Canine leishmaniosi...that is potentially fatal to humans and dogs. Infection with Leishmania infantum is considerably more prevalent than clinical disease, and infected dogs with no signs of disease might, potentially, transmit infection. Diagnosis of asymptomatic infection by serology is insufficient and PCR markedly increases its sensitivity. A new therapy exclusively for canine leishmaniosi...to a sensitive and specific technique for the diagnosis of overt CanL. However, its sensitivity is significantly lower (<30%) in asymptomatic canine infections [12]. Histopathology of tissues with the use of immunohistochemistry (Figure 1) is useful in increasing the sensitivity of detection when a low parasite load is present [13,14].

PCR assays have greatly improved the sensitivity of the parasitological diagnosis of Leishmania infection in dogs. Various canine tissues (including blood, lymph node, bone marrow and spleen) have been used for PCR detection of the parasite. The sensitivity of the PCR protocol is correlated with the number of copies that the amplified DNA region has in the Leishmania parasite. PCR that targets genomic DNA of the internal transcribed spacer 1 (ITS1) region of the ribosomal RNA (rRNA) genes was shown to be highly sensitive in detecting L. infantum DNA in samples from spleen and lymph node aspirates from seropositive dogs or when DNA was purified from conjunctival swabs taken non-invasively. By contrast, sampling blood or the buffy coat (the concentrated white-cell layer from centrifuged blood) from the same dogs was positive in only 17% and 57% of the samples, respectively [6]. The rRNA gene is present in 40–200 copies in Leishmania parasites; by contrast, the kinetoplast DNA (kDNA) minicircle sequence, which is also used for PCR, is present in ~10 000 copies per parasite. PCR based on kDNA has proven more sensitive than genomic DNA for Leishmania detection in canine blood [15,16]. Quantitative real-time PCR is an advanced technique that can detect extremely low levels of parasites.
A study on naturally infected dogs indicated that kDNA real-time PCR of blood or bone marrow is considerably more sensitive than conventional PCR [17,18]. Although conventional PCR of the bone marrow could only detect dogs with a parasite load of >30 parasites per ml, real-time PCR of the same samples detected additional positive dogs with <1 parasite per ml [17].

A serological diagnosis of CanL by detection of antibodies against *Leishmania* is carried out using several methods, including the immunofluorescence antibody test, ELISA, direct agglutination test, western blot and immunochromatography with rapid ‘in-house’ devices [5,9,19]. Cross-reactions with other pathogens, especially *Trypanosoma cruzi* in Central and South America, have been noted, mostly with those tests that use crude *Leishmania* antigens [20]. To overcome this limitation, recombinant polypeptides containing specific epitopes, such as recombinant K39 (rK39), have been adapted for the diagnosis of CanL [20,21]. In a comparison of methods with different antigen and conjugate combinations, rK39 ELISA was highly specific (96%–100%) but lacked sensitivity...
(29%–65%) in the detection of asymptomatic infected dogs [22]. Immunochromatographic qualitative assays based on recombinant or purified antigens have been developed for rapid serological diagnosis, and commercial kits are available but their performance is still not optimal [22].

CanL is a disease in which infection does not necessarily result in clinical illness. Studies carried out in some endemic areas have shown that the majority of the population harbors the parasite (as evidenced by PCR), whereas a much smaller proportion displays clinical signs of disease [2]. Although seropositivity is found in 88%–100% of the symptomatic dogs, it is evident in only 30%–66% of the asymptomatically infected ones [2,20].

Diagnosis is usually carried out for two main reasons: (i) to confirm ‘disease’, (e.g. to find out whether a dog that shows clinical signs that are consistent with CanL has the disease); or (ii) to investigate the presence of ‘infection’ for epidemiological studies, for the prevention of transmission from asymptomatic carriers by blood transfusion, to avoid the importation of infected dogs to non-endemic countries (as required by some governments) or to monitor response to treatment. The ‘disease’ can be investigated in a symptomatic dog by quantitative serological techniques because most symptomatic dogs have high anti-leishmanial-antibody titers [20]. PCR is a possible addition to serology and could be useful if titers are inconclusive or in regions where positive serology might be due to cross-reactivity with trypanosomes. Investigation for ‘infection’ should be handled differently because asymptomatic dogs are often seronegative and have a low parasite load, which might not be detected by some less-sensitive PCR techniques such as those targeting genomic DNA that have a threshold of 2–5 parasites per ml of blood [15] (compared with real-time kDNA PCR that can detect ≤1 per ml of blood [17]). It is, thus, recommended that kDNA PCR is performed from a lymph node, bone marrow and/or spleen and, in addition, that quantitative serology is carried out. Confirmation of a negative status could require repeated testing after three months [23].

