Veterinary Dermatology

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REVIEW OPEN ACCESS

World Association for Veterinary Dermatology Consensus Statement for Diagnosis, and Evidence-Based Clinical Practice Guidelines for Treatment and Prevention of Canine Leishmaniosis

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ABSTRACT

Background: Canine leishmaniosis (CanL) due to *Leishmania infantum* remains common, and veterinarians do not always follow scientifically sound approaches for diagnosis, treatment and prevention.

Objectives: To provide consensus guidelines for diagnosis and evidence-based guidelines for treatment and prevention of CanL.

Methods and Material: Clinical consensus guidelines for the diagnosis were structured based on literature and authors' experience. Three electronic databases were searched for randomised controlled trials, systematic reviews and meta-analyses on treatment and prevention.

Results, Conclusions and Clinical Importance: Diagnosis should be based on compatible clinical signs and/or clinicopathologic abnormalities, exclusion of differentials, demonstration of infection and increased concentration of anti-*Leishmania* IgG (quantitative serology). Euthanasia for public health purposes is not recommended and drugs with anti-*Leishmania* activity should be avoided in subclinically infected dogs. Recommended treatments include meglumine antimoniate-allopurinol (first-line treatment), miltefosine-allopurinol (first-line treatment) and aminosidine-allopurinol (second-line treatment); marbofloxacin may be considered in dogs with advanced chronic kidney disease. In endemic areas, recommended measures for prevention include deltamethrin 4% collar, flumethrin 4.5%-imidacloprid 10% collar or permethrin 50%-imidacloprid 10% spot-on, not using infected blood products for transfusion, not breeding seropositive bitches or dogs with CanL, administration of domperidone (seronegative dogs) and dietary nucleotides-active hexose correlated compound (subclinically infected, seropositive dogs).

Previous Presentations: The evidence-based clinical practice guidelines for treatment and prevention of canine leishmaniosis were presented by the last author, in an invited lecture, at the 10th World Congress of Veterinary Dermatology, Boston, MA, USA, July 2024.

Vaccination with *Li*ESP with MDP may be considered, whereas protein Q vaccine is recommended in areas with very high rates of seroconversion. In non-endemic areas, recommended measures include not using infected blood products for transfusion and removal of infected female dogs from reproduction.

ZUSAMMENFASSUNG

Hintergrund: Die canine Leishmaniose (CanL), verursacht durch *Leishmania infantum* bleibt eine häufige Erkrankung. VeterinärmedizinerInnen verfolgen nicht immer wissenschaftlich fundierte Herangehensweisen zur Diagnose, Behandlung und Vermeidung.

Ziele: Eine Erstellung von Konsensus-Empfehlungen für die Diagnose und Evidenz-basierte Richtlinien für die Behandlung und die Vermeidung von CanL.

Methoden und Materialien: Klinische Konsensus-Empfehlungen für die Diagnose wurden basierend auf Literatur und Erfahrung der AutorInnen strukturiert. Drei elektronische Datenbanken wurden durchsucht, um randomisierte kontrollierte Studien, systematische Reviews und eine Metaanalyse zu Behandlung und Vermeidung zu finden.

Ergebnisse, Schlussfolgerungen und klinische Bedeutung: Die Diagnose sollte auf kompatiblen klinischen Zeichen und/oder klinisch-pathologische Veränderungen, Ausschluss von Differentialdiagnosen, Demonstration der Infektion und Zunahme der anti-*Leishmania* IgG (Quantitative Serologie) basieren. Eine Euthanasie zum Zweck der Volksgesundheit wird nicht empfohlen und Medikamente mit anti-*Leishmania* Aktivität sollten bei subklinisch infizierten Hunden vermieden werden. Empfohlene Behandlungen inkludieren Meglumine Antimonate-Allopurinol (Erstlinientherapie), Miltefosine-Allopurinol (Erstlinientherapie) und Aminoside-Allopurinol (Zweitlinientherapie); Marbofloxacin könnte bei Hunden mit fortgeschrittener chronischer Nierenerkrankung eingesetzt werden. In endemischen Gebieten beinhalten die empfohlenen Maßnahmen zur Vermeidung ein Deltamethrin 4%iges Halsband, Flumethrin 4,5%iges—Imidacloprid 10%iges Halsband oder Permethrin 50%-Imidaclorpid 10% Spot-on. Kein Einsatz infizierter Blutprodukte für Transfusionen, keine Zucht mit seropositiven Hündinnen oder Rüden mit CanL, Verabreichung von Domperidone (seronegative Hunde) und diätetische Nukleotid-aktive Hexose korrelierte Mischungen (subklinisch infizierte, seropositive Hunde). Eine Impfung mit *Li*/ESP mit MDP könnte erwogen werden, während Protein Q Vakzine in Gegenden mit sehr vielen Sero-konvertierten Tieren empfohlen wird. In nicht endemischen Gegenden beinhalten die empfohlenen Maßnahmen infizierte Blutprodukte nicht für Transfusionen einzusetzen und infizierte weibliche Hunde aus der Reproduktion zu nehmen.

摘要

背景: 犬利什曼病(Canine leishmaniosis , CanL)由犬利什曼原虫(Leishmania infantum)引起仍然常见 , 并且兽医在诊断、治疗和预防方面并不总是遵循科学合理的方法。

目标: 为CanL的诊断提供共识指南 , 并为治疗和预防提供循证指南。

方法与材料: 诊断的临床共识指南基于文献和作者的经验制定。通过三大电子数据库检索治疗和预防的随机对照试验、系统综述和荟萃分析。

结果、结论及临床重要性: 诊断应基于相符的临床症状和/或临床病理学异常、排除其他鉴别诊断、感染的证据以及抗利什曼原虫IgG浓度升高(定量血清学)。不建议因公共卫生目的实施安乐死 , 并且在亚临床感染犬中应避免使用抗利什曼原虫活性的药物。推荐治疗包括葡萄糖酸锑胺-别嘌呤醇(首选治疗)、米替福新-别嘌呤醇(首选治疗)和阿米诺昔定-别嘌呤醇(二线治疗) ; 对于慢性肾病晚期的犬可考虑使用马波沙星。在流行区 , 推荐的预防措施包括使用4%氯氰菊酯项圈、4.5%氟氰菊酯-10%吡虫啉项圈或50%氯菊酯-10%吡虫啉滴剂 , 不使用受感染的血液制品进行输血 , 不繁育血清学阳性的母犬或患CanL的犬 , 在血清学阴性犬中使用多潘立酮(domperidone) , 在亚临床感染、血清学阳性犬中使用膳食核苷酸-活性多糖复合物(AHCC)。可考虑使用LiESP与MDP疫苗 , 而在血清转换率极高的地区推荐使用Q蛋白疫苗。在非流行区 , 推荐的预防措施包括不使用受感染的血液制品进行输血 , 并移除受感染的母犬以避免繁殖。

RÉSUMÉ

Contexte: La leishmaniose canine (CanL) due à *Leishmania infantum* demeure courante, et les vétérinaires ne suivent pas toujours des approches scientifiquement fondées pour le diagnostic, le traitement et la prévention.

Objectifs: Fournir des lignes directrices consensuelles pour le diagnostic et des lignes directrices fondées sur des preuves pour le traitement et la prévention de la CanL.

Méthodes et matériel: Les lignes directrices cliniques consensuelles pour le diagnostic ont été structurées sur la base de la littérature et de l'expérience des auteurs. Trois bases de données électroniques ont été consultées pour trouver des essais contrôlés randomisés, des revues systématiques et des méta-analyses sur le traitement et la prévention.

Résultats, conclusions et importance clinique: Le diagnostic doit être basé sur des signes cliniques compatibles et/ou des anomalies clinico-pathologiques, l'exclusion des diagnostics différentiels, la mise en évidence de l'infection et une

concentration accrue d'IgG anti-Leishmania (sérologie quantitative). L'euthanasie à des fins de santé publique n'est pas recommandée et les médicaments ayant une activité anti-*Leishmania* doivent être évités chez les chiens infectés de manière subclinique. Les traitements recommandés comprennent l'antimoniate de méglumine-allopurinol (traitement de première intention), la miltéfosine-allopurinol (traitement de première intention) et l'aminosidine-allopurinol (traitement de deuxième intention) ; la marbofloxacine peut être envisagée chez les chiens atteints d'une maladie rénale chronique avancée. Dans les zones endémiques, les mesures de prévention recommandées comprennent le collier à la deltaméthrine 4 %, le collier à la fluméthrine 4,5 %-imidaclopride 10 % ou le spot-on à la perméthrine 50 %-imidaclopride 10 %, l'interdiction d'utiliser des produits sanguins infectés pour les transfusions, l'interdiction d'élever des chiennes séropositives ou des chiens atteints de CanL, l'administration de dompéridone (chiens séronégatifs) et de nucléotides alimentaires – composé corrélé d'hexose actif (chiens infectés de manière subclinique, séropositifs). La vaccination avec LiESP avec MDP peut être envisagée, tandis que le vaccin protéine Q est recommandé dans les zones où les taux de séroconversion sont très élevés. Dans les zones non endémiques, les mesures recommandées comprennent la non-utilisation de produits sanguins infectés pour les transfusions et le retrait des chiennes infectées de la reproduction.

要約

背景: *Leishmania infantum*による犬リーシュマニア症(CanL)は依然として一般的であり、獣医師は診断、治療、予防のために必ずしも科学的に正しいアプローチに従っていない。

目的: 本研究の目的は、CanL の診断に関するコンセンサス・ガイドライン、および治療と予防に関するエビデンスに基づくガイドラインを提供することであった。

材料と方法: 診断に関する臨床的コンセンサスガイドラインは、文献および著者の経験に基づいて構成した。治療および予防に関するランダム化比較試験、システマティックレビュー、メタアナリシスについて3つの電子データベースを検索した。

結果、結論および臨床的重要性: 診断は、適合する臨床症状および/または臨床病理学的異常、鑑別の除外、感染の証明、および抗リーシュマニアIgG濃度の上昇(定量的血清学的検査)に基づくべきである。公衆衛生目的の安楽死は推奨されず、不顕性感染犬では抗リーシュマニア活性を有する薬剤は避けるべきである。推奨される治療法には、メグルミンアンチモニア-アロプリノール(第一選択治療)、ミルテホシン-アロプリノール(第一選択治療)、アミノシジン-アロプリノール(第二選択治療)があり、慢性腎臓病が進行した犬にはマルボフロキサシンが考慮される。流行地域では、デルタメトリン 4%首輪、フルメトリン 4.5%-イミダクロプリド 10%首輪、ペルメトリン 50%-イミダクロプリド 10%スポットオ ン、感染した血液製剤を輸血に使用しない、血清陽性の雌犬や CanL を持つ犬を繁殖させない、ドンペリドンの投与(血清 陰性犬)、食事性ヌクレオチド-活性ヘキソース相関化合物の投与(不顕性感染、血清陽 性犬)などが推奨される。血清転換率が非常に高い地域ではプロテインQワクチンの接種が推奨される。非流行地域では、感染した血液製剤を輸血に使用しないこと、感染した雌犬を繁殖から排除することなどが推奨されている。

RESUMO

Contexto: A leishmaniose canina (LCCan) causada por *Leishmania infantum* permanece comum, e os veterinários nem sempre seguem abordagens cientificamente sólidas para diagnóstico, tratamento e prevenção.

Objetivos: Fornecer diretrizes de consenso para o diagnóstico e diretrizes baseadas em evidências para o tratamento e prevenção da LCan.

Métodos e material: Diretrizes clínicas de consenso para o diagnóstico foram estruturadas com base na literatura e na experiência dos autores. Três bases de dados eletrônicas foram pesquisadas em busca de ensaios clínicos randomizados, revisões sistemáticas e metanálises sobre tratamento e prevenção.

Resultados, conclusões e importância clínica: O diagnóstico deve ser baseado em sinais clínicos compatíveis e/ou anormalidades clinicopatológicas, exclusão de diagnósticos diferenciais, demonstração de infecção e aumento da concentração de IgG anti-*Leishmania* (sorologia quantitativa). A eutanásia para fins de saúde pública não é recomendada e medicamentos com atividade anti-*Leishmania* devem ser evitados em cães com infecção subclínica. Os tratamentos recomendados incluem antimoniato de meglumina-alopurinol (tratamento de primeira linha), miltefosina-alopurinol (tratamento de primeira linha) e aminosidina-alopurinol (tratamento de segunda linha); marbofloxacino pode ser considerado em cães com doença renal crônica avançada. Em áreas endêmicas, as medidas recomendadas para prevenção incluem coleira de deltametrina 4%, coleira de flumetrina 4,5%-imidacloprida 10% ou permetrina 50%-imidacloprida 10% spot-on, não usar produtos sanguíneos infectados para transfusão, não reproduzir cadelas soropositivas ou cães com CanL, administração de domperidona (cães soronegativos) e composto correlacionado com hexose ativa de nucleotídeos na dieta (cães soropositivos infectados subclinicamente). A vacinação com *Li*ESP com MDP pode ser considerada, enquanto a vacina de proteína Q é recomendada em áreas com taxas muito altas de soroconversão. Em áreas não endêmicas, as medidas recomendadas incluem não usar produtos sanguíneos infectados para transfusão e remover cadelas infectadas da reprodução.

RESUMEN

Introducción: La leishmaniosis canina (CanL) causada por *Leishmania infantum* sigue siendo frecuente, y los veterinarios no siempre aplican enfoques científicamente sólidos para su diagnóstico, tratamiento y prevención.

Objetivos: Proporcionar directrices de consenso para el diagnóstico y directrices basadas en la evidencia para el tratamiento y la prevención de la CanL.

Métodos y material: Las directrices de consenso clínico para el diagnóstico se estructuraron en base a la literatura y la experiencia de los autores. Se realizaron búsquedas en tres bases de datos electrónicas para encontrar ensayos clínicos aleatorios, revisiones sistemáticas y metaanálisis sobre tratamiento y prevención.

Resultados, conclusiones e importancia clínica: El diagnóstico debe basarse en signos clínicos compatibles y/o anomalías clinicopatológicas, la exclusión de diagnóstico diferencial, la demostración de infección y el aumento de la concentración de IgG anti-Leishmania (serología cuantitativa). No se recomienda la eutanasia con fines de salud pública y se debe evitar el uso de fármacos con actividad anti-Leishmania en perros con infección subclínica. Los tratamientos recomendados incluyen antimoniato de meglumina-alopurinol (tratamiento de primera línea), miltefosina-alopurinol (tratamiento de primera línea) y aminosidina-alopurinol (tratamiento de segunda línea); se puede considerar la marbofloxacina en perros con enfermedad renal crónica avanzada. En áreas endémicas, las medidas recomendadas para la prevención incluyen collar de deltametrina 4%, collar de flumetrina 4.5%-imidacloprid 10% o permetrina 50%-imidacloprid 10% spot-on, no usar productos sanguíneos infectados para transfusión, no criar perras seropositivas o perros con CanL, administración de domperidona (perros seronegativos) y compuesto dietético correlacionado con hexosa activa de nucleótidos (perros seropositivos con infección subclínica). Se puede considerar la vacunación con LiESP con MDP, mientras que la vacuna de proteína Q se recomienda en áreas con tasas muy altas de seroconversión. En áreas no endémicas, las medidas recomendadas incluyen no usar productos sanguíneos infectados para transfusión y retirar a las perras infectadas de la reproducción.

1 | Introduction

1.1 | Aetiology

The genus *Leishmania* includes kinetoplastid protozoa transmitted by phlebotomine sand fly vectors of the genus *Lutzomyia* (subdivided into different genera in a recent taxonomic revision) and *Phlebotomus*, in the New and Old World, respectively. These protozoa are the causative agents of leishmaniases, which are diseases with different degrees of severity, affecting several animal species and humans, on most continents [1]. *Leishmania infantum* is usually associated with human visceral leishmaniasis (VL) but may also cause cutaneous lesions [cutaneous leishmaniasis (CL)], which are more typical of other dermotropic species of the genus (e.g., *L. major*, *L. tropica*). Furthermore, some of the latter may invade internal organs (e.g., *L. amazonensis*), and some other species can cause mucocutaneous disease (e.g., *L. braziliensis*). These protozoa are included in the subgenera *Leishmania*, *Viannia* and *Mundinia*, according to the localisation of developmental stages in the digestive tract of their sand fly vectors and to their biochemical and molecular characteristics, whereas *Leishmania* species of lizards are included in the subgenus *Sauroleishmania*. The characterisation of *Leishmania* species and strains should rely on the isolation of the parasite in culture followed by multilocus enzyme electrophoresis or another reference method, such as DNA sequencing, and on the molecular characterisation of specific targets [e.g., kinetoplast DNA (kDNA), intergenic transcribed spacer region-1 (ITS-1), heat shock protein 70 (*hsp70*) gene], according to the aims and the required level of parasite identification.

Leishmaniases are listed among the neglected tropical diseases greatly impacting, in terms of morbidity and mortality, human populations worldwide with a yearly burden of approximately 30,000 cases of VL and of more than 1 million cases of CL (World Health Organization website https://www.who.int/health-topics/leishmaniasis#tab=tab_1 last accessed on December 2024). Human VL is mostly prevalent in developing countries with 95% of cases reported in Brazil, Chad, Eritrea, Ethiopia, India, Iraq, Kenya, Nepal, Somalia, South Sudan, Sudan, Uganda and Yemen. Similarly, more than 90% of CL cases are reported in Afghanistan, Algeria, Brazil, Colombia, Iran, Iraq, Pakistan, Peru, Sri Lanka, Syrian Arab Republic and Yemen. Nonetheless, these diseases are often underreported or not diagnosed at all, in remote rural areas of the world, suggesting that the figures above are probably underestimations.

Like in humans, multiple *Leishmania* species infect dogs, potentially leading to diseases with variable clinical and clinicopathological manifestations. Among them, *L. infantum* is the most important one from a global perspective and can cause a disease characterised by both cutaneous and visceral involvement, called canine leishmaniosis (CanL). Unless otherwise stated, the remaining text of this article will be devoted to CanL due to *L. infantum*.

1.2 | Epidemiology

Leishmaniosis caused by *L. infantum* is probably the most important canine vector-borne disease of zoonotic concern, being prevalent all over the world except Oceania [2, 3]. The infection is distributed, in relationship to the presence of sand fly vectors, in Far East Asia, Africa, Middle East, Europe and Central and South America [4]. Genetic studies suggest that *L. infantum* was introduced to the New World by

the Conquistadores via infected dogs [5], and then the protozoon found suitable vertebrate hosts and proper sand fly vectors to perpetuate. In the last decades, the distribution of CanL has expanded in many geographic areas where it was not previously endemic, such as northern Argentina [6] and in northern regions of Italy and Spain [7–9]. The reasons for this expansion are mainly linked to the fact that sand fly vectors are colonising new ecological niches due to global increase in mean temperatures, the human and animal movements and other human activities (e.g., deforestation, urbanisation) [2, 10, 11]. Also, the lack or inefficacy of control programs (e.g., diagnosis, monitoring and use of appropriate repellents in dogs) as well as the occurrence of reservoir animals other than dogs may represent important factors favouring the presence of new foci of infection [12].

