding area of research in which new and previously known molecules are being tested, including domperidone.
- Immunoprophylaxis. Vaccines can be used in dogs to elicit an adequate immune response that will avoid progression of disease upon infection. Today, this approach is considered a cornerstone for the control of CanL.

Chemotherapy

The World Health Organization (WHO) has suggested reserving antileishmanial drugs used for treatment of humans for exclusive use in human leishmaniosis and not for veterinary purposes, due to suspicion of development of drug resistance from use in animals [39]. Despite this, the first-line treatment for CanL is currently the combined use of leishmanicidal agents used as second-line drugs for humans (e.g., pentavalent antimonials, miltefosine) and allopurinol [21,40,41]. Determination of the best intervention is based on clinical examination, and staging of CanL according to immunodiagnostic test results, clinical signs, and clinicopathological abnormalities [40,42]. Commercially available drugs, dosages, and common side effects are listed in Table 3. It is unlikely that treatment of only dogs with clinical signs would reduce human or CanL prevalence, as infected dogs without clinical signs that would not be treated, and other domestic reservoirs, would maintain disease in the region. Some of these drugs have reported clinical resistance in dogs (Table 3).

The only way to establish whether chemotherapy decreases canine infectiousness and transmission is by xenodiagnosis. This method involves exposing a treated and potentially infected dog to female sand flies over an established period to demonstrate the presence or absence of *Leishmania* parasites within sand flies [43]. Due to the cumbersome nature of this technique, the literature describes only a few studies that have used this methodology to assess infectiousness after treatment. Several published studies indicated that anti-*Leishmania* drugs reduced or completely ablated canine infectiousness for at least 4 months (Table 4). Xenodiagnosis studies are needed to establish whether other drugs, including miltefosine, provide similar effects on transmission.

Table 3. Drugs with Demonstrated Leishmanicidal/Leishmaniostatic Effects in Clinical Canine Leishmaniosis[a]

Drug and dose	Mechanism of action	Duration of rx	Side effects	Time to relapse	Drug resistance
Meglumine antimoniate (50 mg kg/12 h SC or 100 mg/kg/24 h)	Blocking parasite metabolism through inhibition of phosphofructokinase enzyme	4–6 wks	Local pain, pancreatitis, panniculitis nephrotoxicity	6–12 mo	Reported [86,87]
Miltefosine (2 mg/kg/24 h)	Impairment of signaling pathways and cell membrane synthesis	4 wks	Anorexia, vomiting, diarrhea	4–6 mo	Not reported
Paromomycin (4 mg/kg PO)	Interference with mitochondrial pathway	3 wks	Nephrotoxicity Ototoxicity	3–4 mo	Not reported
Allopurinol (10 mg/kg/12 h PO)	Interference with purine pathway	6–24 mo	Xanthinuria, urolithiasis, renal mineralization [88]	Not well documented	Reported [89]
Meglumine antimoniate (50 mg kg/12 h SC or 100 mg/kg/24 h) + allopurinol (10 mg/kg/12 h PO)	Blocking parasite metabolism through inhibition of phosphofructokinase enzyme (MA), Interference with purine pathway (All)	4 wks (MA) 6-12 months (All)	Local pain, pancreatitis, panniculitis Nephrotoxicity (MA), Xanthinuria, urolithiasis, renal mineralization (All)	Not reported	Not reported
Miltefosine (2 mg/kg/24 h) + Allopurinol (10 mg/kg/12 h PO)	Impairment of signaling pathways and cell membrane synthesis (Mil), Interference with purine pathway (All)	4 wks (Mil) 6–12 mo (All)	Anorexia, vomiting, diarrhea (Mil), Xanthinuria, urolithiasis, renal mineralization (All)	Not reported	Not reported
Marbofloxacin (2 mg/kg/24 h)	Inhibition of DNA gyrase	4 wks	Not reported	3–6 mo	Not reported

[a]Abbreviations: rx, treatment; h, hours; wks, weeks; mo, months; PO, *per os*; SC, subcutaneous; mg, milligram; kg, kilogram; MA, meglumine antimoniate; All, allopurinol; Mil, mitofosine.