Update on therapy

Although important breakthroughs have been made in the diagnosis and control of CanL in recent years, progress in treatment of the disease still lags behind. Several drugs used for therapy of the disease are able to improve clinical treatment of the disease still lags behind. Several drugs have been evaluated for CanL therapy, but these have mostly been suggested as additions to be administered in combination with the core drugs or as a second-line therapy for dogs that do not respond well to other medicine (Box 1). Advances in the diagnosis of the disease and chronic renal failure caused by CanL have enabled the early management of renal failure and improvement in the rate of recovery from clinical disease [25]; however, treated dogs continue to harbor infection and be infectious to sand flies [18,26–28].

Box 1. Drugs used in CanL therapy

First-line drugs
- Meglumine antimoniate [24,28].
- Aminosidine [24].
- Meglumine antimoniate in combination with allopurinol [24].
- Amphotericin B [24].

Second-line drugs
- Aminosidine [24].
- Pentamidine [24].
- Metronidazole in combination with enrofloxacin [29].
- Metronidazole in combination with spiramycin [30].
- Miltefosine.
- Ketoconazole [24].

The most commonly used drugs for the treatment of CanL are the pentavalent antimony meglumine antimoniate, which selectively inhibits leishmanial glycosylation and fatty acid oxidation, and allopurinol, which inhibits protein translation by interfering with RNA synthesis. These two medications are frequently used in combination. Studies on treatments with each of these drugs, alone or in combination, have demonstrated that most treated animals might relapse back to a clinical disease [18,26–28]. Amphotericin B, which acts by binding to ergosterol in the parasite’s cell membrane and altering its permeability, is also used, but it is nephrotoxic and might endanger dogs with CanL that already have existing renal pathology. Other drugs reported to have some efficacy against CanL include pentamidine, miltefosine, aminosidine (paramomycin), ketoconazole, metronidazole with spiramycin, and metronidazole with enrofloxacin [24,29,30]. These are currently considered to be second-line drugs, and more extensive clinical studies are necessary to verify their therapeutic effectiveness.

The zoonotic potential of CanL, lack of a parasitological cure, and the reported occurrence of parasitic resistance to pentavalent antimonials, amphotericin B, aminosidine and miltefosine [31] indicate that it would be best to avoid or minimize the use of the same drugs for therapy of CanL and human leishmaniosis. New drugs that belong to different classes should be developed to achieve a clinical cure or remission, in addition to eliminating the infection.

Vaccine development

Effective vaccination against CanL can prevent the disease in dogs and constitutes a major strategy for decreasing the threat of infection to humans. After decades of attempting to produce safe and effective vaccines with limited success, newly developed vaccines are currently in progressive stages of clinical field trials, and one brand is licensed for commercial use in Brazil [32–34]. The adjuvant added to the Leishmania vaccine plays a major part in enticing and corroborating the response to the antigen and its recognition by the immune system. Thus, the choice of adjuvant is extremely important in vaccination against CanL.

Box 2. Classes of candidate vaccines against canine leishmaniosis

- Killed Leishmania [35–38].
- Purified Leishmania fractions [32–34,39–44].
- Recombinant antigens [46].
- DNA vaccines [46–48].

Four classes of candidate Leishmania vaccines have been evaluated in dogs (Box 2). Killed Leishmania vaccines consist of inactivated promastigotes [35–38]. A clinical trial using merthiolate sound-disrupted promastigotes of Leishmania braziliensis with bacilli Calmette-Guerin (BCG) as adjuvant showed good protection against an intravenous L. infantum inoculation [35]. Alum-precipitated Leishmania major with BCG tested in dogs followed serologically for 16 months showed a 69% reduction in the seroconversion rate of vaccinated dogs compared with the controls [37].