New cases of canine infection occur when sand flies are active, that is throughout the year in the New World and from spring to autumn (i.e., from May to October) in the Old World, although there are some endemic areas in Europe, such as Southern Spain, where the transmission season has been expanded to almost 10 months per year, mainly due to climate changes [8]. Sand flies are small, fragile insects that may virtually colonise almost all environments, from forests to human houses and from coastal plains to hilly areas, if proper conditions to complete their biological life cycle (e.g., habitat with organic matter, high humidity) are available [4]. Another important component in the natural transmission chain of *L. infantum* is represented by the presence of potential vertebrate hosts, other than dogs, in different ecotypes [13]. Although dogs are the main peridomestic reservoir of *L. infantum* worldwide, the parasite has been isolated from many other classes of mammals (e.g., rodents, lagomorphs, marsupials, non-human primates and carnivores) [2, 14]. The role played by these animal species as reservoirs of *L. infantum* depends on a complex chain of factors in different ecological contexts.

Dogs and cats have been shown to act as a source of *L. infantum* infection for phlebotomine sand flies, whereas studies about other animal species are scant and their possible role is usually inferred by the delineation of the blood source of infected engorged female sand flies. However, both the black rat (*Rattus rattus*) and the Iberian hare *Lepus granatensis*) have been demonstrated to infect sand flies [15, 16]. A paradigmatic example is represented by the Iberian hare, which has been implicated in the most important outbreak of human leishmaniosis (2009–2012) [17] known in the history of Europe, in people frequenting a suburban park near Madrid, Spain [18]. The prevalence of seropositivity was not increased in dogs from the same area, and conversely, up to 45% of hares sampled were infected by *L. infantum*. In addition, naturally infected hares were infectious to *Phlebotomus perniciosus*, the main vector of *L. infantum* in that area, and this sand fly species showed a high preference to feed from hares under natural conditions. Based on some experiments of sand fly feeding preferences and on the molecular detection of *L. infantum* in red foxes (*Vulpes vulpes*), this animal has also been suggested as a putative reservoir of *L. infantum* [19, 20]. A plethora of other animals such as opossums and wild canids have also been regarded as potential reservoirs of *L. infantum* in Latin America [21, 22]. From a public health perspective, the presence of reservoirs other than dogs could reduce the effectiveness of control programs based on the application of repellents on dogs.

The dynamic of *L. infantum* infection in dogs is multifactorial and it is linked to the vectors (e.g., species composition, density, host preference, length of transmission season) and the presence and availability of infected reservoir hosts, primarily other dogs [23]. In endemic areas, dogs may remain subclinically infected (i.e., not presenting clinical signs or clinicopathologic abnormalities of CanL) for all their life [23], but their infectivity increases when they develop CanL [24].

2 | Pathogenesis and Immunology of Canine Leishmaniosis

In the vertebrate host, amastigote (non-flagellated) forms of *Leishmania* spp. replicate inside macrophages, which are ingested by the sand fly vectors along with their blood meal. Subsequently, they transform to promastigotes and replicate in the gut of the insect until the flagellated metacyclic promastigotes are inoculated to a new receptive host [4]. However, blood transfusion, direct and vertical or venereal transmissions have also been demonstrated as alternative modes of infection, that are particularly important in areas where suitable phlebotomine sand fly vectors are not present [25, 26]. Up to now, natural transmission of *L. infantum* through insects other than sand flies or through arachnids, like ticks, has been speculated but not proven.

Infection is initiated when the female sand fly introduces the metacyclic promastigotes into the superficial dermis of the dog. The promastigotes are lodged in the sand fly's intestinal tract, together with saliva, intestinal microbiota and other products such as promastigote secretory gel (PSG), each of which play a determinative role in the establishment of infection. For example, saliva acts by inhibiting haemostasis, whereas the bacteria of sand fly intestine and PSG act as pro-inflammatory factors [27]. In the next few hours, an acute inflammatory response is triggered at the inoculation site, and neutrophils are the first cells to arrive, attracted by cytokines and chemokines such as IL-1β and CXCL1. After neutrophils, the next cells to become infected are the resident and inflammatory dermal macrophages. Notably, apoptotic parasitised neutrophils are phagocytosed by macrophages, contributing to their high infection rates, in a 'Trojan Horse' model of infection [27]. Then, infected macrophages migrate to the regional lymph nodes, where they initiate the adaptive immune response, and they enter the blood stream (parasitemia) circulating and homing to different internal organs [28]. Bone marrow, spleen, liver and lymph nodes are usually among the first, although, as the disease progresses, most, if not all, parenchymal organs may become infected. In dogs, *L. infantum* has a marked dermotropism and the skin is one of the main target organs. Therefore, excepting the site of inoculation, numerous additional areas of skin infection develop after haematogenous spread, with a multifocal or generalised distribution. The dissemination of the infection to the skin is essential for the transmission of parasites to the sand fly vector and is responsible for most of the skin lesions [29].

The immune responses mounted by the canine host play an important role in the susceptibility to *L. infantum* and development of disease [30]. Interferon-γ (INF-γ) activates macrophages to kill intracellular amastigotes through the production of reactive oxygen species, and this has been shown to be a protective cell-mediated immune pathway which enables the control of infection. Conversely, immune responses that induce the secretion of interleukin-4 and the evolution of B-cells into plasma cells with increased immunoglobulin production are linked to uncontrolled infection and progression to CanL. Research in rodents infected by dermotropic *Leishmania* species, such as *L. major*, has shown that the T-helper 1 (Th1) type of immune response with its associated cytokine cascade led to parasite elimination by activated macrophages and resistance. On the contrary, the T-helper 2 (Th2) immune response led to increased parasite load and production of non-protective anti-*Leishmania* antibodies and finally to disease. Dogs usually develop mixed Th1/Th2 responses, and the balance between them determines the course of infection [28, 30].

The progression from infection to CanL is marked by a depressed cell-mediated immunity and an extreme upregulation of humoral response. In chronic CanL, dogs increasingly express the programmed death-1 (PD-1) cell-surface receptor on their lymphocytes (T-cell exhaustion) and experience diminished lymphocyte proliferation responses upon stimulation, initially with *L. infantum* antigen (parasite-specific suppression of cell-mediated immunity) and later with irrelevant mitogens (generalised suppression of cell-mediated immunity) [28, 31, 32]. At the same time, circulating immune complexes are formed and their deposition in special vascular plexuses mediate some important pathological manifestations of CanL [33], such as glomerulonephritis, uveitis, arthritis and vasculitis [28, 34–36]. In addition, the presence of parasites triggers macrophagic and lymphoplasmacytic inflammation in multiple organs.

Susceptibility or resistance to CanL are also influenced by the dog's genetic makeup. Severe CanL is rare among Ibizan hounds in the Balearic islands of Spain and its prevalence is significantly less common compared to other canine breeds in the same *L. infantum*-endemic islands [37]. It has been shown that the Ibizan hound produces a predominantly cellular response against *L. infantum* while other breeds, that evolved in non-endemic areas, such as the Boxer, Rottweiler and German shepherd dogs, are more susceptible and are overrepresented in CanL surveys [38, 39].

3 | Non-Cutaneous Manifestations of Canine Leishmaniosis

The clinical manifestations of CanL are broad and variable among dogs, mainly due to the differences in their immune responses and the multiplicity of pathogenic mechanisms [28]. In general, CanL is a chronic, multisystemic disease that may affect almost every system and organ, with severity varying from mild and self-limiting to fatal [30].

3.1 | History and Common Clinical Manifestations

The typical history reported by owners of dogs with CanL includes the appearance of skin lesions, ocular abnormalities, weight loss, lethargy, exercise intolerance, lameness and epistaxis. Dogs with chronic kidney disease (CKD) or other internal organ involvement (e.g., liver, gastrointestinal, respiratory system) may be admitted due to additional clinical signs such as polyuria/polydipsia (PU/PD), anorexia, vomiting, diarrhoea, melena or sneezing [40].

On physical examination, the main non-cutaneous findings associated with CanL are peripheral lymphadenomegaly, pale mucous membranes, splenomegaly, ocular lesions, poor body condition, muscle atrophy involving mainly the masticatory muscles, rhinitis and joint swelling [30, 41]. In particular, the prevalence of ocular lesions such as keratoconjunctivitis and uveitis varies from 12% to 71% in different canine populations and studies [42–44].

When muscle atrophy affects mainly the temporal muscles it is attributed to chronic masticatory muscle myositis, whereas it is generalised in cachectic animals [41, 45]. Polymyositis has also been described is some cases [46].

Gastrointestinal manifestations may appear in conjunction with other clinical signs of CanL and more rarely as the only clinical presentation, especially in certain breeds such as the Boxer and German shepherd dog [47]. They include small intestinal diarrhoea, with or without melena and clinical signs due to ulcerative granulomatous colitis and/or lymphoplasmacytic enteritis [48, 49]. Ascites and vomiting due to liver disease are rare [40].

Central nervous system (CNS) inflammation, usually manifested by clinical signs of encephalitis, has been described in CanL [50, 51]. Some inflammatory lesions are characterised by the presence of abundant T lymphocytes and mononuclear cells and may be due to co-infections with pathogens such as *Toxoplasma gondii* and *Neospora caninum* [52, 53]. In other cases, damage of CNS vascular bed leads to infarctions [54]. Blood–brain barrier compromise has been demonstrated and may explain high levels of anti-*L. infantum* IgG in the cerebrospinal fluid [55, 56]. However, *L. infantum* has also been detected in the CNS of dogs with CanL but no neurological signs, which implies that the mere presence of the parasite does not necessarily mean that it is responsible for these signs [57, 58].

The respiratory system may also be affected. Chronic rhinitis is common [59], whereas chronic interstitial pneumonia has been detected histopathologically but without associated clinical signs [60]. Although rare, some dogs with CanL and heart disease have been described, with evidence of myocarditis and local presence of the parasite [61, 62]. The disease may also affect the male and female reproductive system. Males may present low semen quality with reduced progressive motility and increased number of spermatozoa with morphological abnormalities. Semen quality appears to be partially restored after long-term allopurinol administration [63]. In females with CanL placentitis due to *L. infantum* has been described after abortion [64].

3.2 | Clinicopathologic Abnormalities

About 63% of the dogs admitted with CanL are anaemic, usually with mild-to-moderate non-regenerative anaemia, and 25% have

lymphopenia [36]. Normocytic-normochromic non-regenerative anaemia develops as in other chronic debilitating inflammatory diseases which affect haematopoiesis and worsens when CKD develops. Haemorrhage and haemolysis may contribute to anaemia in some dogs. Mild to moderate thrombocytopenia is common. Anti-platelet antibodies are present in some dogs with CanL, opsonizing the thrombocytes and decreasing their lifespan in the circulation. In addition, the decreased clotting capacity of platelets (thrombocytopathy), along with vasculitis and serum hyperviscosity, can result in bleeding tendency, which, in combination with ulcerative rhinitis, explains why epistaxis may occur [65, 66].

The most common serum biochemical findings are hyperproteinaemia, hyperglobulinaemia (mainly due to the increased antibody production) and hypoalbuminaemia (due to glomerular loss and inflammation) [30, 36, 67]. Also, positive acute phase proteins, such as C-reactive protein (CRP) and ferritin, are increased whereas not only albumins but also paraoxonase 1 (PON1), another negative acute phase protein, is decreased [68]. Exceptionally elevated activities of liver enzymes are found in a minority of dogs, whereas azotemia, proteinuria [increased urine protein/creatinine ratio (UPC)] and low urine specific gravity may be found in cases with CKD, mainly due to immune complex deposition on the glomeruli [34]. Proteinuria, which may initially be reversible, is often present long before CKD deteriorates enough to result in increased blood creatinine, symmetric dimethylarginine (SDMA), urea and inorganic phosphorous concentrations, at which stage the prognosis declines. Indeed, CKD is the primary cause of natural death in CanL [23, 69].

4 | Cutaneous Manifestations of Canine Leishmaniosis

Although CanL is a systemic disease, it commonly causes skin lesions which are reported in 56%–90% of the cases, and in some dogs they can be the only abnormalities observed [70–72]. The skin lesions in CanL are characterised by extreme pleomorphism, variable severity and are uncommonly associated with pruritus. The clinical pleomorphism is reflected in the histopathological features as well. It is partially unknown why the disease can have so many different cutaneous presentations, however the interaction between the host immune system and the parasite is suspected to play a role in this [70–72].

Cutaneous manifestations of CanL can be divided into typical and atypical. Typical clinical patterns are highly suggestive of CanL, and include exfoliative (scaling) dermatitis, ulcerative dermatitis affecting bony prominences, papular dermatitis and onychogryphosis. Atypical clinical patterns are less specific and less suggestive of CanL and may mimic many other diseases. These include pustular dermatitis, nodular dermatitis, ulcerative dermatitis other than ulcers on bony prominences and footpad and/or nasal hyperkeratosis [71, 72]. Affected dogs may present with a single clinical pattern or more than one. Clinical patterns of CanL are the same in endemic and non-endemic areas, however prevalence of the different presentations may vary [73].

Differential diagnoses for each clinical pattern and useful clinical hints ('clinical pearls') to help the clinician are summarised in Table 1. Clinical pearls are defined as practical medical tips based on experience and personal observations [74]. The most important determinant of validity of a clinical pearl is the number of observations: the more numerous the observations, the greater their diagnostic value. However, considering that most pearls are personal opinions and not evidence-based data, they should be used with caution.

4.1 | Exfoliative Dermatitis

Exfoliative (scaling) dermatitis (Figure 1) is the most common dermatological presentation of CanL, and its prevalence has been reported to be between 45.7% and 98.7% [70]. It is characterised by large, dry, whitish scales, often described as asbestos-like, variably adherent to the underlying skin. When scales are very adherent, they may be more easily palpated than seen, and their removal can leave an erosion. Along with the scales, follicular casts, partial alopecia or both may be present. Pruritus is usually absent unless there is secondary bacterial infection. Lesions may initially involve the face and ear pinnae, with a symmetrical distribution around the eyes and then extend to the hairy surfaces of the trunk and limbs. Distribution may be localised, regional or generalised and symmetrical or asymmetrical [70–72].

Histopathologically, this pattern is characterised by epidermal and follicular orthokeratotic hyperkeratosis and a perivascular to interstitial infiltrate in the superficial and mid dermis (Figure 2a), with or without inflammation and destruction of the sebaceous glands (sebaceous adenitis). The dermal infiltrate may also be more intense, from nodular to diffuse and may involve the panniculus (pyogranulomatous to granulomatous panniculitis). The predominant inflammatory cells are macrophages, plasma cells and lymphocytes; less commonly neutrophils, eosinophils and mast cells are found, whereas multinucleated giant cells are rare. Variable numbers of amastigotes may be identified in biopsies of dogs presenting with exfoliative dermatitis [75, 76].

Idiopathic sebaceous adenitis is the main differential when follicular casts are observed during clinical examination. Histopathologically, involvement of the sebaceous glands in the inflammatory process can be observed in half of skin biopsies obtained from areas with exfoliative dermatitis [70, 76]. Sebaceous adenitis associated with CanL is characterised by a multinodular to diffuse dermal inflammatory infiltrate not limited to the perifollicular dermis [77]. It has been suggested that when no inflammatory infiltrate is observed in the dermis but it is strictly centred on the sebaceous glands, idiopathic sebaceous adenitis is more likely than CanL, even in endemic areas [77].

4.2 | Ulcerative Dermatitis

Ulcerative dermatitis is the second most frequent cutaneous manifestation of CanL after exfoliative dermatitis [70]. The ulcerative lesions of CanL are commonly grouped together, despite different clinical presentations being observed, reasonably

TABLE 1 | Differential diagnoses for each cutaneous clinical pattern of CanL and useful clinical hints ('clinical pearls').

Clinical pattern			Main clinical differentials[a]	Clinical pearls
Exfoliative dermatitis			Idiopathic sebaceous adenitis, superficial bacterial folliculitis, exfoliative superficial pyoderma, dermatophytosis, demodicosis, cheyletiellosis, CETCL, ECLE, ichthyosis, secondary dry seborrhoea (e.g., due to hypothyroidism), idiopathic dermatosis of pinnal margins (localised), zinc responsive dermatitis	Large, dry, silvery or asbestos-like scales, variably adherent to the underlying skin. Initial localisation may be periocular (butterfly sign), on dorsal nose, ear pinnae and/or elbows
Ulcerative dermatitis on sites subjected to trauma			Localised deep bacterial or fungal infections, decubital ulcers, vasculitis, acral lick dermatitis, superficial necrolytic dermatitis, neoplasms	Well demarcated, deepened, non-bleeding, chronic ulcers with sharp raised margins. Acral lick dermatitis-like lesions will persist despite treatment of secondary infection and prevention of self-trauma with mechanical barriers
Ulcerative dermatitis on body extremities			Vasculitis of any cause (systemic infection including other vector-borne diseases, autoimmune and immune mediated diseases like SLE, adverse reactions to vaccines or drugs, idiopathy etc.), ischaemic dermatopathies (e.g., dermatomyositis), other vascular diseases (e.g., proliferative thrombovascular necrosis of ear pinnae), fly bite dermatitis, frostbite	Clinicopathological abnormalities suggestive of CanL and elevated levels of circulating anti-*Leishmania* antibodies are normally present
Ulcerative dermatitis of nasal planum and/or mucocutaneous junctions	Predominant nasal planum	Without involvement of nasal orifices and alar folds	Nasal LE, CETCL, actinic dermatosis, uveodermatologic syndrome, dermatomyositis, SLE	In CanL depigmentation follows inflammation (unlike actinic dermatosis), the lesions do not necessarily appear first on the dorsal planum (unlike nasal LE) and normally do not involve the lips (unlike CETCL)
		With involvement of nasal orifices and alar folds	In addition to the above, consider mucocutaneous pyoderma, mucocutaneous LE, adverse drug reactions, EM, TEN, pemphigus vulgaris, paraneoplastic pemphigus, autoimmune or hereditary subepidermal bullous dermatoses	In CanL, anal and genital mucocutaneous junctions are infrequently affected and mottled dyspigmentation next to ulcers is uncommon (unlike mucocutaneous LE). In CanL concurrent mucosal lesions are less common compared to TEN, pemphigus vulgaris, paraneoplastic pemphigus, autoimmune or hereditary subepidermal bullous dermatoses
		With hairy dorsal nose involvement	In addition to the above, pemphigus erythematosus and foliaceus	Greyish scales and crusts predominate, and no papules-pustules are normally observed
	Predominant mucocutaneous junctions		Mucocutaneous pyoderma, mucocutaneous LE, adverse drug reactions, EM, TEN, CETCL, pemphigus vulgaris, paraneoplastic pemphigus, autoimmune or hereditary subepidermal bullous dermatoses, zinc responsive dermatosis, superficial necrolytic dermatitis	See above-mentioned clinical pearls of ulcerative dermatitis of the nasal planum with involvement of nasal orifices and alar folds

(Continues)

TABLE 1 | (Continued)

Clinical pattern	Main clinical differentials[a]	Clinical pearls
Papular dermatitis	Reactions to arthropod stings/bites, sterile granuloma-pyogranuloma syndrome, reactive histiocytosis, neoplasia, canine leproid granuloma, bacterial folliculitis	Typically located on the concave surface of the pinnae; erythematous papules that rapidly (within days) evolve to umbilicated papules ('volcano sign')
Cutaneous and mucocutaneous nodular dermatitis	Infectious granulomas (mycobacteriosis, deep mycoses), neoplasia (mast cell tumour, lymphoma), sterile granulomatous dermatitis (sterile granuloma-pyogranuloma syndrome), reactive histiocytosis, eosinophilic granuloma, calcinosis circumscripta, amyloidosis	Often accompanied by clinicopathologic findings of CanL. Ulcerated nodules commonly show an interwoven 'granulation tissue' in the centre
Pustular dermatitis	Pemphigus foliaceus, other immune-mediated superficial pustular diseases (adverse drug reaction, neutrophilic or eosinophilic sterile pustular dermatoses), superficial pyoderma, pustular dermatophytosis	Rule out differentials such as superficial pyoderma and dermatophytosis first. Often accompanied by clinicopathologic findings of CanL
Footpad and/or nasal hyperkeratosis	Zinc-responsive dermatosis, hereditary nasal parakeratosis of Labrador Retrievers, idiopathic nasodigital hyperkeratosis, hereditary digital hyperkeratosis, psoriasiform dermatitis of the paw pads, pemphigus foliaceus, distemper, superficial necrolytic dermatitis, ichthyosis	Often accompanied by other clinicopathologic manifestations of CanL. Rarely exudative
Onychogryphosis	Onychomycosis, onychogryphosis of the geriatric dog, lupoid onychitis	Excessive growth and abnormal curvature of multiple or all claws. Concurrent exfoliative or ulcerative dermatitis

Abbreviations: CanL, canine leishmaniosis due to *L. infantum*; CETCL, cutaneous epitheliotropic T cell lymphoma; ECLE, exfoliative cutaneous lupus erythematosus; EM, erythema multiforme; LE, lupus erythematosus; SLE, systemic lupus erythematosus; TEN, toxic epidermal necrolysis.
[a]Differential diagnoses for each cutaneous clinical pattern must be tailored to the clinical context.