Immunotherapy

As *Leishmania* spp. live intracellularly within phagocytic cells, the mechanisms of disease relate directly to dysfunction of macrophages, dendritic cells, and other immune cells. Current research efforts are targeted at finding therapeutic agents that maintain strong, balanced, parasite-targeted, cell-mediated immunity through protective Th1-type immune responses. Currently, immunomodulatory drugs are used as adjunctive treatment to conventional therapy, with the aim of getting better and more sustained clinical improvement and reducing the dose of anti-*Leishmania* drugs, therefore lowering the risk of developing resistance and adverse side

Table 4. Reduction of Infectiousness in Treated Dogs Evaluated by Xenodiagnosis

Dog sample size	Clinical improvement	Treatment protocol	Infectiousness to sand flies	Parasite burden (tissue and method)	Refs
2	Yes	Antimonials	Reduction	Not assessed	[36]
4	Yes	Antimonials + allopurinol	Lack of infectiousness	No change	[90]
10	Yes	Antimonials	Reduction	No change (popliteal lymph)	[37]
36	Yes	Antimonials (liposomal formulation)	Reduction	Reduction (bone marrow)	[91]
26	Yes	Antimonials +allopurinol, antimonials, allopurinol	Reduction	Reduction (bone marrow)	[38]
52	Yes	Liposomal antimonials, allopurinol, liposomal antimonials + allopurinol	Reduction	Reduction (bone marrow)	[92]

effects. As in dogs, human visceral leishmaniosis (VL) manifests through interleukin (IL)-10- and transforming growth factor (TGF)-β-mediated immunosuppression [44]. Anti-IL-10 monoclonal antibodies in murine models allowed a 10-fold reduction in the effective dose of antimonial compared with drug alone [45]. Only modest immune improvement was seen after antibody blockade of IL-10 in an *ex vivo* study of $CD4^+$ T cells from *L. infantum*-infected dogs [46]. The use of Toll-like receptor (TLR) agonists, such as Pam3Cys (a TLR-2/1 ligand) together with miltefosine, indicated that inclusion of a TLR agonist required a lower miltefosine dose in mice to promote a decreased parasite load [47]. Unfortunately, very few of these treatments have been tested so far in dogs with CanL.

Immunomodulators assessed to date in CanL are a dopamine D2 receptor antagonist, domperidone [48], and a protein aggregate of magnesium–ammonium phospholinoleate–palmitoleate anhydride (P-MAPA) [49]. Domperidone treatment prevented development of clinical disease [50], but efficacy data supporting treatment of sick dogs are limited at best [48] or discouraging [51]. Domperidone is safe, and side effects are rare. P-MAPA induced stimulation of cellular immunity and no toxic effects in dogs. When CanL sick dogs were treated with P-MAPA, clinical signs improved, skin parasite load decreased, and dogs had decreased IL-10 serum levels with increased IL-2 and IFN-γ levels [48]. Immunotherapy is an active area of investigation with the potential of additional products to be on the market soon. Despite this, the only well-documented safe treatment at present is domperidone, at the recommended dose, to prevent clinical disease progression in conjunction with conventional anti-*Leishmania* chemotherapy – with the understanding that its efficacy is, at best, controversial. The ability of the canine immune system to respond to infection changes over the course of progressive leishmaniosis [46]. A decision to use immunotherapeutic agents should be made recognizing that clinically ill animals may have a limited or exhausted T cell response – therefore immunomodulatory therapy must always be made in conjunction with chemotherapy and will have limited efficacy when the parasite load is high.