Purified Leishmania fractions vaccines include components of whole cultured parasites or their excretory–secretory (ES) macromolecules. The main representative of this group of antigens is the glycoprotein GP63-enriched fraction, also known as the ‘fucose mannose ligand’ (FML), of Leishmania donovani. An FML-based vaccine was evaluated in Brazil in three field studies [32,39,40] of 24, 48 and 11 months, respectively. It showed an 80% vaccine efficacy rate [32] and was licensed in Brazil as the first commercial vaccine for CanL (Leishmune®). The FML vaccine has also been proposed for immune therapy of infected dogs and as a transmission-blocking vaccine [40–42]. However, separating naturally infected and FML-vaccinated dogs is difficult and has led to reluctance to use this vaccine among some veterinarians in Brazil, where seropositive dogs are culled [43]. In addition, for further critical evaluation of this vaccine, data are needed on the direct effects of the FML vaccine on reducing the infectiousness of vaccinated dogs to sand flies and on reducing disease in human populations in regions where dogs were vaccinated. A second vaccine based on purified parasite fractions has been studied in France, where good protection against L. infantum experimental infection has been obtained. This vaccine contains an ES antigen purified from defined-medium culture supernatant of L. infantum promastigotes (LiESAp) with muramyl dipeptide as adjuvant [34]. Evaluation of the LiESAp vaccine for prevention of natural infection in a double-blinded field trial has indicated a high efficacy rate [44].

Recombinant Leishmania antigens consist of immunogenic proteins derived from cloned Leishmania genes and purified from transfected expression vectors. A vaccine based on a multisubunit recombinant Leishmania polypeptide known as Leish-111f failed to prevent infection and progression of disease in vaccinated dogs [45].

DNA vaccines against CanL comprise an alternative to vaccination with protein antigens. A vaccination trial was carried out in two groups of dogs using a plasmid carrying the gene for LACK antigen (DNA-LACK), or DNA-LACK followed by a booster of recombinant vaccinia virus containing the same gene. A 60% protection of dogs was obtained in the second group after 17 months of follow-up [46]. A second study employed a combination of DNA and protein immunization with L. infantum cysteine proteinases type I (CPB) and type II (CPA). After challenge and 12 months of follow-up, the bone marrow of ten vaccinated dogs was negative for Leishmania amastigotes [47]. An additional study with multiantigenic plasmid DNA encoding four proteins failed to show protection against L. infantum experimental challenge [48].

Although progress has been made in CanL vaccination in the past decade, the challenge of producing optimal vaccines against this disease has not been met and more research is needed for identifying vaccine targets and immune-stimulating yet safe adjuvants.

Prevention and control of vectors

The prevention of sand-fly bites breaks the transmission cycle of Leishmania and prevents infection from taking place. Several chemical compounds have been shown to have a repelling or insecticidal effect on sand flies with a variable degree of efficacy [49–53], which depends on factors such as the insecticide mode of action, ability to spread and remain sustained in the skin and the specific susceptibility of the biting sand-fly species. The pyrethroids are currently the most widely used insecticides because of their effectiveness against sand flies and low toxicity to the canine host.

Dog collars impregnated with deltamethrin have been shown to possess repellent and insecticidal effects against sand flies; the effects of the collars last for more than six months [49]. Deltamethrin collars are effective against a variety of sand-fly species in different environments in Europe, Asia and South America [54–57]. A matched-cluster randomized trial from Iran indicated that collaring of dogs in intervention and control villages significantly reduced the occurrence of seroconversion in dogs and in children living in the intervention villages [56]. This study proved that protecting a major proportion of the canine population in an endemic focus with an efficient insecticide is effective in reducing infection in humans.

Topical application of spot-on and spray formulations of other pyrethroids have also been reported to be effective against sand flies but for shorter durations than collars [50]. The spray combination of permethrin and pyriproxifen has immediate repellent activity and protects for >21 days [51], and the spot-on combination of permethrin and imidacloprid has a repellent effect that starts 24 hours after application and lasts at least 21 days [52,53]. The permethrin–imidacloprid spot-on combination was successful in protecting kennelled dogs in southern Italy from CanL when applied every two weeks [52]. Protection with topical insecticides is, therefore, a valuable tool that could be integrated successfully in control programs for CanL and used in addition to vaccination.

The periodic use of drugs against leishmaniosis for prevention of transmission has not proven successful. Administration of allopurinol daily for one week every month to dogs in a controlled field study in an endemic area in Greece did not prevent either new infection with L. infantum or the progression of existing infection to becoming symptomatic in other dogs [58]. Other trials that evaluated the infectiousness of dogs with CanL to sand flies after treatment have found that, although infectious-
ness can decrease temporarily, it is eventually resumed [27]. Additional control measures against leishmaniosis aimed at vectors in kennels and homes include spraying, protective windows, door and kennel nets, and curtains treated with residual pyrethroids [59].