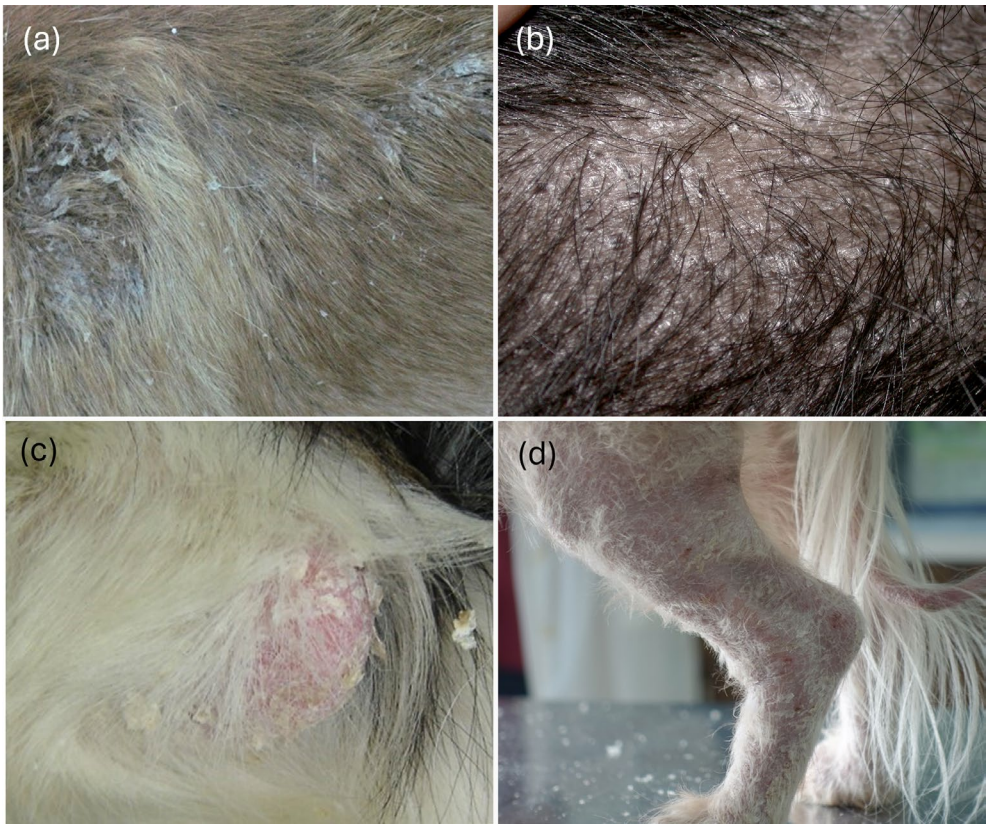


FIGURE 1 | Examples of exfoliative (scaling) dermatitis. (a) Large, greyish scales adherent to the hair coat. (b) Small, whitish scales on the surface of the skin and hypotrichosis. (c) Large scales, erythema and hypotrichosis. (d) Scales, erythema, small erosions and alopecia/hypotrichosis.

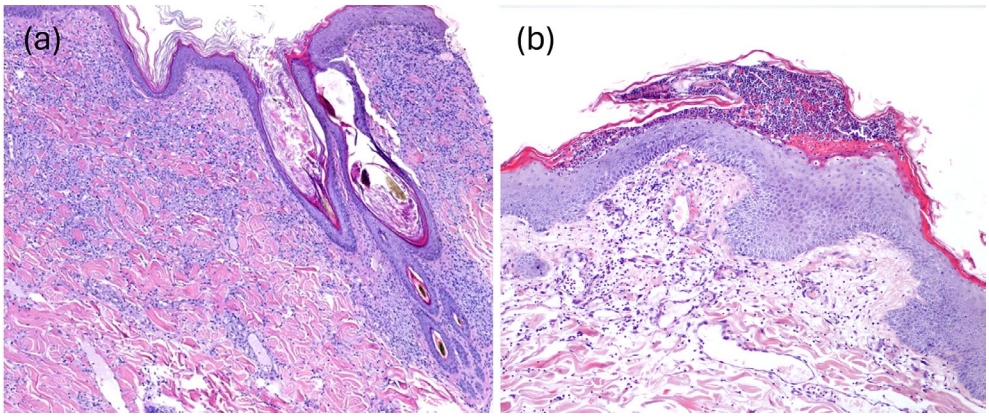


FIGURE 2 | Examples of histopathology findings from skin lesions of dogs with leishmaniosis. (a) Histopathology from lesions of exfoliative dermatitis. Haematoxylin and eosin (courtesy: F. Abramo). (b) Histopathology from lesions of pustular dermatitis. Haematoxylin and eosin (courtesy: F. Abramo).

suggestive of a different underlying pathogenesis. Clinical patterns include ulcers (i) on sites subjected to trauma, like bony prominences-pressure points and sites of pre-existing wounds, (ii) on body extremities (paws, apex of ear pinnae, nose, tip of tail) and (iii) on nasal planum and/or mucocutaneous junctions. In all cases they are non-pruritic, variably painful and chronic and frequently do not respond to antibiotics. Ulcers on bony prominences, unlike other ulcerative patterns, represent a relatively striking clinical presentation of CanL; the remaining ulcerative patterns are more likely to overlap, both clinically and histologically, with other diseases [72].

4.2.1 | Ulcerative Dermatitis on Sites Subjected to Trauma

a. On bony prominences-pressure points (Figure 3): ulcers appear as chronic, indolent and deep, with sharp raised borders. These commonly affect carpal and tarsal joints or the ischiatic tuberosities. They can be solitary or multiple, and unilateral or bilateral. It has been hypothesised that continued pressure causes secondary inflammation with subsequent infiltration of infected macrophages, which in turn results in more severe inflammation and ulcer development [70].

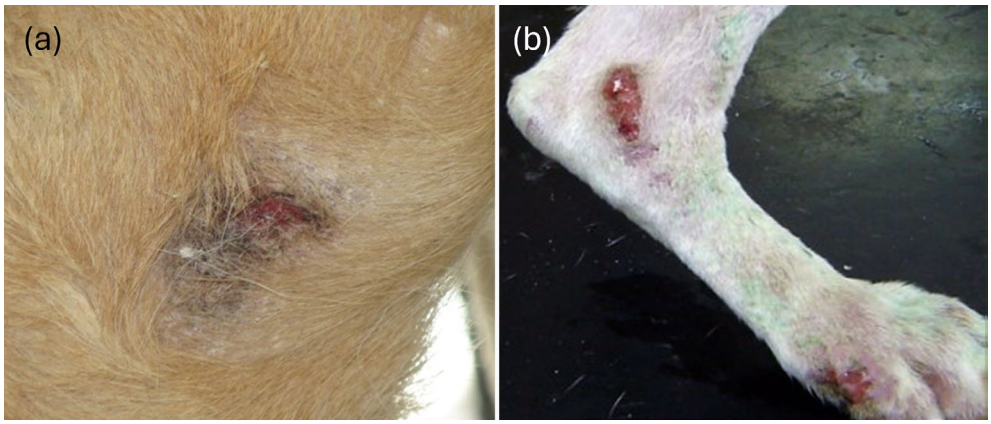


FIGURE 3 | Examples of ulcerative dermatitis on bony prominences and pressure points. (a) Ulcer on the elbow, hypotrichosis and scales due to concurrent exfoliative dermatitis. (b) Ulcers on the bony prominences of the hind leg.

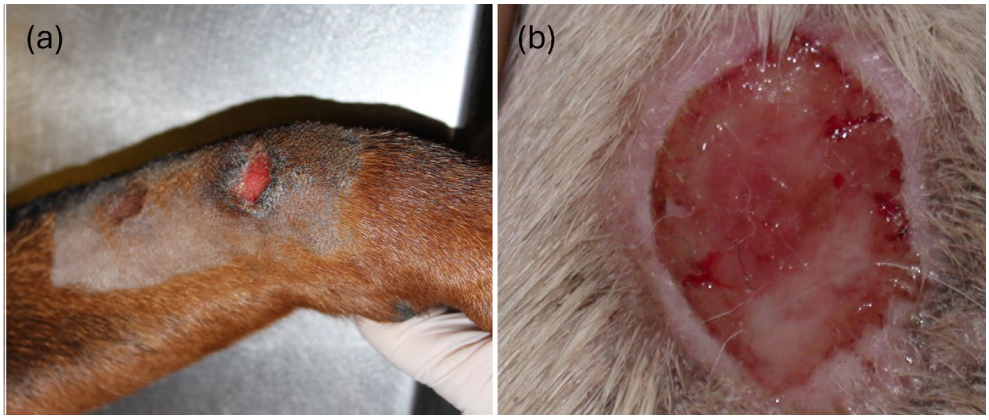


FIGURE 4 | Examples of ulcerative dermatitis on sites of pre-existing wounds. (a) Ulcer on the carpal area (the leg has been clipped). (b) Deep indolent ulcer with raised borders on the area of pre-existing acral lick dermatitis.

b. On the site of pre-existing wounds: less frequently, persistent ulcers have been reported at sites of pre-existing injury with loss of skin integrity, such as surgical wounds [78] or in lesions of acral lick dermatitis [79] (Figure 4). Ulcerated nodules and plaques may also develop in some cases. It has been hypothesised that these lesions are the consequence of the influx of infected macrophages at the site of trauma during the normal wound healing process. The extracellular release of parasites perpetuates inflammation, interferes with healing, and explains the chronicity of the lesions. A similar pathogenetic mechanism has also been described in people who have presumably been infected while travelling to endemic areas and, within weeks to months after their return, develop CL with ulcers at the site of even minor mechanical trauma (such as insect bites, tattoos or shaving cuts) [80, 81]. The peculiarity of these lesions is that the initial injury causing disruption of skin integrity is irrelevant to the parasite [82].

Dermatopathological examination of biopsies taken from ulcers at sites subjected to trauma often shows epidermal hyperplasia with ulceration and a periadnexal to diffuse neutrophilic-macrophagic dermal infiltrate with variable numbers of amastigotes [70, 72].

4.2.2 | Ulcerative Dermatitis on Body Extremities

In this case the pathogenesis of ulcers is attributed to cutaneous vasculitis with deposition of circulating immune complexes in the vessel wall. The characteristic lesions consist of occasionally bleeding ulcers covered by haemorrhagic crusts, typically located on the margins of the pinnae and less commonly on the tip of the tail, digits and paw pads (Figure 5). Occasionally, onychomadesis with subsequent onychodystrophy can be observed, resulting from vascular damage to the nail matrix [72]. Infrequently, in addition to or in lieu of lesions indicative of vasculitis, signs of ischaemic dermatopathy can be present, such as multifocal alopecic areas characterised by cutaneous atrophy, scaling, hypo- or hyper-pigmentation distributed mainly on the head and distal legs [72].

Although the type and distribution of these lesions strongly suggest vascular damage, this is rarely documented because they are not frequently subjected to dermatopathological examination owing to difficulty in collecting skin biopsies from these locations. In addition, vascular inflammation may be temporary and does not affect all vessels, complicating its documentation. However, even if vascular damage is confirmed histologically, it would still be difficult to attribute a causal role to the parasite

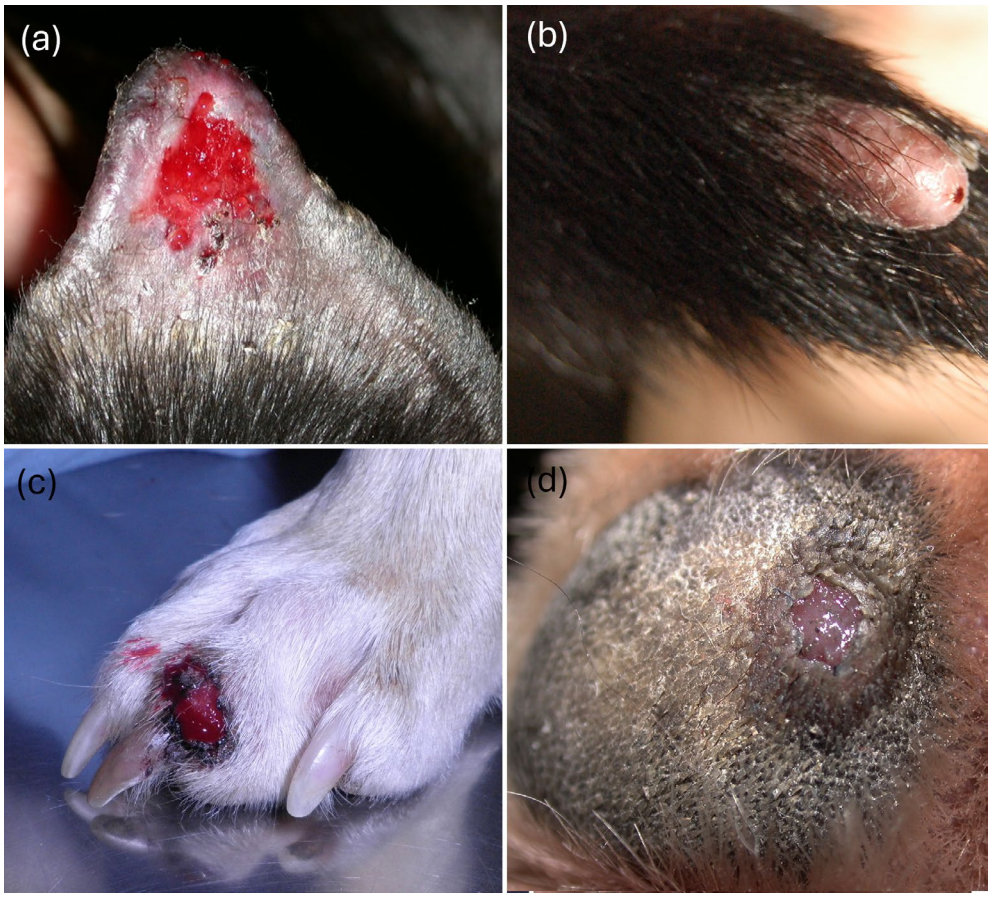


FIGURE 5 | Examples of ulcerative dermatitis on body extremities. (a) Wedge-shaped ulcer near the tip of ear pinnae, alopecia and scales due to concurrent exfoliative dermatitis. (b) Small ulcer and alopecia on the tip of the tail. (c) Bleeding ulcer on a digit. (d) Shallow ulcer on a paw pad.

as its presence within lesions may not be demonstrable, even by molecular techniques. The lesions are induced by the deposition of circulating immune complexes rather than direct presence of the organism. The diagnosis of leishmaniosis in these cases is mostly based on the remaining clinical signs, suggestive clinicopathologic changes, exclusion of differentials, demonstration of the infection and of elevated levels of circulating anti-*Leishmania* antibodies [33].

4.2.3 | Ulcerative Dermatitis of the Nasal Planum and/or Mucocutaneous Junctions

Dermatitis of the nasal planum caused by CanL is characterised by erosions, ulcers, crusts, scales and variable depigmentation, with possible swelling and loss of the typical cobblestone architecture (Figure 6a) [72, 83]. These lesions can also involve the nasal orifices and alar folds and may be accompanied by scales and crusts on the haired skin caudal to the planum. Discoid lupus erythematosus (DLE) is the main differential, both clinically and histologically [83]. A retrospective study comparing histopathological and immunopathological features of nasal planar dermatitis in 20 dogs with DLE or CanL showed a band-like lymphoplasmacytic dermatitis at the dermo-epidermal junction, with basal cell vacuolation and occasional apoptosis in both diseases. However, a nodular-to-diffuse superficial and/or deep infiltrates composed of macrophages, lymphocytes and plasma cells was visible only in CanL [83]. Amastigotes were not seen in any of the haematoxylin–eosin (H&E)–stained sections, denoting that the number of parasites is low. CD20-positive lymphocytes predominated over CD3-positive T cells in both diseases, but the percentage of dermal Mac387-positive macrophages was significantly higher in CanL compared to DLE [83].