Immunoprophylaxis

Protective and effective vaccines constitute an essential tool for preventing infectious diseases and are therefore important for the prevention of CanL. Understanding the characteristics of protective immunity is the first requirement for the evaluation of an anti-*Leishmania* vaccine candidate. Immunity to all *Leishmania* species, as intracellular pathogens, is dependent upon producing a Th1-type cellular immune response, including IL-12 production by dendritic cells and macrophages, efficient antigen presentation, and subsequent IFN-γ production from T cells [52,53]. Innate immune system-based clearance of *Leishmania* requires intracellular killing via the production of oxygen radicals within infected macrophage phagolysosomes. Critical innate responses are mediated through the detection of parasite surface molecules, or pathogen-associated molecular patterns (PAMPs), detected by pattern-recognition receptors (PRRs), including Toll-like receptors, NOD-like receptors, and others [54]. PAMPs are key components in many current *Leishmania* vaccines as antigens, adjuvants, or both. This response is enhanced by IFN–γ stimulation of phagocytes by natural killer (NK) cells early after *Leishmania* infection and by IFN-γ from $CD4^+$ T cells in more established infections [55]. Defining the elements of cell-mediated immunity, including cytokine production, that provides protective immunity against *Leishmania* spp. in dogs is at least partially understood as requiring high IFN – γ production by $CD4^+$ T cells, with detectable but low levels of IL-10 and IL-4 production by these cells. Establishing how to produce protective immunity through vaccination has been more elusive [53,56]. Vaccination as immunotherapy for CanL has had conflicting results [57,58]. These studies demonstrated success in mounting a balanced Th1-type response from canine cells following TLR agonist supplementation, indicating that vaccination can be successful, but inclusion of a TLR4 agonist within subunit vaccine formulation was not successful in halting disease progression [59,60]. Perhaps more importantly, healthy yet

infected dogs progressing to clinical VL have T-cell exhaustion [46], which interferes with vaccine/immunotherapeutic responses [58]. $CD4^+$ T lymphocytes from dogs with clinical CanL had significantly reduced proliferation and IFNγ-production to vaccine antigens compared to those from healthy dogs. It is important to consider targeted vaccine formulation able to recover exhausted T cell responses before consideration of any vaccine formulation as an immunotherapy vs. prophylaxis for leishmaniosis.

The ability of a vaccine to maintain a low parasite load is essential for limiting transmission from dogs to people as an *L. infantum* reservoir. The cumulative work of many research groups has led to multiple lead vaccine/adjuvant combinations potentially capable of producing a cell-mediated immune response against infection by *Leishmania* [61].

No vaccines for human VL are available at present due to the variability in species of *Leishmania* that affect people, lack of success in producing effective vaccines, and the high cost associated with the development of a product for people without much ability to afford it [62]. In contrast, four commercial vaccine products (Leishmune®; Leish-Tec®; CaniLeish®; Letifend®) have been licensed and marketed for the control of CanL, the first two in Brazil, the others in Europe. Leishmune® was withdrawn from the market under the decision of the Brazilian health authorities and therefore will not be discussed in depth.

The three currently licensed anti-*Leishmania* vaccines for veterinary use include a recombinant single-protein antigen (Leish-Tec®), a secreted/excreted antigen (Canileish®), and a recombinant polypeptide antigen (Letifend®). All *Leishmania* vaccine antigens and adjuvants tested in dogs under field conditions with natural exposure to infected sand flies with published efficacy are provided (Table 5). This list demonstrates that few trials for CanL vaccines have been performed under field conditions. Experimental challenge does not reproduce the course of natural infection. This is probably due to differences when using an injection of cultured promastigotes, instead of repetitive small-volume infectious bites, which includes immunomodulatory sand fly salivary antigens [63] that may modulate immune responses. Multiple studies in mouse models demonstrated that route, dose, and components of the injection

Table 5. Anti-*Leishmania* Vaccines Tested in Field Trials with Natural Exposure to Vector

Composition		Dogs (*n*) (breed)	Follow up (months)	Country	Vaccine efficacy (VE)	Primary outcome	Refs
Antigen	Adjuvant						
Fucose-mannose ligand (FML)	QuilA	85 mixed	48	Brazil	80%	Clinical disease	[93]
Chimeric MML	MPL-SE or Adjuprime	45 naïve Beagles	24	Italy	0%	Active infection[a]	[60]
LiESP	MDP	340 mixed	24	France	92%	Clinical disease	[64]
LiESP	QA-21	90 naïve Beagles	24	Italy/Spain	68.4%	Active infection	[66]
Gentamicin-attenuated *Leishmania*	None	103 naïve German Shepherd cross	24	Iran	92%	Clinical disease	[94]
A2	Saponin	847 mixed	18	Brazil	71.4%	Parasite detection	[72]
Q-protein	None	549 kennelled mixed	24	France/ Spain	72%	Clinical disease	[69]

[a]Active infection was defined as the detection of parasite growth in tissue culture from PCR-positive dogs, shortly followed by the elevation of IFAT titers.

greatly influence the immune response to *Leishmania* challenge. These challenges therefore make translating these findings into real life settings difficult. A further limitation to the development of a robust canine vaccine against CanL is insufficient knowledge of adjuvant efficacy and safety in dogs.