Update on public health issues
Human visceral leishmaniosis caused by L. infantum is a potentially fatal zoonotic disease that affects mainly young children and adults suffering from AIDS [60]. Transmission of L. infantum from dogs or wildlife animal reservoirs via sand flies is the main route for human infection. People in endemic regions share the same habitat and are frequently in close physical contact with infected dogs, whether they have a symptomatic disease or, as is more common, harbor infection asymptptomatically. Several studies have investigated the association between canine and human leishmaniosis in the same region and examined to what degree infection in dogs increases the risk of human disease. Multi-level modeling of a disease outbreak, which included >1200 human cases, in the city of Teresina (Brazil) during 1993–1996 revealed that the increasing prevalence of canine infection foreshadowed a rise in the incidence of human disease. Furthermore, poor socioeconomic conditions were found to have an amplifying effect on the association between canine infection and human disease [61]. In the city of Belo Horizonte (Brazil), 4673 seropositive dogs and 64 human patients were detected during one year and analyzed using spatial analysis technology. Of the human cases, 84% were correlated with canine infection, and ~70% of all human and canine cases were detected at high altitudes of 780–880 m above sea level, where a high density of the sand-fly vector Lutzomyia longipalpis was found [62]. In Iran, where the disease is found mostly in rural areas, seropositivity among children was found to increase with village dog density and dog ownership was a significant risk factor for child seropositivity [63].

In southern Europe, where human disease is often sporadic and the ratio between clinically affected humans and infected dogs is low, the ownership of dogs is usually not perceived as being associated with an increased risk of leishmaniosis for humans. Despite this, studies from Europe have established linkage between human and canine infection at the population level. In the Alpujarra region in southern Spain, 33% of the schoolchildren tested by the Leishmanin skin test were positive, indicating exposure to infection, and a close relationship was observed between the canine seroprevalence in different bioclimatic zones and the percentage of schoolchildren who tested positive [64]. Human and canine infections in Greece, like human and canine infections in Spain, also present a related pattern [65].

What methods of intervention should be used for the control of CanL and prevention of human disease? Dog culling is practiced in Brazil, and seropositive dogs are eliminated as part of a control program. Killing infected dogs that are pets would be unacceptable in countries in which the dog is considered a part of the family, and its effectiveness in the control of infection is disputed [66–69]. The main reasons for the failure of dog culling in Brazil in stopping the spread of infection are the high incidence of infection and infectiousness of dogs to sand flies, the presence of wildlife reservoirs, the insensitivity of serological methods to detect infection, the inability to reach and test all the canine population, the time delay between diagnosis and culling, and the rapid replacement of culled dogs by new susceptible pups [67,69]. The future for CanL control should be an integrated approach to prevention. It could include population vaccination against L. infantum with an effective canine vaccine and the use of deltamethrin collars or other long-acting topical insecticide applications. Topical insecticides can prevent new infections and reduce sand-fly feeding on dogs that are already infected, whereas a vaccine would prevent the establishment of infection introduced by bites from those sand flies that have escaped the insecticide effect. This approach would depend on the availability of safe, of efficient and economic vaccines, and of stable insecticides that would remain active for 3–6 months.

Efforts should be made to prevent CanL from entering new countries or regions where conditions for transmission exist. This can be implemented by testing for infection using sensitive methods and preventing the movement of infected animals. Dogs visiting endemic areas should be protected by insecticides or vaccines. Parasitologists, veterinarians, public health officials, environmental agency wardens and dog owners should be kept updated on the risk of zoonotic leishmaniosis. In addition, interdisciplinarily integrated monitoring systems should be maintained to convey information on the disease in a rapid and standard manner [70].

Summary
Recent intensive research on the different aspects of CanL has improved our understanding of the epidemiology, pathogenesis, diagnosis, therapy and prevention of this infection. Despite this, CanL continues to be a challenging zoonosis that is spreading and creating new frontiers. The development of sensitive molecular diagnostic techniques has improved the detection of asymptomatic infection, but optimal treatment of disease and therapeutic elimination of infection have not been accomplished. Furthermore, progress has been made in the prevention of canine infection by reducing sand-fly bites by using topical insecticides and vaccination, but these are not yet practiced on a large scale and have not been included in official control programs to reduce canine and human disease. Future efforts should be aimed at further improving these new advances and applying them.

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