Variably depigmented, non-pruritic, scaling, crusting, erosive and ulcerative lesions of other mucocutaneous junctions may also be seen in CanL [71]. All mucocutaneous junctions can be affected; however, in addition to the nasal planum, the medial canthus of the eyes and lips appear to be more frequently involved (Figure 6b–d). Histopathological findings of mucocutaneous lesions in dogs with leishmaniosis caused by *L. infantum* have not been reported, however they are likely to resemble those observed in mucocutaneous pyoderma This is characterised by a perivascular to interstitial or band-like infiltrate, predominantly plasmacytic, at the dermo-epidermal junction, accompanied by lymphocytes, macrophages and neutrophils [72]. Mucocutaneous pyoderma histologically overlaps not only with the mucocutaneous lesions of CanL but also with both chronic DLE and mucocutaneous lupus erythematosus, all of which can be accompanied by bacterial superinfection [84, 85]. Therefore, whenever mucocutaneous pyoderma is included

FIGURE 6 | (a) Ulcerative dermatitis of nasal planum. (b) Ulcerative dermatitis involving the mucocutaneous junctions of the nasal philtrum. (c) Shallow ulcers on the medial and the lateral canthus. (d) Ulcers on the lips.

in the differential diagnosis, specific antimicrobial treatment prior to biopsy is recommended [72, 85].

4.3 | Papular Dermatitis

In endemic regions, this is a very characteristic primary cutaneous manifestation of *L. infantum* infection. Although the exact prevalence is unknown, it is very common (54%) in resistant breeds, such as the Ibizan hound [86]. Lesions start as raised erythematous papules in sparsely haired skin, such as the inner pinnae, eyelids, dorsal part of the nose, lips, caudal abdomen and medial thighs (Figure 7). Sometimes, the papules coalesce to reach a final size of a small plaque. A crust develops in the centre of each papule, covering an ulcer with a raised edge and umbilicated appearance [71]. This clinical appearance is similar to the one observed in the localised form of human CL, known as the 'volcano sign'. It is highly suspected that papules develop at the site of *Leishmania* inoculation in dogs with strong cell-mediated immunity against *L. infantum* [71]. Evidence for this hypothesis is the lesion distribution, reduced parasite dissemination to internal organs, low parasite load in lesional skin, lack of other clinicopathological abnormalities, low or negative specific antibody levels, positive results of the leishmanin skin test (LST), high expression of IFN-γ in blood and lesional skin and spontaneous resolution over 3–5 months [87–90]. The histopathological picture of papular dermatitis is dominated by a nodular to diffuse granulomatous dermatitis, without multinucleated giant cells and usually with few parasites [89].

4.4 | Cutaneous and Mucocutaneous Nodular Dermatitis

Cutaneous and mucocutaneous nodular dermatitis is a relatively uncommon clinical presentation, with a prevalence of up to 12% [70]. It is described more frequently in the Boxer breed. Clinically, it is characterised by single or multiple plaques or nodules of variable size (1–10 cm), usually located on the head, distal limbs, and thorax (Figure 8). Unlike papular dermatitis, these lesions are localised on haired areas. They may sometimes ulcerate. Less commonly, nodules have been noted on mucocutaneous junctions and mucous membranes, such as the oral or genital mucosa [91]. Nodules are attributed to the spread of the parasite to the skin or mucous membranes via the lympho-haematogenous pathway in moderately to severely affected dogs. Dermatopathological examination reveals a diffuse granulomatous or pyogranulomatous dermatitis, sometimes with multinucleated giant cells and with a variable but frequently high number of amastigotes [92, 93].

4.5 | Pustular Dermatitis

Pustular dermatitis is an uncommon presentation of CanL, with a prevalence ranging between 1% and 13% of the cases [70]. Typical lesions are variable-sized pustules surrounded by an erythematous halo and admixed with erythematous papules, epidermal collarettes and crusts, all representing various stages of lesion evolution. Lesions may show a polycyclic or arciform

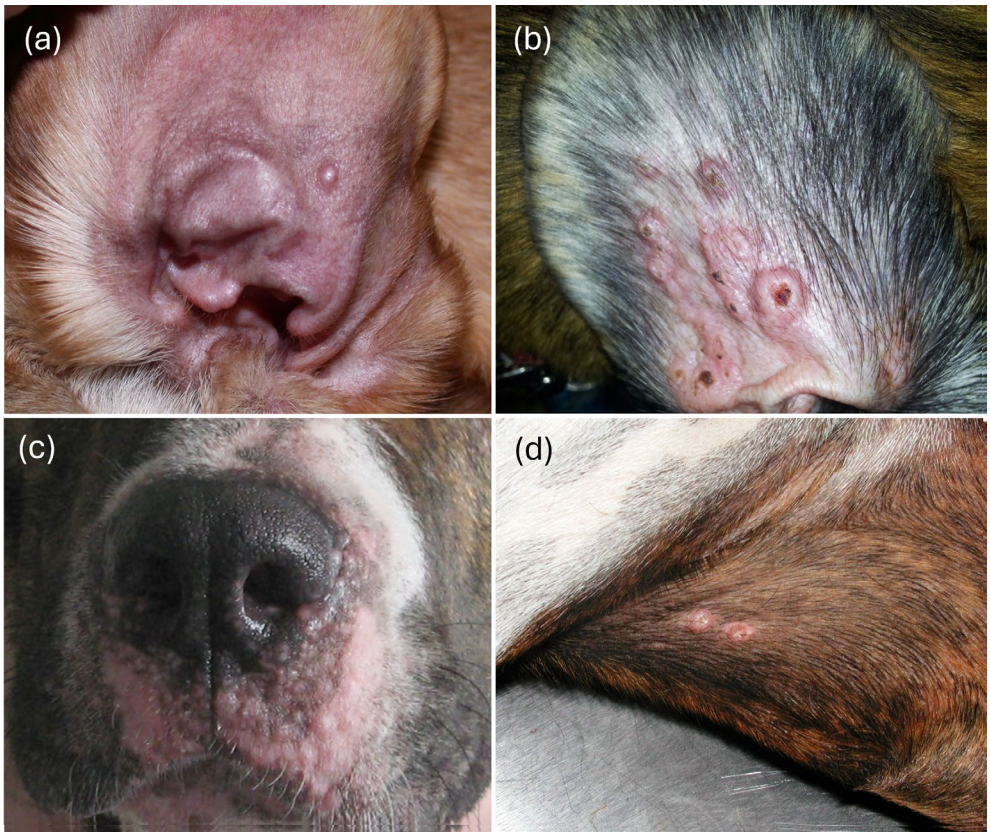


FIGURE 7 | Examples of papular dermatitis. (a) Single papule on the ear pinnae. (b) Multiple, umbilicated, and sometimes crusted papules on the ear pinnae. (c) Multiple papules on the upper lip. (d) Two papules on the ventral chest.

FIGURE 8 | Examples of nodular dermatitis. (a) Multiple nodules on the eyelids. (b) Multiple nodules on the upper and the lower lip. (c) Ulcerated nodule on the nasal philtrum. (d) Ulcerated nodule on a digit.

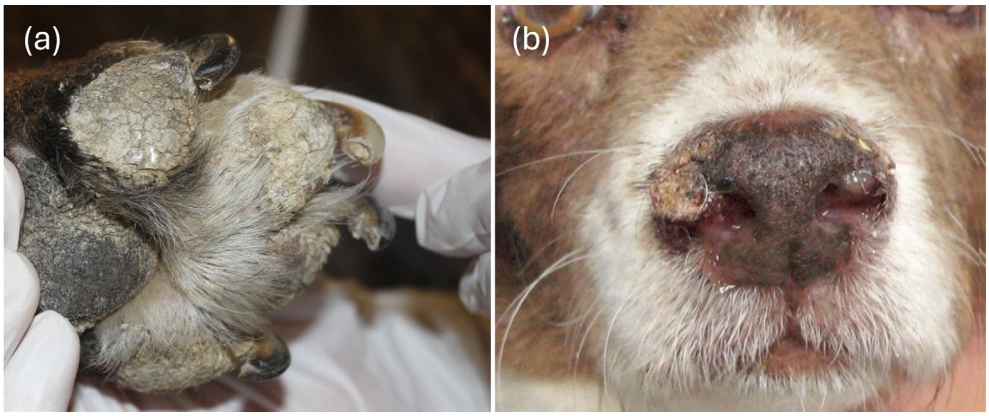


FIGURE 9 | Hyperkeratosis of (a) the footpads and (b) the nasal planum.

configuration and multifocal alopecia occasionally may be present. Pustules occur in a generalised, symmetrical distribution involving both densely and sparsely haired areas. Pruritus is variable but often severe [71, 72, 94, 95]. Affected dogs may also show systemic signs such as anorexia and fever.

A recent study showed that in endemic areas there is a statistically significant association between CanL and this uncommon clinical presentation [95], but their aetiopathogenetic relationship remains unclear. Two hypotheses have been proposed: (i) an immune-mediated pustular dermatitis develops in a dog infected by *L. infantum* and the ensuing dysregulation of the immune system causes the infection to progress to CanL; and (ii) the immune-mediated pustular dermatitis may occur secondary to CanL-induced immunologic abnormalities. Moreover, in some cases, the possibility of an adverse drug reaction as underlying cause of pustular dermatitis cannot be ruled out [94, 95].

On histopathological examination, subcorneal or intraepidermal, variable sized pustules containing neutrophils and occasionally few acantholytic cells, associated with spongiosis and neutrophilic exocytosis are observed (Figure 2b). In the superficial dermis, there is a mild to moderate, perivascular to interstitial dermatitis. Amastigotes may be occasionally identified in the dermis underneath the pustules, but not within the pustules, by means of immunohistochemistry (IHC) [94, 95].

4.6 | Footpad and/or Nasal Hyperkeratosis

Footpad and/or nasal hyperkeratosis is characterised by greyish, thick and dry scales (Figure 9). These are strongly adherent to the underlying epidermis and sometimes are accompanied by deep fissures, which can be painful, especially when located on the paw pads [70]. This dermatological problem is often associated with other clinical manifestations of CanL in moderately to severely affected dogs [70]. Recently, it has been suggested that the combination of alopecia and nasal hyperkeratosis showed the greater positive likelihood ratio to increase the pre-test probability of CanL [96]. Histopathologic findings seen in the hyperkeratotic footpads include epidermal hyperplasia with hyperkeratosis, epidermal hypermelanosis, melanin incontinence, perivascular to interstitial and, less commonly, nodular to diffuse dermatitis [97]. The main inflammatory cells are

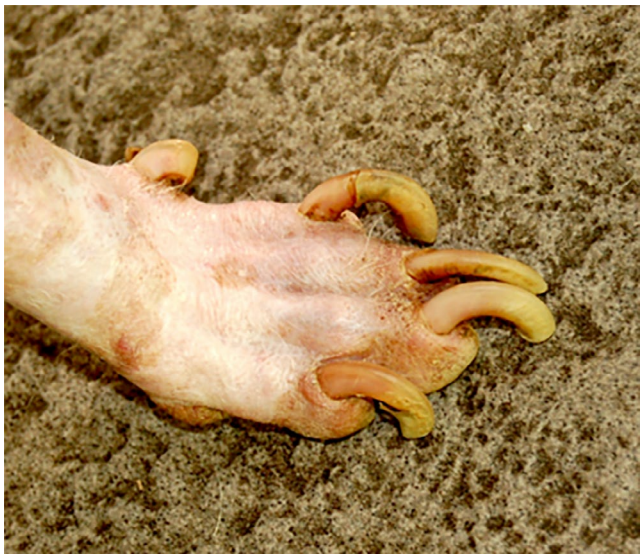


FIGURE 10 | Onychogryphosis.

macrophages and to a lesser extent lymphocytes and plasma cells, whereas fewer neutrophils, eosinophils and mast cells may be present [97].

4.7 | Onychogryphosis

Onychogryphosis is common in CanL, with a reported prevalence between 43.4% and 54.4% [70]. It is a tardive, chronic sign of CanL, and it is clinically characterised by excessive growth and abnormal curvature of the nails (Figure 10). Rarely, it is the only clinical sign, because in most dogs with CanL it is accompanied by exfoliative and/or ulcerative dermatitis. Onychogryphosis, as well as footpad and/or nasal hyperkeratosis, may represent a localised form of exfoliative dermatitis [34, 98].

Histopathological findings are non-specific. Onychogryphosis is characterised by lymphocytic exocytosis, mild to severe lichenoid mononuclear dermatitis with or without hydropic degeneration of basal keratinocytes, dermo-epidermal clefting and pigmentary incontinence [98]. Amastigotes cannot be found, at least in H&E–stained preparations [98].

5 | Diagnosis of Canine Leishmaniosis

The investigation of infection by *L. infantum* and/or for CanL is performed for different indications and reasons, including suspected CanL in dogs with compatible clinical signs, evaluation of blood donors and breeding stock, health check of clinically healthy dogs, importation or exportation of dogs, monitoring dogs during treatment and epidemiological surveys.

Several types of tests are available ranging from techniques to visualise the parasites, to the detection of antibodies against *Leishmania* and to the molecular detection of parasite DNA [23]. These techniques have different sensitivities and specificities. Some are more useful for particular purposes, such as the diagnosis of CanL in dogs with compatible signs admitted for veterinary care, whereas others are more valuable for the detection of subclinical infection in blood donors or apparently healthy dogs that are undergoing a health check. The diagnostic approach to a dog suspect of CanL should include, at minimum, complete blood count, serum biochemical profile (including protein electrophoresis), urinalysis (including UPC) and one or more of the following tests [99, 100].

5.1 | Microscopy and Histopathology

Leishmania amastigotes can be demonstrated by microscopic examination of smears from the skin, lymph nodes, spleen, bone marrow, joint fluid, abdominal fluid or other fluids, tissues and affected organs. The preparations should be stained with Romanowsky type stains, such as Giemsa or Diff Quik. Amastigotes are round to oval, 2.5–5 μm long and 1.5–2 μm wide, and possess a nucleus and a rod-shaped, darker staining kinetoplast that is visible in the cytoplasm separately from the nucleus. The diagnostic sensitivity of microscopy depends on the parasitic load in the target tissue, the quality of the preparations, the experience of the examiner and the number of microscopic fields that are examined. In general, the sensitivity is much higher in dogs with CanL compared to subclinically infected dogs. For example, the sensitivity of lymph node cytology in dogs with CanL was found to be 84% or 93% after the examination of 100 or 1000 microscopic fields, whereas the relevant figures for subclinically infected dogs were 13% and 26%, respectively [101]. Amastigotes may also be viewed in histopathological and/or IHC examination of skin and other organs (see Diagnostic approach to the skin lesions of dogs with leishmaniosis) [99].

5.2 | Serology

Several serological methods for the detection of anti-*Leishmania* antibodies are available. These include quantitative tests, such as the indirect immunofluorescence assay (IFA), enzyme-linked immunosorbent assay (ELISA) and direct agglutination test (DAT), as well as qualitative commercial kits. The latter provide only a positive or negative result, and when positive, they should be followed by a quantitative test, which will provide a titre that is important for treatment monitoring (successful treatment is typically associated with a decrease of antibody levels over time, whereas unsuccessful treatment or relapses are associated with increased antibody levels) [102]. All tests employ whole parasites or recombinant antigens for the detection of *Leishmania*-specific IgG. In general, good sensitivities and specificities are gained with most serological assays for the diagnosis of CanL, whereas subclinically infected dogs are often seronegative or have low antibody levels [99]. In regions where multiple *Leishmania* species and/or *Trypanosoma* spp. co-exist, serological cross-reactions may occur [103, 104].

5.3 | Molecular Tests [Polymerase Chain Reaction]

These tests allow the diagnosis of infection with *Leishmania* spp. by the detection of the parasite's kinetoplast DNA (kDNA). Many assays that target different sequences of genomic or kDNA have been developed, and, generally, those targeting kDNA are the most sensitive. PCR can be performed on DNA extracted from tissues, blood, other fluids such as cerebrospinal or synovial liquid or even from histopathologic specimens. The biological samples that are characterised by the higher sensitivity are bone marrow, lymph node and spleen aspirates, as well as conjunctival swabs, with the latter being the only samples that are obtained non-invasively. PCR using blood and other body fluids is considered less sensitive and dogs with CanL can be negative [105]. On the contrary, PCR of bone marrow and lymph nodes is typically positive in dogs with CanL and can also be used for detection of *Leishmania* in subclinically infected seronegative dogs [99, 100].

5.3.1 | Consensus Statement

The diagnosis of CanL is based on the compatible clinical signs and/or clinicopathologic abnormalities, exclusion of as many major differentials as feasible, the demonstration of infection and the increased concentration of anti-*Leishmania* IgG in serum (quantitative serology). Both PCR and serology, in combination, can detect subclinical infection in blood donors, breeding dogs, dogs being imported to non-endemic countries and in epidemiological studies, whereas serology is adequate for health checks.

5.4 | Staging of Canine Leishmaniosis

Two main non-validated systems have been proposed for staging of CanL [99, 106, 107]. The clinical staging system for CanL provided by the LeishVet group can be found at https://www.leishvet.org/fact-sheet/clinical-staging/. It divides the disease into four stages based on clinical signs, clinicopathologic abnormalities and level of anti-*Leishmania* antibodies. This system is helpful for decisions on the most suitable treatment for each dog, and for consideration of a prognosis. The clinical stage may change if the dog improves or deteriorates [99, 100].