Field trials are difficult to perform as there is low compliance from dog owners to use their dogs for experimental purposes. In addition, the legal requirements to euthanize experimentally infected dogs at the end of each trial, particularly in Europe, place a large emotional toll on those performing such trials. Other complicating factors to field vaccine trials are the annual variability in infection rate due to changes in weather, sand fly abundance, and the necessity for a long follow-up to have measurable disease outcomes [56].

A commercially available vaccine based on purified excreted promastigote antigen of *L. infantum* (LiESAp) was employed in a 24-month field trial using muramil dipeptide (MDP) adjuvant [64]. Study results were complicated by a weak transmission season. The definitive commercial vaccine based on the same antigen was registered as Canileish® in several European countries in 2011, with purified extract of *Quillaja saponaria* (QA-21), an adjuvant similar to saponin. The indication of CaniLeish® is for immunization of *Leishmania*-seronegative dogs more than 6 months old inducing immunity lasting at least 1 year [65]. Oliva *et al.* [66] described results of a randomized double-blind, placebo-controlled trial which assessed the field efficacy of Canileish® (Table 5). Ninety naive beagles were exposed to natural *L. infantum* infection during two consecutive transmission seasons in two highly endemic areas in the Mediterranean basin. Studies of experimentally infected beagles demonstrated that, after vaccination, dogs produced a mixed type 1 cell-mediated immune response within 3 weeks of first/prime vaccination [64,67]. Some dogs developed infection despite vaccination and were infectious to sand flies at a decreased rate compared to nonvaccinated dogs [68].

The vaccine registered in Brazil (LeishTec®) is based on a recombinant *Leishmania* amastigote antigen (A2) combined with saponin adjuvant [69]. An experimental trial included 14 dogs (7 controls) experimentally infected with promastigotes after which vaccinated dogs showed partial clinical protection [69]. Additional studies identified a humoral response in vaccinated dogs and lack of interference of vaccine-derived antibodies with quantitative diagnostic [70]. An 18-month field trial performed in *Leishmania*-endemic areas of Brazil included 847 dogs [70]. This study confirmed efficacy of Leishtec® in prevention of disease, similar to other CanL-licensed vaccines against CanL. Further studies are needed to establish the epidemiological role of vaccinated dogs in preventing infection of additional dogs and people, and the true efficacy and safety of vaccines, including LeishTec®, which is hampered by the conflict between canine *Leishmania* vaccination and the evolution of policies regarding culling of dogs with leishmaniosis in Brazil.

In 2016, Letifend® was licensed for veterinary use in Europe (www.ema.europa.eu/). Letifend® contains 'protein Q' antigen, a recombinant protein made fusing parts of the *L. infantum* Lip0, Lip2a, Lip2b, and histone H2A proteins [71]. Unlike other marketed vaccines, this vaccine does not contain an adjuvant. Another difference to previous vaccines is the regimen of vaccination, including a single injection. The efficacy of Letifend® was assessed in a 24-month field study in France and Spain, following 549 seronegative dogs, half of which were vaccinated. At the end of the study there were 8 confirmed cases of leishmaniosis in the vaccinated group and 19 cases in the placebo group, showing efficacy in reducing CanL. Based on the European Union licencing database (EPAR), few side effects occurred; the most common was scratching at the injection site, which resolved within 4 h. A previous study demonstrated that Q protein was able to protect dogs against an experimental infection by *L. infantum*, and that dogs vaccinated with this protein can be diagnostically distinguished from naturally infected dogs [72].

There are very few published data regarding the Phase IV postmarket efficacy of these vaccines. Their safety profiles indicate adverse events of variable grades of severity, which occurred mainly in response to saponin adjuvant. Large-scale, longer-duration, field trials in naturally infected dogs are underway to determine the long-term efficacy, tolerance, and safety of these vaccines, as reported [73]. Current studies sufficiently support the use of vaccination to prevent CanL. However, methodological shortcomings, including low-infection rates leading to low numbers of infected animals and other limitations within these publications, should be considered when assessing their conclusions.