6 | Diagnostic Approach to the Skin Lesions of Dogs With Leishmaniosis

A dog with CanL may present either with no macroscopic skin lesions or skin lesions that are directly or indirectly caused by CanL or skin lesions due to coincidental diseases [70]. The precise diagnosis of the cause of skin lesions in a dog with CanL

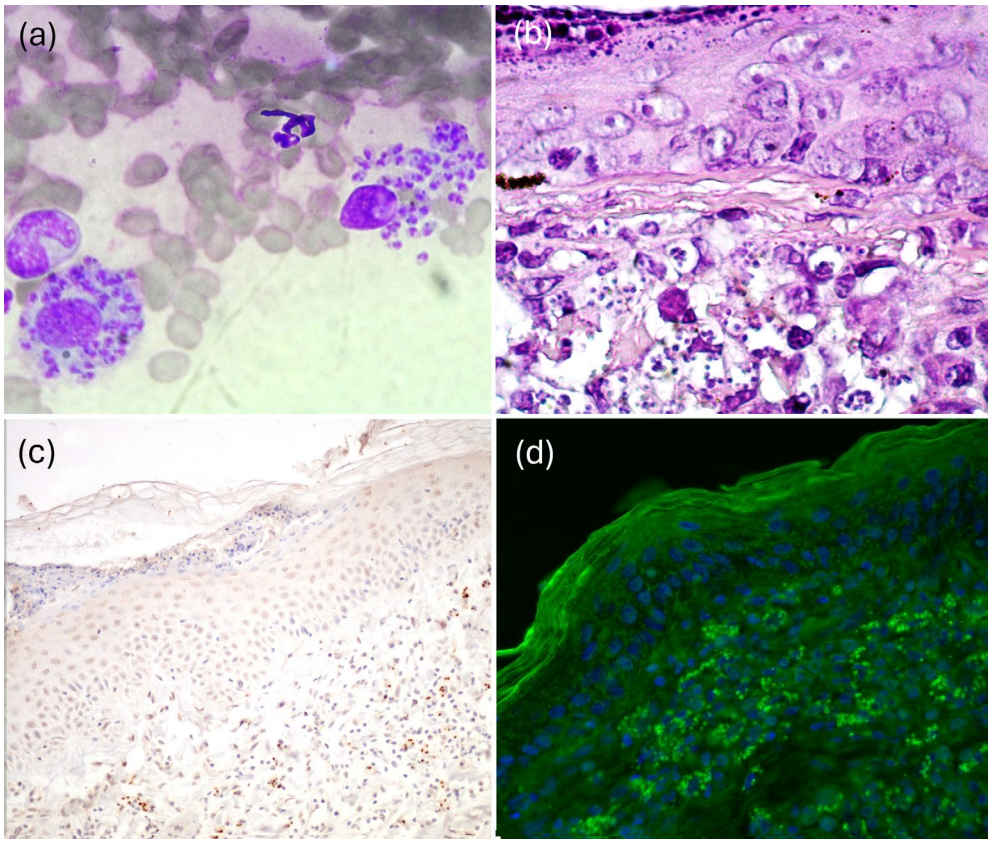


FIGURE 11 | (a) Cutaneous cytology showing numerous amastigotes. Diff Quik. (b) Cutaneous histopathology with numerous amastigotes. Haematoxylin and eosin. (c) Cutaneous immunohistochemistry with numerous amastigotes. Streptavidin-biotin (courtesy: F. Abramo); (d) direct immunofluorescence in a skin biopsy sample from a dog with exfoliative dermatitis. Each fluorescing dot in the dermis represents an amastigote. FITC, fluoresceine isothiocyanate/DAPI.

is of major importance for the overall management and prognosis. As an example, treatment and prognosis of a dog with CanL and scaling will be vastly different if the scaling is due to CanL-associated exfoliative dermatitis compared to scaling due to concurrent epitheliotropic T cell lymphoma. Unfortunately, the polymorphism of CanL-associated skin lesions results in an extensive list of differentials (Table 1) that becomes even longer when different types of skin lesions are present in the same dog (e.g., when a dog with CanL shows exfoliative dermatitis, ulcerative dermatitis and footpad hyperkeratosis) [70].

The laboratory examinations that can be used to diagnose CanL-associated skin lesions include cytology, histopathology, IHC, direct immunofluorescence (DIF) and PCR. The necessity to undertake some or all these examinations depends on the certainty of CanL diagnosis, the macroscopic appearance of skin lesions, the clinical experience of the veterinary surgeon and the geographical area. For example, after a definitive diagnosis of CanL, few to no additional examinations may be needed for a dog that lives in an endemic area and presents the typical lesions of CanL-associated exfoliative dermatitis. On the contrary, if the diagnosis of CanL is not certain (e.g., it is based only on positive qualitative serology), the clinical presentation is not typical and/or the dog lives in a non-endemic area, an extensive diagnostic investigation should follow to confirm that the skin lesions are indeed due to CanL and not to another concurrent disease.

Cutaneous cytology can demonstrate the inflammatory component of CanL skin lesions (typically macrophagic or purulent-macrophagic), confirm the presence of intracellular or extracellular *Leishmania* amastigotes (Figure 11a), and help to exclude or confirm some differentials, such as superficial and deep bacterial infections, deep fungal infections, pemphigus foliaceus, neoplastic and non-neoplastic tumours. Samples can be obtained from areas with exfoliative dermatitis after gentle lifting the scales and crusts, from the border of ulcers, from papules or nodules after fine-needle puncture or fine-needle aspiration and from skin biopsy samples (imprint smears) [108–110]. When microscopy is performed by experienced examiners, the detection of amastigotes has a specificity of 100% [111–113], but the sensitivity also depends on the time devoted to review of the slide (i.e., number of fields examined), and the type of macroscopic lesions [101]. In CanL, the diagnostic sensitivity of cutaneous cytology varies from 62% to 100% [113, 114]. It may be higher in exfoliative compared to ulcerative dermatitis [108] (in the latter it is up to 36%) [115], in the papular form ranges from 33% to 62% [87, 88] and in the nodular form it is typically very high (close to 100%) [108].

Histopathological examination of skin biopsies (Figure 11b) will show CanL-associated lesions in the epidermis, dermis and panniculus, characterise the type and distribution of the inflammatory infiltrate [which may vary depending on the macroscopic

lesions (reviewed in [70])] and help to exclude or confirm many differentials (Table 1). However, it may be difficult to visualise the organisms, especially when their numbers are low, and it is almost impossible to definitively identify them as *Leishmania* amastigotes because their characteristic features (cell membrane, nucleus, kinetoplast) are not readily visible [116, 117]. Using Giemsa instead of H&E stain may help in the identification of the parasites [118]. In general, presence of presumed amastigotes is reported in 7%–75% of dogs with various skin lesions due to CanL [89, 97, 117, 119–123], and in 9% of dogs with papular dermatitis [89].

Immunohistochemistry (Figure 11c) is a useful adjunct to histopathology because it can detect low number of organisms [122, 124], prove their identity as *Leishmania* amastigotes, and, thanks to the counterstain, show their localisation within inflammatory foci. Although the specificity of meticulously standardised IHC protocols is high, there are still some doubtful cases where dye precipitates cannot be easily differentiated from low numbers of amastigotes [125]. Sensitivity depends on the parasitic density [126], may be higher than that of cytology [117], varies from 18% to 100% in dogs with CanL and various skin lesions [89, 117, 118, 122, 123, 127], and has been reported to be 31% in exfoliative dermatitis [76] and 82%–100% in papular dermatitis [87, 89].

Direct immunofluorescence (Figure 11d) has been proposed as an alternative to IHC. Due to the lack of background staining it permits more accurate measurement of the parasitic density in the skin [97] but gives no information about the location of the parasites in relation to the areas of inflammation. Although in the single published study on this topic the sensitivity of DIF was 100% [97], it was not compared with the sensitivity of IHC. The specificity of DIF is unknown, and currently it is not commercially available.

Skin PCR [conventional PCR, nested PCR, real-time quantitative PCR (qPCR)] is probably the most sensitive test for the detection [88, 89, 113, 117, 125, 127–129], and the most accurate test for the quantification (qPCR) of *Leishmania* DNA [130–133], that presumably [134–136], but not necessarily [137, 138], corresponds to the presence of live amastigotes. However, it gives no information on the location of amastigotes in the skin, and it may be positive in transiently infected dogs (e.g., if the biopsy sample was obtained from an area recently bitten by an infected vector), in subclinically infected dogs and in dogs with parasitemia (due to the inevitable presence of blood in the biopsy material) [139, 140]. Alternatively, skin scrapings can be used instead of biopsy samples, but in this case the sensitivity of PCR may be significantly lower [141].

In conclusion, minimally invasive, low-cost diagnostic tests, such as cytology and parasitological examinations, should be performed in every dog with suspected CanL and skin lesions; they may strengthen (or weaken) the possibility that the skin lesions are due to CanL (e.g., cytology) and help to confirm or exclude other diseases that may co-exist (e.g., demodicosis, pemphigus foliaceus, pyoderma). More invasive and costly examinations (skin biopsy for histopathology, IHC or qPCR) should be considered when the diagnosis of CanL is not definitive (i.e., it is based only on positive qualitative serology), the skin lesions are the only findings (i.e., dogs without systemic signs and clinicopathological abnormalities) especially in non-endemic areas, and the macroscopic appearance of skin lesions is not typical of CanL and/or is compatible with concurrent skin diseases.

6.1 | Case Example 1

Penny is a 5-year-old, spayed-female, Jack Russell terrier, which 5 months prior to presentation had developed areas of partial alopecia extending progressively from the head to the dorsum, and which were initially accompanied by moderate pruritus. Glucocorticoids and oclacitinib improved pruritus but not alopecia. The dog was otherwise healthy and a rapid immunochromatographic test for CanL was negative. Penny lived indoors and outdoors in the garden, and regularly received imidacloprid and permethrin spot on.

General physical examination showed no abnormalities and on dermatologic examination areas of partial alopecia were observed on the head, dorsal trunk and outer surface of the hind limbs (Figure 12). The cutaneous problem was defined as multifocal partial alopecia and the diagnostic hypotheses included demodicosis, idiopathic sebaceous adenitis, CanL, hypothyroidism and (less likely), immune mediated-autoimmune folliculitis.

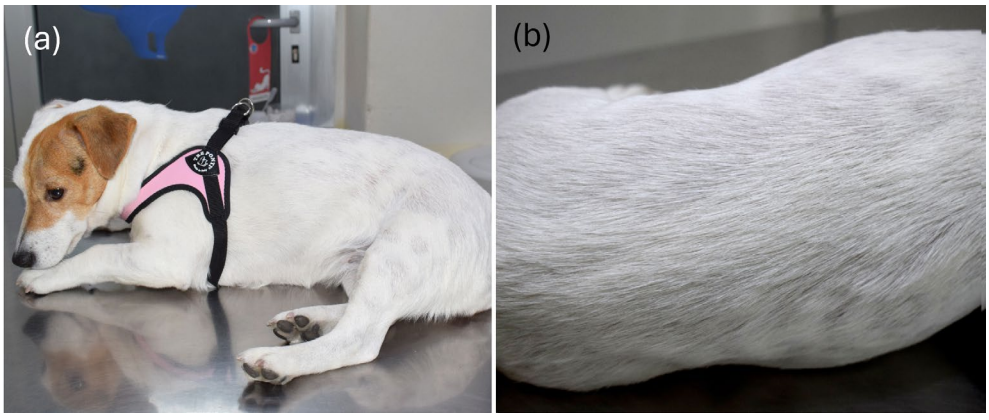


FIGURE 12 | Multifocal areas of partial alopecia on the (a) head, trunk and outer surface of hind limbs, and (b) dorsal trunk.

No parasites were observed on microscopic examination of skin scrapings and plucked hairs. Complete blood count, biochemistry profile with serum protein electrophoresis, urinalysis, quantitative serological test (ELISA) for CanL and measurement of serum total T4 revealed only mild hyperglobulinsemia [3.6 g/dL; reference range (RR): 2.7–3.5 g/dL] and a low-positive ELISA (antibody level 14.8%; positivity cut-off > 11%, with values between 11% and 30% considered as low-positive).

A bone marrow qPCR was performed to identify and quantify *Leishmania* parasites, and multiple skin biopsies were taken to confirm or rule out sebaceous adenitis. Bone marrow qPCR was negative and the main cutaneous histopathological lesions were periadnexal, histiocytic and lymphoplasmacytic infiltrates with few neutrophils, associated with absence of sebaceous glands and mild-to-moderate basketweave and lamellar orthokeratotic hyperkeratosis with follicular keratosis. The lesions were compatible with idiopathic sebaceous adenitis. However, since the dog lived in an area endemic for leishmaniosis and CanL can cause sebaceous adenitis, and because of the presence of rare small granulomatous foci in the panniculus, skin PCR for *Leishmania* was performed. The PCR result was negative.

It was concluded that Penny was a seropositive dog with idiopathic sebaceous adenitis; however, it was considered essential to periodically monitor her over time by serologic testing, especially if ciclosporin was to be administered for the management of idiopathic sebaceous adenitis.

Seropositive but PCR-negative dogs which reside in or have visited an area where sand fly vectors are present, but which have no clinical signs or clinicopathologic changes, must be monitored over time. Recently, it has been reported that nearly one quarter of clinically healthy seropositive dogs living in an endemic area will become seronegative by the end of the next non-transmission season [142]. These dogs likely represent a heterogeneous group, including infected dogs with a low parasite load that is non-detectable by PCR (even in tissues where their numbers are normally high, such as bone marrow and skin) and dogs with a transient infection. Also, seropositivity in dogs in which infection cannot be demonstrated has been attributed to nonspecific false-positive reactions [104, 140, 143, 144], and to cross-reactivity due to infection by other trypanosomatids, including pathogenic (e.g., *L. braziliensis*) and non-pathogenic (e.g., *L. tarentolae*) *Leishmania* species [145, 146]. On the contrary, evidence for cross-reactivity with other pathogens (e.g., *Ehrlichia canis*, *Toxoplasma gondii*, *Neospora caninum* and *Babesia canis*) is weak and speculative.

6.2 | Case Example 2

Nina is an 8-month-old, spayed-female, Labrador retriever, which for about 2 months prior to presentation showed papules on the inner side of both ear pinnae. These lesions had grown larger in recent days. Nina was initially treated with a cream containing gentamicin and dexamethasone without improvement. She was otherwise healthy, lived indoors and outdoors in a house, regularly received a spot-on product containing fipronil and was wearing a deltamethrin-impregnated collar.

General physical examination showed no abnormalities and dermatologic examination revealed multiple, whitish, 3–6 mm papules on each ear pinna (Figure 13). The cutaneous problem was defined as persistent papular dermatitis on both inner ear pinnae and the diagnostic hypotheses included CanL, insect bites and canine leproid granuloma syndrome.

Cytologic examination of samples obtained by fine-needle puncture revealed scarce lymphocytes and macrophages containing few *Leishmania* amastigotes in their cytoplasm (Figure 14). Complete blood count, biochemistry profile with serum protein electrophoresis and urinalysis show no alterations, and quantitative serological test (ELISA) for leishmaniosis was negative (13.2 ELISA units; positivity cut-off > 35). If amastigotes had not been detected on cytological examination, one of the following could have been performed: (a) *Leishmania* qPCR from material

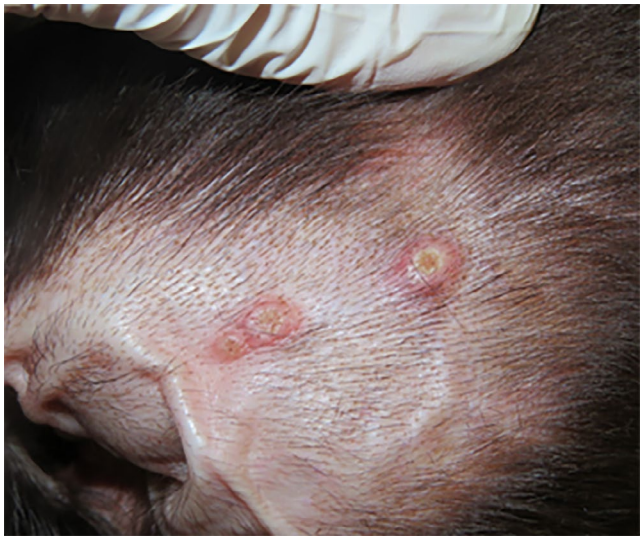


FIGURE 13 | Three papules on the concave surface of the ear pinnae.

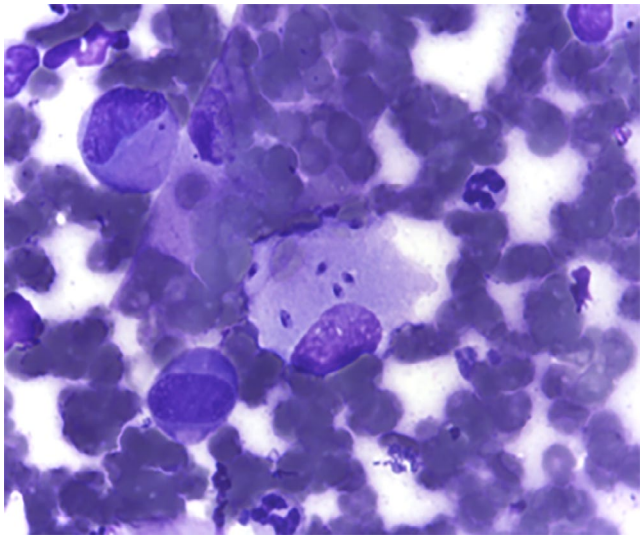


FIGURE 14 | Macrophages and neutrophils in the cytology smear from a papule. At least five *Leishmania* amastigotes are present in the cytoplasm of one macrophage at the centre of the picture.

collected through impression, fine-needle puncture or scraping from the surface of the papules or (b) skin biopsy for histopathology followed, if necessary, by IHC or qPCR.

Nina was affected by a mild form of CanL, namely papular dermatitis. Information regarding the treatment and outcome of this form is scarce. However, the prognosis is good, even without treatment. Nonetheless, physical examination and humoral immune response monitoring is needed to detect early disease progression.

Nina was left untreated. Ten days after diagnosis papules evolved to crusted papules with an umbilicated appearance (Figure 15). Twenty-five days after diagnosis the lesions were regressing and were completely resolved by Day 37 (Figure 16). One month after diagnosis the results of haematology, serum biochemistry and urinalysis were within reference ranges. The ELISA was still negative and remained so 1 year after diagnosis and upon subsequent annual rechecks for the following 4.5 years.

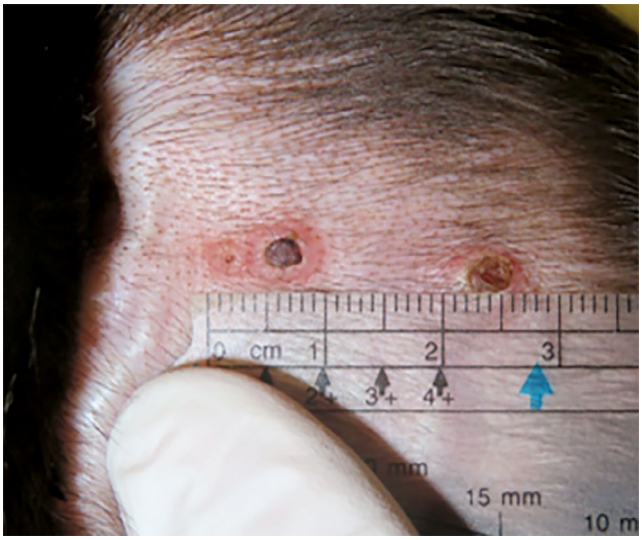


FIGURE 15 | Same lesions like on Figure 13 evolving to crusted papules within 10 days.

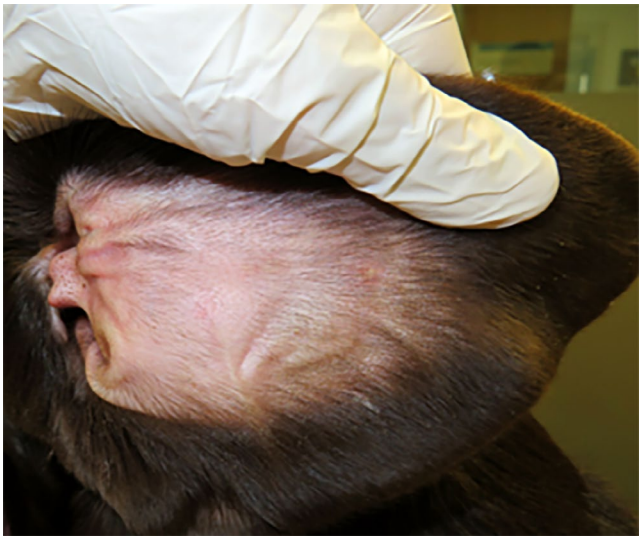


FIGURE 16 | Complete regression of the lesions on Figure 13 within 37 days.

6.3 | Case Example 3

DJ is an 8-year-old, neutered-male, American Staffordshire terrier with generalised skin lesions present for approximately 1 year. DJ was initially diagnosed with atopic dermatitis, with episodes of pruritus exacerbated during summer months, and was receiving allergen-specific immunotherapy. A recent deterioration of skin lesions was witnessed, and the dog was treated with oral cephalexin and oclacitinib without improvement. Complete blood count and biochemistry profile with serum protein electrophoresis were performed 15 days prior to consultation and revealed hypoalbuminaemia (2.4 g/dL; RR: 2.87–4.76 g/dL), hyperbetaglobulinaemia (2.4 g/dL; RR: 0.72–1.80 g/dL), hypergammaglobulinaemia (2.4 g/dL; RR: 0.28–1.57 g/dL) and a mild increase in aspartate aminotransferase activity (130 IU/L; RR: 16–89 UI/L). Quantitative serology for CanL (ELISA) was $R = 1.82$ ($R > 1.8$ = very high positive). The dog lived in an apartment with no other pets and regularly received oral afoxolaner.

General physical examination showed moderate popliteal lymphadenomegaly, and dermatologic examination revealed skin lesions mainly on the head, ear pinnae and limbs. Alopecia with fine whitish scales was observed on both pinnae (Figure 17a), periocular region (Figure 17b), elbows and tarsal regions (Figure 17c). Ulcers were observed on the inferior lip and tongue margins (Figure 17d). The cutaneous problems were defined as exfoliative (scaling) dermatitis and mucocutaneous ulcerative dermatitis. The diagnostic hypotheses included CanL and less likely, a combination of CanL with atopic dermatitis, demodicosis, exfoliative cutaneous lupus erythematous, idiopathic sebaceous adenitis and/or hypothyroidism; for all of these differentials, the possibility of secondary bacterial infection was also considered.

Microscopic examination of plucked hairs was negative for mites. Cytological examination of impression smears from the skin underneath scales revealed neutrophils with intracellular cocci and a few macrophages. Urinalysis revealed urine specific gravity of 1040 and proteinuria (UPC: 2.64; RR < 0.2).

It was concluded that DJ was affected by a severe clinical form of CanL associated with secondary bacterial infection. The dog was treated with meglumine antimoniate, at 100 mg/kg (divided every 12 h), subcutaneously (SC) and with allopurinol at 10 mg/kg, twice daily and bathed twice a week with chlorhexidine shampoo. One month later, dermatological examination revealed scarring alopecia on the lips and periocular region and mild interdigital erythema. Biochemistry profile with serum protein electrophoresis revealed mild hypoalbuminaemia (2.67 g/dL; RR: 2.93–4.12 g/dL), hypergammaglobulinaemia (1.53 g/dL; RR: 0.24–0.86 g/dL) and improved proteinuria (UPC: 1.69).

7 | Systematic Review on Treatment and Prevention of Canine Leishmaniosis

A systematic review of all active drug or placebo-controlled, randomised controlled trials (RCTs) and of recent (i.e., published between 2018 and 2022) systematic reviews or meta-analyses on the treatment and prevention of CanL was performed. To this aim, RCTs, systematic reviews and meta-analyses on the efficacy of one or more therapeutic and/or preventive interventions in dogs

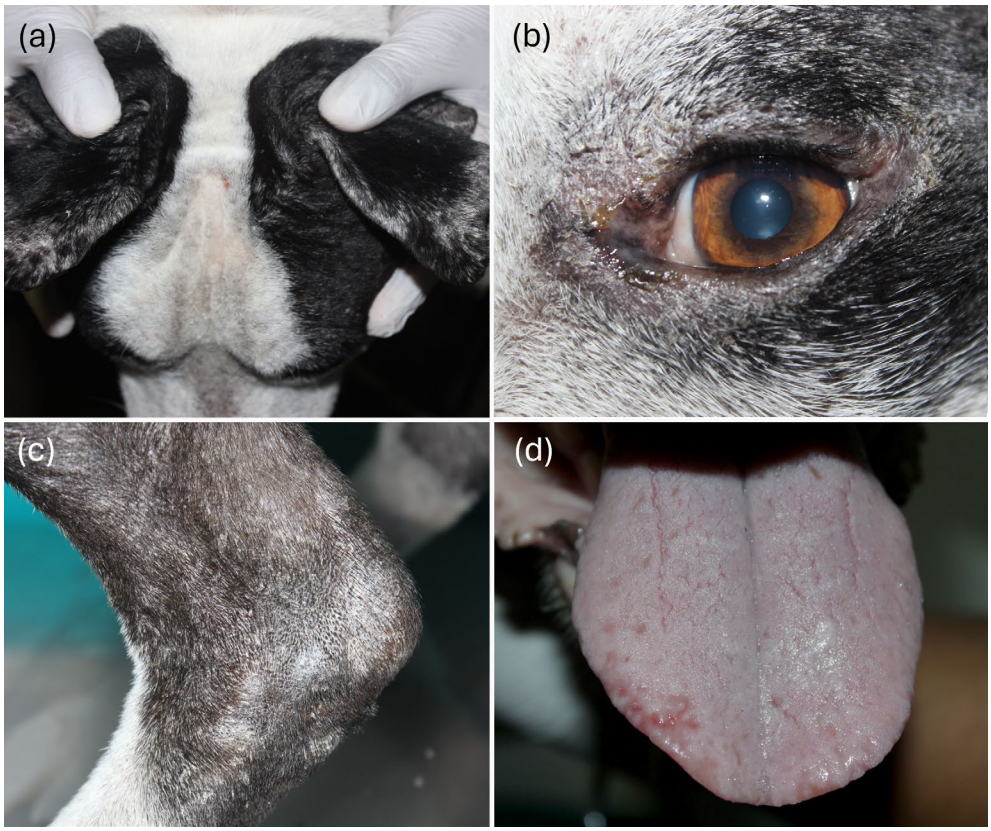


FIGURE 17 | (a) Bilateral alopecia and fine scales on the convex aspect of ear pinnae. (b) Scales on periocular skin. (c) Hypotrichosis and scales on tarsal skin. (d) Ulcers on the margins of the tongue.

with natural CanL, published in peer-reviewed journals in any language (articles published in non-English language were to have an English abstract to be considered), were evaluated.

To be eligible for inclusion, RCTs on the *treatment* of CanL had to include naturally infected dogs presenting with clinical signs and/or common and clinically important laboratory abnormalities (i.e., anaemia, hypoalbuminaemia, hyperglobulinaemia, proteinuria) compatible with the disease. In addition, the diagnosis of CanL had to be confirmed by demonstration of the infection (e.g., by molecular tests, cytology, histopathology and/or immunohistochemistry) and/or by positive serology. RCTs including both dogs with CanL and subclinically infected dogs were eligible if the results for the former could be clearly differentiated from the results for the latter dogs. RCTs on the *prevention* of CanL had to include dogs with no evidence of infection and/or subclinically infected dogs presenting no clinical signs or laboratory abnormalities compatible with CanL. RCTs including both subclinically infected dogs and dogs with CanL were eligible if the results for the former could be clearly differentiated from the results for the latter dogs.

Relevant articles were searched in Medline (via PubMed), Thomson Reuter's Web of Science and CAB Abstract (via EBSCO host) on 11 January 2023 using the following search string: "(dog OR dogs OR canine) AND (leishman*) AND (treatment OR therapy OR trial OR prevent* OR antimon* OR meglumin* OR stibogluconate* OR allopurinol* OR miltefosin* OR aminosidin* OR paromomycin* OR amphotericin* OR pentamidin* OR ketoconazol* OR itraconazol* OR fluconazol* OR metronidazol* OR azole OR spiramycin* OR terbinafin* OR diminazen* OR phosphocholin* OR furazolidon* OR sitamaquin* OR fluoroquinolon* OR enrofloxacin* OR marbofloxacin* OR trifluralin* OR bisabolol OR levamisol* OR mycobacterium OR interferon* OR interleukin* OR impromun* OR domperidon* OR glucocorticoid* OR corticosteroid* OR predniso* OR antimicrobial peptide OR cecropin OR melittin OR insecticid* OR repellent OR mesh net OR pyrethroid* OR deltamethrin* OR permethrin* OR flumethrin* OR cyhalothrin* OR oil OR citronella OR deet OR fenthion* OR diazinon* OR pyriprol* OR fipronil* OR imidacloprid* OR metaflumizon* OR amitraz OR spinosad* OR isoxazolin* OR afoxolaner OR fluralaner OR lotilaner OR sarolaner OR pheromon* OR vaccin* OR canileish OR letifend OR leishvaccine OR leishmune OR leish-tec OR leishtec)". As the previous systematic review included all relevant articles that were published between 1980 and 2004 [147], our search was limited to articles published before 1980 and after 2004 and included publications available as early-view articles published electronically ahead of printing, in 2022. The search results were tabulated and cross-checked by two authors. The titles, abstracts and when necessary, the full texts of these articles were scrutinised independently by two authors to identify those fulfilling the above eligibility criteria.

For each of the therapeutic interventions, data were initially extracted and tabulated by one author and were then cross-checked by another author. Data of interest included the following: (i) number of dogs; (ii) clinical status/severity of CanL (using any classification/scoring system); (iii) method of

diagnosis of CanL; (iv) dosage regimen (dose, route of administration, frequency of administration, duration); (v) the percentage (%) of dogs that achieved clinical cure (defined as absence of clinical signs) and the time to achieve clinical cure; (vi) the % of dogs that achieved clinical improvement without cure, the time to achieve clinical improvement and the degree of clinical improvement (using any clinical scoring system); (vii) the % of dogs that achieved either clinical cure or improvement and the time to achieve clinical cure or improvement; (viii) the % of dogs that dropped out of the study (for RCTs without intention-to-treat analysis); (ix) the % of dogs that died or were euthanised; (x) the % of dogs that relapsed after treatment discontinuation and the time to relapse; (xi) the % of dogs with normalisation of clinically important laboratory abnormalities [i.e., anaemia, thrombocytopenia, increased total proteins, decreased albumins, increased globulins, decreased albumin/globulin ratio, protein electrophoresis abnormalities, increased blood urea nitrogen (BUN), increased creatinine, increased SDMA, increased inorganic phosphorus, increased CRP or other acute phase proteins, proteinuria] and the time for normalisation; (xii) the % of dogs with improvement without normalisation of laboratory abnormalities and the time for improvement; (xiii) the % of dogs with either normalisation or improvement of laboratory abnormalities and the time for normalisation or improvement; (xiv) changes (% increase or decrease) in red blood cell parameters, serum proteins, indicators of renal function and acute phase proteins; (xv) the % of dogs with reappearance of laboratory abnormalities after treatment discontinuation and the time for reappearance; (xvi) the reduction of parasitic load, the examined organ(s) or tissue(s), the method and the time of evaluation; (xvii) the % of dogs with absence of parasites, the examined organ(s) or tissue(s), the method and the time of evaluation; (xviii) the increase of parasitic load after treatment discontinuation, the examined organ(s) or tissue(s), the method and the time of evaluation; (xix) the changes in the number of immune cells and/or of lymphocyte subpopulations and the induction of cell-mediated immunity, including the methods used for these assays and the time of evaluation; (xx) the reduction of antibody titre or optical density (OD), the method and the time of evaluation; (xxi) the % of seropositive dogs that became seronegative, the method and the time of evaluation; (xxii) the increase of antibody titre or OD after treatment discontinuation, the method and the time of evaluation; (xxiii) the reduction of parasite transmission to sand flies, the method and the time of evaluation; and (xxiv) the adverse effects of the treatment, their frequency and their severity.

For each of the preventive interventions, data were initially extracted and tabulated by one author and then they were cross-checked by another author. Data of interest included the following: (i) number of dogs; (ii) clinical status of dogs: subclinical infection and/or seropositivity or no evidence of infection, including the method(s) of evaluation; (iii) dosage regimen (dose, route of administration, frequency of administration) if applicable; (iv) the % of subclinically infected dogs that did not develop CanL and/or did not develop clinical signs of CanL and/or did not develop laboratory abnormalities of CanL; (v) the % of seropositive dogs and the % of seronegative dogs that did not develop CanL and/or did not develop clinical signs of CanL and/or did not develop laboratory abnormalities of CanL; (vi) the % of dogs with no evidence of infection that did not develop CanL and/or did not develop clinical signs of CanL and/or did not develop laboratory abnormalities of CanL; (vii) the severity of clinical signs and laboratory abnormalities of CanL (using any classification/scoring system) for those dogs that developed the disease; (viii) the immunological changes, such as changes in the number of immune cells and/or of lymphocyte subpopulations and the induction of cell-mediated immunity, including the method of evaluation (e.g., leishmanin skin test, lymphocyte proliferation assays, cytokine production by PBMCs, leishmanicidal activity of macrophages); (ix) the reduction of parasitic load of subclinically infected dogs, the examined organ(s) or tissue(s), the method and the time of evaluation; (x) the % of subclinically infected dogs and the % of seronegative dogs with absence of parasites, the examined organ(s) or tissue(s), the method and the time of evaluation; (xi) the % of dogs with absence of parasites both before and after the intervention, the examined organ(s) or tissue(s), the method and the times of evaluation; (xii) the reduction of antibody titre or OD of seropositive dogs, the method and the time of evaluation; (xiii) the % of seropositive dogs that became seronegative, the method and the time of evaluation; (xiv) the % of seronegative dogs that remained seronegative, the method and the times of evaluation; (xv) the induction of vaccine-induced antibodies; (xvi) the reduction of parasite transmission to sand flies, the method and the time of evaluation; and (xvii) the adverse effects of the treatment, their frequency and their severity.

7.1 | Evaluation of Quality of Randomised Controlled Trials

The quality of each RCT was evaluated according to Olivry and Bizikova [148] with some modifications. For each RCT on therapeutic interventions, the following parameters were assessed as 'adequate', 'unclear' or 'inadequate': (i) degree of certainty that all dogs present CanL and no concurrent diseases (Table 2); (ii) method of generation of randomisation sequences; (iii) method of concealment of allocation to treatment groups; and (iv) inclusion of cases lost to follow-up in ITT analyses. For each RCT on preventive interventions, the following parameters were assessed as 'adequate', 'unclear' or 'inadequate': (i) degree of certainty that all dogs did not present CanL (Table 3); (ii) method of generation of randomisation sequences; (iii) method of concealment of allocation to treatment groups; and (iv) inclusion of cases lost to follow-up in ITT analyses.

Each RCT on therapeutic or preventive interventions was rated as follows: (i) high quality when all four parameters were assessed as adequate; (ii) intermediate quality when at least one parameter was assessed as adequate and the remaining parameters as unclear and/or inadequate; and (iii) low quality when all four parameters were assessed as unclear and/or inadequate.

7.2 | Evaluation of Level of Evidence and Consistency Among Studies

The level of evidence (LoE) was evaluated according to Ebell et al. [149] as simplified by Bond et al. [150] as follows: (i) good quality, patient-oriented when based on high-quality RCTs or

TABLE 2 | Assessment of the degree of certainty that all dogs enrolled in a therapeutic RCT present CanL and no concurrent diseases.

Adequate	(All clinical signs and all laboratory abnormalities compatible with CanL) AND (detection of parasite or parasite DNA and/or positive serology) AND (exclusion, with reasonable certainty, of major differentials and concurrent diseases)
Unclear	(Clinical signs not typical of CanL without exclusion of more common differentials and without demonstration of parasite in the affected organs) OR (laboratory abnormalities not typical of CanL without exclusion of more common differentials) OR (negative results for the detection of parasite and/or parasite DNA in some, but not all, dogs) OR (no exclusion, with reasonable certainty, of major differentials and concurrent diseases)
Inadequate	(Clinical signs or laboratory abnormalities that cannot be explained by CanL) OR (no tests to detect the parasite or parasite DNA) OR (no serology)

Abbreviations: CanL, canine leishmaniosis due to *L. infantum*; RCT, randomised controlled trial.

TABLE 3 | Assessment of the degree of certainty that all dogs enrolled in a preventive RCT did not present CanL.

Adequate	[No clinical signs and no laboratory abnormalities (i.e., anaemia, hyperproteinaemia, hypoalbuminaemia, hyperglobulinaemia, proteinuria) compatible with CanL] OR (negative results for the detection of parasite DNA and negative serology)
Unclear	(Both molecular tests and serology not performed AND no clinical signs compatible with CanL, but presence of laboratory abnormalities compatible with CanL that were not attributed to another disease) OR (haematology and/or biochemistry and/or urinalysis not performed)
Inadequate	Clinical signs and/or laboratory abnormalities compatible with CanL without negative molecular tests and negative serology

Abbreviations: CanL, canine leishmaniosis due to *L. infantum*; RCT, randomised controlled trial.

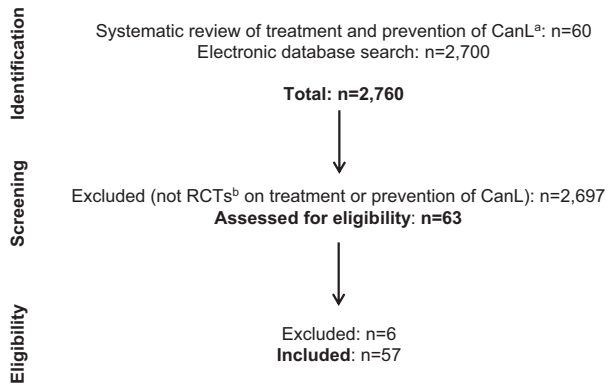
meta-analysis of consistent RCTs; (ii) limited quality, patient-oriented when based on lower quality clinical trials, cohort studies or case–control studies; and (iii) other evidence when based on consensus, usual practice, disease-oriented evidence or case series. For interventions that have been tested in more than one RCT, the consistency of the results across studies was evaluated according to Ebell et al. [149] as follows: (i) consistent when most studies found similar or at least coherent (i.e., differences were explainable) results OR when results are based on high-quality and up-to-date systematic reviews or meta-analyses; and (ii) inconsistent when there is considerable variation among study results and lack of coherence OR when high-quality and up-to-date systematic reviews or meta-analyses do not find consistent evidence.

7.3 | Strength of Recommendation

Based on the quality of RCTs, LoE and consistency among studies, the strength of recommendation (SORT) was characterised [149, 150] as (i) strong when based on consistent and good quality patient-oriented evidence; (ii) moderate when based on inconsistent or limited quality patient-oriented evidence; or (iii) weak when based on consensus, usual practice, disease-oriented evidence, or case series.