Current CanL vaccines do not prevent establishment of infection. Vaccination therefore possibly maintains subclinical infection in clinically healthy dogs. These infected healthy animals can be a source of infection for dogs and for humans. Both clinically healthy infected dogs and sick dogs have been shown to be infectious to sand fly vectors, but infectiousness is higher from seropositive dogs with overt clinical disease [74]. Vaccines may modulate the outcome of infection and also reduce infectiousness to sand flies. The difficulty of large-scale xenodiagnosis studies has not allowed for determination of a definitive answer regarding the role of vaccinated dog populations in transmission, to date. Due to limitations of currently licensed products, vaccination has to be considered as a part of a comprehensive control program for CanL, in which concurrent use of repellents against sand flies should be used on vaccinated dogs to decrease potential transmission (Table 6).

As anti-*Leishmania* vaccination is not a core vaccination for dogs, the decision to vaccinate should be based upon the following: (i) individual benefit/risk to the dog; (ii) age, breed, life-style or use, habitat, reproductive status; and (iii) owner compliance. These factors will determine whether vaccine use is appropriate. If vaccination is elected, quantitative serology (immunofluorescence antibody test [IFAT], enzyme-linked immunosorbent assay [ELISA]) must be negative prior to vaccination based on the manufacturer's recommendations. A negative qualitative rapid test requires confirmation by a quantitative method [40].

Other Measures for Control of CanL

Euthanasia

There is no justification for mandated euthanasia of infected and/or sick dogs [34]. In addition to being ethically questionable, several studies have demonstrated its lack of efficacy. In China and Brazil, massive canine culling failed to alter the incidence of human VL [75]. In Brazil, indiscriminate culling of dogs has not led to a reduction in the incidence of human VL. Brazilians, like people everywhere, are motivated to protect and keep their pet dogs healthy, as supported by numerous reports [34,75,76]. A study of Brazilian dog owners indicated that they are attached to their pets, and are motivated to spend a substantial portion of their earnings for topical insecticides instead of culling [77]. Via Brasileish, veterinarians and scientists from across Brazil are pressuring authorities to stop dog culling in Brazil [78]. In China, this measure is currently not enforced, and it is no longer mandated to sacrifice sick dogs, although cases do have to be reported and treatment controlled by a veterinarian [79]. The WHO recommends obligatory euthanasia for stray or wild dogs, and veterinary treatment and follow-up of owned dogs was found to be positive for *L. infantum* [39]. In many environments, the dog is not the only species that serves as a potential reservoir for *L. infantum* [80,81]. Euthanasia of infected dogs does not result in efficient control of the source of human VL.

Leishmaniosis Prevention by Restricting Breeding of Infected Dogs and Other Routes of Transmission

Previous studies have underscored that infected dams, whether clinical or without clinical signs of CanL, transmit *L. infantum* to their offspring [82–84]. Transmission appears to be primarily transplacental, and not transmammary or transvaginal [83]. The likelihood of infection appears

Table 6. Preventative Recommendations Based on Risk of *L. infantum* Infection.

Geographic area	Clinical status	Different scenarios	Travel history	Lifestyle	Preventative applications	Additional recommendations
Nonendemic areas	Any	0	Local (negligible)	Any	None	Avoid breeding with, or blood transfusion from dogs belonging to scenarios 3–5 (and 1–2, if possible)
		1	Occasional travel to endemic fringe or endemic areas	Any	Repellents: Cover the entire period of travelling/exposure including the delay for activity	See scenario 0 If travel once and less than 3 weeks, topical insecticide spot-on formulations applied at least 2 days before travelling/ exposure. For longer periods of travel, repeated spot on or collars. Test for *L. infantum* infection (6 months post- travel, via quantitative serology)
		2	Frequent (or long) travel to endemic fringe/ endemic areas	Breeding, frequently outdoors	Repellents: cover the period of travelling including the delay for activity Vaccination[c] (optional)	See scenario 0 If long or frequent trips preventative measures should be the same as for Scenario 4 Test for *L. infantum* infection (6 months post last travel, via quantitative serology)
		3	Re-homing from an endemic area	Any	None	Test for *L. infantum* infection via quantitative serology If positive, do not breed, consider treatment (staging); ectoparasite control Testing of other household dogs
Endemic and fringe areas	Any Seronegative	4	Outdoors		Repellents all year round or during the known sand flies season. Vaccination[c] (optimal)	Domperidone could be considered (if not vaccinated) Periodic testing if breeding or blood donor
		5	Indoors		Repellents as in 4. Vaccination[c] (optional)	Domperidone could be considered (if not vaccinated) Periodic testing if breeding or blood donor
	Seropositive Healthy[a]	6a	Any		Repellents all year round	Do not use for breeding or as blood donor Periodic check Test other household dogs.
	Seropositive Sick[b]	6b				Do not use for breeding or blood transfusion to other dogs. Staging Treatment as needed. Test other household dogs.