7.4 | Eligible Randomised Controlled Trials

The previous systematic review included 60 references [147] and search of the three electronic databases yielded 2700 additional articles, resulting in a total of 2760 articles. At initial screening 2697 were excluded, and 63 articles were considered further. Of these 63 articles, six were excluded for one of the following reasons: systematic review evaluating only one RCT that has been included as separate publication ($n = 1$), RCT on the treatment of CanL not including dogs with clinical signs and/or laboratory abnormalities due to CanL ($n = 1$), RCT on the treatment of CanL including both dogs with CanL and subclinically infected dogs because the results for the former could be clearly differentiated from the results for the latter dogs ($n = 1$) and RCTs on the treatment or prevention of CanL not reporting data of interest for this systematic review ($n = 3$). Therefore, 57 articles (55 RCTs and two meta-analyses) were evaluated (Figure 18).



[a] CanL: canine leishmaniosis due to *L. infantum*

[b] RCTs randomized controlled trials

FIGURE 18 | Flow diagram of the selection of eligible randomised control trials and meta-analyses.

8 | Treatment of Canine Leishmaniosis

8.1 | Indications for Euthanasia (Instead of Treatment)

Euthanasia of dogs with CanL (and, even more, of subclinically infected seropositive dogs) has been advocated, and is still enforced by the legislation of some countries, as an effective measure to decrease the incidence of human VL due to *L. infantum*. The rationale behind this approach is that in endemic areas, dogs are the major peridomestic reservoir of the parasite and the primary source of its transmission to vectors. There is some evidence in favour of the efficacy of this strategy in a few countries, like China, after periods of massive eradication of all dogs (irrespective of their infectious and serology status) combined with extensive use of environmental insecticides [151]. However, this approach could possibly block the transmission cycle of *L. infantum* only if seropositive dogs were the sole reservoir capable to transmit the parasite, if all seropositive dogs could be identified and euthanised, and if euthanised dogs were not replaced by new ones that may also become reservoirs. Nowadays, it is clear that none of these conditions holds true: other domestic animals (e.g., cats) [152–155] and wildlife can infect sand fly vectors and can sustain or greatly amplify parasite transmission (e.g., hares and rabbits in Madrid) [17, 18], some subclinically infected seronegative dogs may also transmit *L. infantum* [156, 157] and euthanasia of owned dogs is commonly declined by their owners [157]. Moreover, identification of seropositive stray dogs, which are numerous in many endemic areas, is impractical and labor-intensive; [156] massive euthanasia of dogs is, at minimum, ethically questionable and opposed to the role of these animals in modern society; [158] and the euthanized dogs are frequently replaced by new ones [159, 160]. All the above explain the results of epidemiological models showing that euthanasia is the least effective measure for the control of human VL due to *L. infantum*, the reoccurrence of the disease in China and the limited efficacy of the euthanasia plus environmental insecticide-based VL control programme in Brazil [158, 161–165].

A second argument in favour of euthanasia is that the alternative option, namely the treatment of dogs with CanL, will induce drug-resistant strains of *L. infantum* that may result in cases of human VL unresponsive to the same and to cross-resistant drugs. However, in addition to the great reduction of infectivity to sand flies during treatment of CanL [166], the avoidance of first-line drugs for treatment of human VL in the same area along with the systematic use of insect repellents and administration of isoxazolines during and after treatment, makes the justification of this argument very difficult.

Finally, it has been proposed that euthanasia of dogs with CanL (or of all infected dogs) living in non-endemic areas may prevent the establishment of the infection [167]. If such an area is close to an endemic one, the environmental conditions are favourable for sand fly vectors, and there is an adequate number of susceptible dogs, spread of the infection seems unavoidable [7–9]. On the other hand, in the absence of sand fly vectors, infection can be eradicated if infected dogs are removed from the breeding stock and are not used as blood donors [25, 26].

Despite the limited efficacy of euthanasia in terms of public health and of geographical containment of the infection, there are circumstances where it is indicated or may be considered on ethical grounds, such as the following: (a) inability to offer optimal treatment and to systematically apply transmission blocking measures (e.g., owner's refusal due to financial or other reasons, stray dogs) to dogs with CanL at a stage where self-cure is unlikely; (b) poor prognosis, usually due to advanced CKD and less commonly due to liver failure; and (c) poor quality of life despite treatment.

Conclusion: Euthanasia of dogs with CanL cannot be recommended as a tool to decrease the incidence of human VL due to *L. infantum*, to avoid induction of drug-resistant parasites and to block the expansion of endemic areas (SORT: moderate). However, euthanasia of individual dogs with CanL may be considered if proper treatment cannot be administered and prognosis or their quality of life is poor (SORT: weak).

8.2 | Indications and Aims of Treatment

With the exceptions listed in the previous section, all dogs with CanL should be treated with drugs having direct anti-*Leishmania* activity, with immunostimulants or with their combination. Routine treatment of subclinically infected dogs, especially those with high or increasing antibody titres, has also been proposed because of a perceived high risk to develop CanL [168]. This practice is discouraged as the widespread use of drugs with direct anti-*Leishmania* activity promotes drug resistance [169], and the majority of subclinically infected seropositive dogs will not develop CanL, at least during the next 8–12 months [142, 170]. However, administration of immunomodulators, like domperidone or dietary nucleotides plus active hexose correlated compound (AHCC) that cannot induce drug-resistance and are generally safe, should be considered [170, 171], in addition to the regular clinical and clinicopathological monitoring (more frequently than every 4 months, especially for dogs with moderate-to-high antibody titres), and use of insect repellents with proven efficacy against sand flies biting [107, 170, 172].

Treatment of CanL should lead to clinical cure (resolution of clinical signs and important clinicopathologic abnormalities, such as anaemia and proteinuria), halt the progression and, if possible, reverse organ damage, reduce parasitic load, avoid the induction of drug-resistant strains of *L. infantum*, minimise the infectivity of dogs to sand flies, promote a strong and long-lasting *Leishmania*-specific cell-mediated immunity that will prevent disease recurrence and be safe [173]. Complete elimination of the parasite (parasitological cure) is rarely, if ever, achieved, is meaningless in endemic areas because exposure to new sand fly bites cannot be completely avoided, and may not be prudent, as a small number of viable parasites may contribute to immunological memory [174].

To this aim, the ideal drug (or drug combination) for the treatment of CanL should be highly effective and safe (based on the results of RCTs in dogs with natural CanL), administered orally (to permit outpatient treatment and to avoid injection site adverse effects), reasonably priced, registered for the treatment of CanL and not be a first-line drug for the treatment of human VL

in the same area. Unfortunately, a drug or a drug combination with all these attributes does not exist.

Conclusion: Drugs with direct anti-*Leishmania* activity and/or immunostimulants should be used for the treatment of dogs with CanL. Administration of drugs with direct anti-*Leishmania* activity should be avoided in subclinically infected dogs, irrespectively of the results of serology (SORT: weak). The aim of the treatment is not the parasitological cure, but the induction of *Leishmania*-specific cell-mediated immunity (SORT: weak).

8.3 | Drugs With Direct Anti-Leishmania Activity

The drugs with direct anti-*Leishmania* activity that are used more commonly for the treatment of CanL include pentavalent antimonials, particularly meglumine antimoniate and non-antimonial drugs including miltefosine and allopurinol.

8.3.1 | Meglumine Antimoniate

Despite using pentavalent antimonials for more than 70 years, the exact mechanism of their action remains unclear. After entering cells, including host macrophages and *Leishmania* amastigotes, pentavalent antimony (Sb^V) is reduced to the active trivalent form that causes amastigote apoptosis, probably through inhibition of ATP and GTP synthesis, change of the structure and function of glucosomes with ensuing disturbance of glucose and fatty acid metabolism, inhibition of the citric acid cycle and inhibition of trypanothione reductase with subsequent thiol loss [175–177]. Furthermore, antimonials may have additional indirect anti-*Leishmania* effects, including increased macrophage and neutrophil phagocytic and leishmanicidal activity [178].

After SC administration in dogs, meglumine antimoniate is completely absorbed, reaches maximum plasma concentrations within 2–4 h, and is quickly eliminated with the urine, having a serum half-life of approximately 2 h [179]. However, antimony accumulates in macrophages where it remains for at least 3 days [180], and this permits administration once per day. The recommended dose of meglumine antimoniate is 100 mg/kg (corresponding to 28.3 mg antimony/kg) SC, once daily or divided in two daily doses, for 28 days (4 weeks) [147, 179, 181–184]. A dose decrement may be indicated in dogs with CKD due to the anticipated reduced excretion of the drug [185].

The efficacy and safety of meglumine antimoniate in dogs with CanL have been evaluated in eight RCTs (Table S1) [181, 184, 186–191]. In these studies, two routes of drug administration [SC and intravenous(IV)] were compared [184]; meglumine antimoniate was compared to miltefosine [181], aminosidine [186], MTC-305 (an *O*-alkyl-hydroxamate derivative) [187], (−)-a-bisabolol [188], a vaccine containing LiF2 (a purified fraction of *L. infantum* promastigotes) [190], two vaccines containing the adjuvant MPL-SE plus recombinant antigens (Leish-110f [189] or Leish-111f) [191] and to placebo [189, 191]. Meglumine antimoniate monotherapy was compared to its combination with aminosidine [186], MTC-305 [187], LiF2 [190], Leish-110f with MPL-SE [189], Leish111f with MPL-SE [191] and MPL-SE [189]. In six of these studies the daily dose of the drug was 100–106 mg/kg, the duration of treatment ranged from 20 to 28 days, and in two of them a second treatment 'cycle' was administered 1 month after the end of the first one [187, 188]. In another study the second 'cycle' was administered if there was not a complete remission 3 weeks after the end of the first one [184]. In the remaining two RCTs, meglumine antimoniate was administered at 20 mg/kg, once daily for 30 days [191] or at 300 mg/kg every second day for a total of 20 administrations (i.e., 40 days) [190]. The number of dogs with CanL treated with meglumine antimoniate varied from six [187–189] to 57 [181]. The confirmation of CanL diagnosis was based on serology (6/8) [181, 184, 186–188, 191] and/or on the demonstration of parasite by microscopy and/or culture (6/8) [181, 184, 186, 189–191] and/or on the demonstration of parasitic DNA with molecular methods (3/8) [181, 187, 188]. The severity of CanL is reported only in one study that included dogs at LeishVet stages I, II and III [187], whereas in two other studies, serum creatinine concentration within reference range was an inclusion criterion [181, 184]. The quality of these studies is intermediate [181, 186–188, 190, 191] or low [184, 189].

According to the results of these studies, the efficacy varies dependent mainly on the time of evaluation. When assessed at the end of treatment period, clinical cure or improvement was witnessed in 81%–100% of dogs [184, 186]; when assessed 2 weeks after the end of treatment, total clinical score was significantly (by 63.4%) lower compared to pre-treatment score [181]. When assessed 4–5 months after the end of treatment, clinical cure or improvement were recorded in 33.3%–100% of dogs [187–191] but the total clinical score was numerically higher than before treatment [187, 188] and 0%–33.3% of dogs had died of CanL [187–191]. When assessed 3 years after the end of treatment, clinical cure or improvement were recorded in 63.6%, but 26.7% of dogs had died of CanL [191]. The difference between early and late efficacy occurred because up to 74.3% of the responders relapsed 40 days to 44 months after the end of the treatment period [184, 186–190].

Data which can be extracted from these RCT's on the evolution of clinically important laboratory parameters, as assessed during and after meglumine antimoniate administration, are limited. Haematocrit increased significantly at the end or 1 month after the end of treatment [184, 189], albumin concentration increased significantly at the end of treatment [189], gamma-globulin concentration was within reference range 5 months after the end of treatment [189], serum creatinine concentration did not increase [184] or was higher at the end of 2 weeks after the end of treatment compared to time 0 in 10%–10.8% of the dogs [181, 186] or increased significantly [188], and the prevalence of proteinuria was significantly lower at the end of treatment [184].

A reduction of parasitic load, based on bone marrow [181, 186, 190] and/or lymph node microscopy [186] at the end of treatment [186] and after 14 [181], 40 [186], 50 [190], 100 [186] or 140 days [190] was a uniform finding in the 3 RCTs where this parameter was evaluated. Of the dogs with positive microscopy and/or culture (bone marrow, lymph node, skin) before treatment, 80%–100% and 37.5%–75% were negative for the same examinations at the end [184, 186] or 1–5 months after the end of treatment [186, 189, 190], respectively. However, in

parallel with the clinical relapses after treatment discontinuation, bone marrow and lymph node qPCR changed from negative at 1 month to positive at 4 months after the end of treatment in 33.3% of the dogs, whereas only 16.7% of them were negative [187, 188]. In addition, bone marrow and lymph node parasitic load 5 months after the end of treatment were no longer lower than on Day 0 [186]. Also, 100% (4/4) of the dogs that survived 5 months after the end of treatment were infectious to sand flies [189].

Meglumine antimoniate administration enhanced the parasiticidal activity of macrophages, and this effect was more pronounced in dogs with lower parasitic burden after treatment [190], whereas *Leishmania*-specific antibody concentrations remained unchanged [186], decreased [181, 189] or initially decreased and then increased [187, 188], depending on the duration of the RCT and the timepoint(s) of evaluation.

The prevalence of adverse drug reactions was 9.5%–66.7% [181, 184, 187], but none of them was severe [186]. Reported adverse effects include injection site reactions, sometimes associated with local oedema and lameness [181, 184], depression [181], lethargy [181], anorexia [181], weight loss [181, 187], vomiting [181] and diarrhoea [181]. Less common adverse effects reported in non-RCTs include hyperthermia, acute pancreatitis, deterioration of kidney function, uveitis, arthritis and leukopenia [192–196]. However, it is unclear if some of these clinical signs could be due to parasite-mediated effects such as massive death of parasites with subsequent release of antigens that trigger or exacerbate immune-complex mediated pathologies, such as uveitis and polyarthritis.

Finally, although not examined in these RCTs, it is well established that the widespread use [197] and the repeated administration of meglumine antimoniate to the same dog [198, 199] promote drug resistance in *L. infantum*. Despite these drawbacks, meglumine antimoniate monotherapy has been proposed for dogs at LeishVet stage I of CanL [88, 99]. This probably stems from dogs with papular dermatitis and no additional clinical or laboratory abnormalities, where strong *Leishmania*-specific cell-mediated immunity exists. These dogs may respond to this treatment without relapsing or progressing to a more advanced stage of the disease. However, they may also self-cure or may respond to topical treatment (see below) [200], and in the absence of RCTs showing an advantage of parenteral meglumine antimoniate administration, this suggestion cannot be supported.

Conclusion: Meglumine antimoniate monotherapy cannot be recommended for the treatment of CanL because of frequent relapses which may lead to the death of the dog, or necessitate repeated administration that promotes drug resistance (SORT: strong).

Compared to the free drug, *liposomal* formulations of *meglumine antimoniate* offer the theoretical advantage of longer half-life and increased antimony concentrations in target organs, like liver and spleen [201–206]. They have been evaluated in three RCTs (Table S2) [202, 207, 208]. In all of them, the drug was administered at the dose of 23 mg/kg (corresponding to 6.5 mg antimony/kg) IV every 4 days, for a total of four [202] or six [207, 208] injections. In these studies, liposomal meglumine antimoniate was compared to allopurinol [208], anti-canine interleukin-10 receptor (IL-10R) monoclonal antibody [207], placebo [202, 208] and to liposomal meglumine antimoniate–allopurinol combination [208]. The number of dogs treated with liposomal meglumine antimoniate varied from eight [208] to 12 [202], the diagnosis of CanL was confirmed by serology (3/3) [202, 207, 208], bone marrow culture (1/3) [207] and bone marrow PCR (1/3) [208], and the severity of CanL was not reported in one RCT [207], another study included 1 of 8 Stage I dogs, 2 of 8 Stage II dogs, 3 of 8 Stage III dogs and 2 of 8 dogs of unknown stage classified with a modified LeishVet staging algorithm [208], and the third RCT enrolled four 'asymptomatic', four 'oligosymptomatic' and four 'symptomatic' dogs [202]. The quality of these studies is intermediate [207] or low [202, 208].

The results of these RCTs on the efficacy of liposomal meglumine antimoniate are inconsistent: in one study, none of the dogs responded, 33.3% were euthanised due to CanL and 4.5 months after the end of treatment the clinical score was higher compared to Day 0 [202]. In another study, there was a non-significant decrease of clinical score at 10 and at 70 days after the end of treatment that was followed by deterioration and, 3 months later, the clinical score was only 2.5% lower than before treatment [207]. And in the third study, 62.5% of the dogs showed clinical improvement at 6 months, whereas 25% had died of unrelated reasons [208]. The evolution of laboratory parameters is presented in only one RCT [207], where 10 days after the end of treatment PCV was significantly higher compared to time 0, and there were no significant changes in platelet count, as well as in total protein, globulin, urea nitrogen and creatinine concentrations.

Comparisons of qPCR results on parasitic load before and after treatment are somewhat inconsistent: at 4 months it was decreased in the bone marrow and spleen [208], at 5 months it was not decreased in the bone marrow [207] and at 6 months it was decreased in the spleen and skin, but not in the bone marrow [208]. Also, all surviving dogs had positive bone marrow culture 4.5 months after the end of treatment [202]. A reduction of infectivity to *L. longipalpis* was recorded in one study: although the decrease of the prevalence of dogs positive in xenodiagnosis was not significant (from 50% before treatment to 16.7% at 4 months and to 33.3% at 6 months after the end of treatment), the percentage of infected sand flies was significantly lower at both post-treatment time points compared to baseline [208]; this finding was further strengthened by the results of another RCT where the percentage of infected sand flies was significantly lower among those fed on treated dogs 4.5 months after the end of treatment, compared to those fed on placebo-treated dogs [202].

Seventy days after the end of treatment, in addition to the increased peripheral blood lymphocyte subpopulations (CD3+, CD4+, INF-γ-positive CD4+, CD8+ and CD21+ cells), *Leishmania* antigen-induced proliferation of CD4+ cells was significantly higher compared to baseline [207]. However, at the same time point, the numbers of interleukin-4 (IL-4)-positive CD4+ cells were also higher compared to time 0, and 3 months later, IL-10 production by PBMCs after stimulation with *Leishmania* antigen increased despite progressive clinical deterioration [207]. Also, the levels of *Leishmania*-specific IgG 6 months after the end of treatment did not differ from baseline [208].

Although liposomal meglumine antimoniate is considered safer than the free drug due to the lower total dose of antimony [202], transient adverse effects occurred in up to 90% of the dogs during and soon after the IV administration, and they included tachycardia, tachypnoea, salivation, vomiting, defecation, urination, prostration, mydriasis followed by miosis, ataxia and tremors [202, 207].

Conclusion: Liposomal formulations of meglumine antimoniate monotherapy cannot be recommended for the treatment of CanL because of the frequent relapses, which may lead to the death of the dog (SORT: moderate).