[a]Healthy: a dog without any clinical signs or clinicopathological abnormalities.
[b]Sick: a dog with clinical and/or clinicopathological abnormalities.
[c]Vaccination recommended for the following dogs. Age: the best candidate would be a young dog living outdoors. Breed: vaccination on most susceptible breeds appears at first as a factor for choosing vaccination, although no information is available on the specific efficacy of vaccines in these breeds as compared to other breeds. Travelling, rehoming, sale, transfer of infected dogs or their infectious reproductive materials for breeding should not be allowed from endemic into nonendemic areas.

to be mediated by the health status of the bitch at the time of pregnancy, but further studies are needed to determine the relative risk of transmission from an infected bitch to its pups based on its clinical status and current treatments. In addition, there is evidence of sexual transmission from sire to dam and subsequent transmission to the resultant puppies [85]. Based on these

findings, the recommended practice is to test any dog that is to be bred prior to breeding, using an accurate quantitative serological test [42], and not to breed any dog that is test positive. Vertical or sexual transmission is probably not rare, but as there is no marker to separate vertical from vector-borne infection, it is impossible to differentiate transmission routes in endemic areas. Nonvectorial routes of transmission are of major importance in nonendemic areas and in kennels where vectorial transmission is absent or limited [83,85]. To date, the efficacy of treating prior to, or during, pregnancy or breeding to prevent vertical *Leishmania* transmission has not been scientifically evaluated. Similarly, the ability of vaccination to prevent vertical transmission is not known. Studies are underway to understand how to prevent this means of *L. infantum* transmission.

Finally, the control of transmission by blood transfusion from dog donors is practiced by rigorously testing of donors prior to blood use. This is another way to avoid the dissemination of *L. infantum* [42].

Concluding Remarks

Given the sufficient tolerance and safety margins of available insecticides and vaccines, specific recommendations can be made to optimise control for desired repellent effect as appropriate in local settings when evaluating the best course for *Leishmania* control of dogs. To meet current needs in preventing the spread of leishmaniosis, future research into the control of CanL should include: (i) discovery and evaluation of new topical insecticides with demonstrated ability to reduce CanL transmission; (ii) development of better vaccines with a good safety profile to better protect dogs over longer periods of time; (iii) xenodiagnosis vector infection rate studies, to establish comparative effectiveness of all the active ingredients of insecticides and chemotherapies used to treat dogs as well for vaccines (see Outstanding Questions). Implementation of these future findings related to the impact of infection control measures on transmission will aid in determining the best practices to decrease transmission between dogs and their human counterparts. No control measure should be considered as an isolated action but instead should form part of a comprehensive prevention program for leishmaniosis in dogs living in, or travelling to, an endemic area, whether they are healthy, infected but clinically healthy, or infected and sick.

Outstanding Questions

What is the ability of natural compounds to provide long-term repellency and insecticidal efficacy against sand flies?

What is the effect of resistance to additional drugs in the current CanL repertoire?

Based on current technologies, what might be a simple test that can act as an alternative to xenodiagnosis to assess the infectiousness of dogs and other infected animals?

What is the role of immunomodulators in treatment regimens for leishmaniosis, and can they be effective as solo therapy?

How can additional vaccine targets be identified that prevent *Leishmania* infection and/or block transmission, as these would be great tools for veterinary and public health?

What are model systems that avoid the use of animals or humans for assessment of vaccine efficacy to reduce the ethical burden of this research?

How can parasite nonvectorial transmission be optimally evaluated in both nonendemic and endemic areas?

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