A RCT that was not included in our systematic review because it was published after 2022, examined the efficacy and safety of a topical formulation of meglumine antimoniate (30% in pluronic F-124), sprayed twice daily for 1 month over the skin lesions of dogs with the papular form of CanL, that (with very few exceptions) was not accompanied by other clinical signs or laboratory abnormalities. The results were promising because there were no adverse effects and a complete response to treatment was recorded in 70% of the dogs [200].

8.3.2 | Miltefosine

Miltefosine is a repurposed drug that was initially developed as antineoplastic agent [209] and later approved for the treatment of CanL. In some countries it is approved for the treatment of human VL and CL. Miltefosine accumulates inside macrophages and causes apoptosis-like death of amastigotes through interference with multiple metabolic pathways, including those responsible for lipid and ATP synthesis and calcium homeostasis [210]. Also, it has indirect anti-*Leishmania* effects by activating macrophages, T lymphocytes and Th1-like immune responses [211]. After oral administration (in the food) at the registered dose of 2 mg/kg once daily, it is well absorbed and accumulates very slowly in the body due to the long half-life. The duration of treatment is 4 weeks.

The efficacy and safety of miltefosine in dogs with CanL have been evaluated in one RCT (Table S3) of 6-week duration (drug administration during the first 4 weeks and 2-week follow-up) [181]. In this study, miltefosine was compared to meglumine antimoniate; 46 miltefosine-treated dogs were eligible for the evaluation of efficacy and 55 for the evaluation of safety. For confirmation of CanL, diagnosis was based on serology and/or bone marrow microscopy and/or bone marrow PCR and a serum creatinine concentration within reference range was an inclusion criterion. The quality of the study is intermediate. At 6 weeks, total clinical score was significantly decreased (by 51.1%) and in 52.2% of the dogs it was ≥ 60% lower than baseline; however, 23.3% of the initially enrolled dogs had been lost to follow-up and intention-to-treat (ITT) analysis was not performed. Considering laboratory parameters, none of the dogs had higher creatinine concentration at study conclusion compared to time 0. The parasitic load was probably reduced, because only 10% of the 30 dogs with positive bone marrow microscopy before treatment were still positive at the end of the trial. The effect of treatment on parasite transmission to sand flies or on *Leishmania*-specific cell mediated immunity was not evaluated and a ≥ 2-fold decrease of IFA titre was recorded in 9.1% of the dogs. The prevalence of adverse drug reactions was 30.9%, including depression or lethargy (3.6%), anorexia, vomiting and/or diarrhoea (30.9%) and polyuria-polydipsia (1.8%) [181].

In other studies, including one RCT published after 2022 [212] and five open trials [213–217], the clinical efficacy was variable: 1 month [213, 217] and 2 months [212] after end of treatment, 20% of dogs did not present clinical signs and at 23 months, 50% of dogs were considered clinically cured [214]; compared to time 0, the clinical score was [217] or was not [212] significantly lower at the end of treatment period, it was significantly lower after 2 [216] and 4 weeks [217] and it was [216] or was not [212] significantly lower after 2 months; by the end of treatment period, 90% of dogs showed clinical improvement [215], and after 2 weeks 50% of dogs had > 75% reduction of clinical score compared to baseline [213, 217]; however, 2 months and 2 years after end of treatment, 20% [212] and 14.3% [214], of dogs, respectively, had died or were euthanised due to CanL. Of the clinically important laboratory parameters, 1 month after end of treatment there was no change in haematocrit, platelet count, total protein, beta-1 globulin, beta-2 globulin and gamma-globulin concentration [217], but albumin concentration and albumin/globulin ratio were significantly higher compared to time 0 [217]. Short-term data on parasitic load are conflicting, with some studies showing a reduction [214–216], but the RCT showing lack of change at the end of treatment period and 2 months later [212]. Nevertheless, long-term monitoring showed that parasitic load started to increase [215]. The percentage of treated dogs that were infectious to sand flies was decreased 2 months after end of treatment (from 51.4% to 25.7%) [216], whereas, at the same time point, IFA titre did not differ significantly from baseline [212]. Subsequently, antibody levels decreased only to increase again 10 months after end of treatment [214].

Induction of miltefosine-resistant strains of *L. infantum* is to be expected due to the long half-life that exposes surviving parasites to subtherapeutic levels of drug after treatment discontinuation, and it has been confirmed in a dog treated with the miltefosine–allopurinol combination [218].

Like for meglumine antimoniate, miltefosine monotherapy for dogs at LeishVet stage I of CanL [99] cannot be adopted without further studies.

Conclusion: Miltefosine monotherapy cannot be recommended for the treatment of CanL because of limited data on long-term efficacy along with the conflicting results of non-controlled trials (SORT: moderate).

8.3.3 | Allopurinol

Allopurinol is parasitostatic for *L. infantum* promastigotes and intracellular amastigotes, an effect mediated by interruption of parasite purine salvage pathway and protein synthesis [219, 220]. The recommended dose is 10 mg/kg, orally, twice daily for at least 6–12 months [221].

The efficacy and safety of allopurinol have been evaluated in five RCTs (Table S4) [208, 221–224]. In these studies, allopurinol

was compared to liposomal meglumine antimoniate [208], placebo [208, 221] or no treatment [222, 224], and allopurinol monotherapy was compared to its combination with meglumine antimoniate [223], liposomal meglumine antimoniate [208, 224] or a defined subunit vaccine (Leish-F2) formulated with second-generation lipid adjuvant in stable emulsion (SLA-SE) [222]. The daily dose of the drug varied from 20 to 60 mg/kg, that were either administered once daily [208, 222] or were split and given twice daily [221, 223, 224] for 60–140 days. The number of allopurinol-treated dogs varied from six [222, 223] to 37 [221], and the confirmation of CanL diagnosis was based on serology (4/5) [208, 221, 223, 224] and/or the demonstration of parasite by microscopy (1/5) [223] and/or the demonstration of parasitic DNA in bone marrow with molecular methods (3/5) [208, 221, 222, 224]. The severity of CanL is reported in two studies: in one of them, CanL was classified as Stage I (6.3%), Stage II (56.3%) or Stage III (37.5%) using a modification of the LeishVet classification system [208], whereas, in the second study, CanL was classified as Stage I (12.5%), Stage II (75%) or Stage III (12.5%) using a clinicopathological scoring system from 0 to IV [224]; in two additional studies, dogs with CanL and CKD [221, 223] or liver insufficiency [223] were excluded. The quality of these five studies is intermediate [221–223] or low [208, 224].

Complete clinical cure was rare (2.7% of dogs after 4 months of treatment [221], and 6.3% of dogs 2 months after treatment discontinuation) [208], but clinical improvement was seen after 20 days [223] and in at least 25% of dogs between the end of treatment and the following 2 months [208]. Also, at the end of a 4-month treatment period severity of 10 clinical signs of CanL was significantly lower compared to time 0 [221], whereas 2 months or 9 months after the end of a 140-day or a 3-month treatment period, respectively, clinical score was significantly lower than in the placebo groups [208, 222]. However, clinicopathological score on treatment day 130 did not differ between allopurinol-treated and untreated dogs [224], 42.9% of dogs relapsed within a 4-month period after treatment discontinuation [224], and 8.1%–12.5% of the dogs died due to CanL [221, 224].

Prevalence of some clinicopathologic abnormalities, such as anaemia (after 1 month [223] and 4 months [221] of treatment), hyperproteinsemia, hyperglobulinsemia, decreased albumin/globulin ratio and increased inorganic phosphorous (after 4 months of treatment) [221], was significantly lower than on time 0; on the contrary, there was no significant change in the prevalence of hypoalbuminsemia, increased BUN, increased creatinine and proteinuria [221]. Allopurinol administration for 2 months resulted in a significant decrease of total protein, CRP and ceruloplasmin concentration and in non-significant changes in albumin, alpha-2 and gamma-2 globulins [223]. Also, after 130 days of treatment, BUN and creatinine concentrations did not differ from their values on Day 0 [224].

Data on the efficacy of allopurinol in terms of parasitic load reduction are conflicting. Semi-quantitative microscopic examination of lymph node and bone marrow smears showed a significantly lower number of amastigotes after 4 months of treatment but all dogs were bone marrow PCR-positive [221]. The latter was also found after drug administration for 130 days, based on bone marrow, liver and spleen qPCR and on skin IHC, but parasitic density was not lower than on Day 0 [224]. When allopurinol was administered for 140 days and the results of qPCR were compared between baseline and 2 months after treatment discontinuation, skin but not bone marrow parasitic density had decreased significantly [208]. Finally, when it was administered for 3 months, and bone marrow qPCR was performed during treatment (Day 63) as well as 3 and 9 months after treatment discontinuation, parasitic density was higher at the latter two time points and all dogs were qPCR-positive at 9 months [222]. Allopurinol treatment reduces infectivity of dogs to sand flies: 56.3% (9/16) dogs with CanL were infectious to *L. longipalpis* before treatment versus 0% (0/16) at the end of a 140-day treatment period and 6.3% (1/16) 2 months after treatment discontinuation; the percentage of infected sand flies was significantly lower at the latter time point compared to baseline [208].

There are no data on the effect of allopurinol on *Leishmania*-specific cell-mediated immunity and the available information on humoral immunity is inconsistent. In one study, administration for 4 months resulted in significant reduction of IFA titres and ELISA ODs, and 5.9% of dogs became negative in both tests [221]. On the contrary, in two other studies, reduction of IFA titres or ELISA ODs was not significant at either the end of allopurinol administration for 130–140 days or 2–4 months after drug withdrawal [208, 224].

In two RCT no adverse effects were noticed [221, 222], but in another study, allopurinol administration at much higher than the recommended dose (30 mg/kg, twice daily) for 130 days resulted in kidney xanthine deposits in half of the dogs [224]. Xanthinuria, that can also lead to lithiasis in renal pelvis and/or urinary bladder, is a well-known adverse effect of allopurinol and may be prevented by feeding a low-protein, low-purine diet for the whole period of drug administration [225].

Long-term allopurinol administration probably promotes the development of resistant strains of *L. infantum* due to their positive selection under drug pressure, and this has been linked to relapses of CanL [169].

The recommendation of using allopurinol monotherapy in dogs at LeishVet stage I of CanL (mainly dogs with papular dermatitis) [99] cannot be adopted for the same reasons given for meglumine antimoniate and miltefosine. Due to lack of relevant studies, it is much more difficult to give evidence-based recommendations in favour [99] or against allopurinol single-agent therapy for dogs at Stage IV of CanL (CKD stage III–IV or extreme proteinuria with or without thromboembolism). If treatment is attempted despite poor prognosis, the initial aim should be to halt the deterioration of and to improve kidney function [172]; subsequent anti-*Leishmania* treatment should be effective enough to reduce parasitic burden and the deposition of immune-complexes in the glomeruli, and allopurinol does not seem to fulfil this criterion.

Conclusion: Allopurinol monotherapy cannot be recommended for the treatment of CanL because of the limited efficacy in terms of clinical improvement and amelioration of clinicopathologic abnormalities (SORT: strong) and the inconsistent efficacy for the reduction of parasitic load (SORT: moderate).

8.3.4 | Aminosidine (Paromomycin)

Aminosidine is an aminoglycoside antibiotic with broad antiprotozoal activity that is not shared with the other drugs of the same class. After binding to 30S ribosomal subunit of the parasite, it inhibits protein synthesis and subsequently blocks energy production and alters membrane permeability, leading to death [226, 227]. The recommended dose is 15 mg/kg, SC, once daily, for at least 3 weeks [228, 229].

The efficacy and safety of aminosidine have been evaluated in a single RCT (Table S5) [186], where it was compared to its combination with meglumine antimoniate and to meglumine antimoniate monotherapy. Eleven dogs with CanL of unknown severity, confirmed by serology and microscopy or culture, were treated with aminosidine for 21 days; the quality of the study is intermediate. Unfortunately, the daily dose of the drug (7 mg/kg, split into two daily SC injections) was approximately half the recommended one, thus limiting the relevance of the results.

Ten of the 11 dogs (90.9%) responded to the treatment, and their response was considered complete (1/10), good (5/10) or moderate (4/10). However, 8 of 10 (80%) relapsed within approximately 40 (4/8) or 160 (4/8) days after treatment discontinuation. Bone marrow microscopy showed a significant reduction of parasitic load at the end of treatment, and after 40 and 100 days compared to Day 0, but the significance was lost at 160 days, and there was no similar change in lymph nodes. In total, both bone marrow and lymph node microscopy were negative for *Leishmania* amastigotes in 54.5% (6/11) of the dogs at the end of treatment and after 40 days, in 45.5% (5/11) after 100 days and in only 18.2% (2/11) after 160 days, and there were no significant changes in IFA titre at any of the above time points. There were no serious adverse effects and only 1 of 11 (9.1%) dogs had a transient increase in BUN and creatinine concentrations [186], which is similar to the results of an open study using the recommended dose (15 mg/kg, SC, once daily) of the drug [229].

No information on the evolution of important clinicopathologic abnormalities, the infectivity to sand flies or the possible changes in cell-mediated immune response against the parasite, during and after treatment, is provided by the RCT. However, an open trial using the recommended dose of aminosidine showed a significant increase of haematocrit and Hb 3 months after the end of treatment [229], and another study using a lower dose (5 mg/kg, SC, twice daily, for 28 days) showed a tendency for increased albumin/globulin ratio and decreased proteinuria [230].

There are no studies on the induction of resistant strains of the parasite during aminosidine treatment, but there are some data supporting that antimony-resistant *L. infantum* can show cross-resistance to aminosidine [199]. Finally, being an aminoglycoside, aminosidine may promote bacterial resistance.

Conclusion: Aminosidine (paromomycin) cannot be recommended for the treatment of CanL because of the relapses after treatment discontinuation (SORT: moderate).

8.3.5 | Marbofloxacin

Marbofloxacin is a third-generation fluoroquinolone that inhibits *Leishmania* topoisomerases and subsequently interferes with the replication of parasite DNA. Moreover, it increases tumour necrosis factor-a (TNF-α) and nitrogen dioxide production by infected macrophages [231]. The recommended dosage regimen for the treatment of CanL is 2 mg/kg orally once daily for 10–40 (usually 20–28) days [232].

The efficacy and safety of marbofloxacin have been evaluated in one RCT (Table S6) [232], where a comparison was made among different treatment durations (10, 20, 28 and 40 days), at the recommended daily dose of 2 mg/kg. Twenty-four dogs with CanL of unknown severity, confirmed by lymph node microscopy and culture, were included, and the quality of the study is intermediate.

Twelve weeks after treatment initiation 66.7% (16/24) of the dogs were considered clinically cured, and 8.3% (2/24) improved. However, five dogs relapsed within 9 months from the beginning of treatment. Lymph node microscopy showed a significantly lower parasitic density at 12 weeks compared to time 0 and none of the dogs presented adverse effects [232].

No information on the evolution of important clinicopathologic abnormalities, the infectivity to sand flies or the possible changes in cell-mediated and humoral immune responses against the parasite is provided by the RCT. However, in an open trial of dogs with CanL and CKD [International Renal Interest Society (IRIS) stage I (39.3%), II (21.4%), III (28.6%) or IV (10.7%)], there were no significant changes in haematocrit, BUN, creatinine, inorganic phosphorus and UPC, whereas albumins increased and globulins decreased at the end of the 4-week treatment period [233]. In another open trial where 61 dogs with CanL received marbofloxacin for 4 weeks, ELISA ODs did not differ between time 0 and 3 months later [213]. Finally, being a fluoroquinolone, marbofloxacin can promote bacterial resistance and for this reason it is considered a second-tier antimicrobial [234].

Conclusion: Marbofloxacin cannot be recommended for the treatment of CanL because of the relapses after treatment discontinuation (SORT: moderate), the paucity of information on critical features of this treatment, such as the effect on infectivity to sand flies and on parasite-specific cell-mediated immunity, and the risk to induce bacterial resistance (SORT: weak). Administration of marbofloxacin should be considered in dogs with CanL and concurrent bacterial infections if the responsible organisms are resistant to first-tier antibacterials and susceptible to marbofloxacin, and perhaps in dogs with CanL and CKD IRIS stage III or IV (SORT: weak).

8.3.6 | Metronidazole

After enzymatic activation, metronidazole produces toxic metabolites causing damage to *Leishmania* DNA. The dose that was used is 25 mg/kg orally once daily for 90 days, and the veterinary product that has been administered to dogs with

CanL contains also spiramycin (150,000 UI/kg orally once daily) [235].

One RCT (Table S7) [235] compared the efficacy and safety of metronidazole (with spiramycin) in 13 dogs with CanL of unknown severity, with that of meglumine antimoniate–allopurinol combination. The diagnosis of CanL was confirmed by serology, microscopy and/or PCR and the quality of the study is intermediate.

By the end of the 3-month treatment period, the clinicopathological score was significantly lower compared to Day 0; specifically, it was decreased in 83.3% (10/12) of the dogs, increased in 16.7% (2/12) and one dog had been removed from the study because it developed pemphigus foliaceus. Clinical improvement occurred after 15–45 (median 15) days, but in 33.3% (3/9) of dogs it was followed by an increase of clinicopathological score, beginning at 30–60 days after treatment discontinuation. The only available specific information on the evolution of clinicopathologic abnormalities is that BUN increased in 7.7% (1/13) of the dogs. Four months after treatment discontinuation, bone marrow, lymph node and/or blood PCR was positive in all dogs and ELISA ODs were like those before treatment. Apart from the dog with pemphigus foliaceus, no other adverse effects were recorded. No data are available on the infectivity of treated dogs to sand flies or possible changes of their *Leishmania*-specific cell-mediated immunity [235].

Conclusion: Metronidazole cannot be recommended for the treatment of CanL because of the frequent relapses after treatment discontinuation (SORT: moderate) and the limited information on some critical features of this treatment, such as the evolution of clinicopathologic abnormalities, infectivity to sand flies and *Leishmania*-specific cell-mediated immunity; moreover, concerns arise from the long-term administration of an antibacterial agent (SORT: weak), as for marbofloxacin.

8.3.7 | O-Alkyl-Hydroxamate (MTC-305)

This drug is a vorinostat derivative that inhibits histone deacetylases. It has been administered at 3.75 mg/kg SC once daily in two cycles, each with a 4-week duration, that were separated by 1 month without treatment, and has been evaluated in a single RCT (Table S8) [187]. In that study, it was given to six dogs with CanL (LeishVet stage I–III), the diagnosis of which was confirmed by serology and qPCR and was compared to meglumine antimoniate monotherapy and to its combination with meglumine antimoniate. The quality of the study is intermediate.

At 7 months (4 months after the end of the 3-month treatment period) none of the dogs had a clinical score of zero. However, clinical improvement occurred between 2 and 4 months, and by 7 months the clinical score decreased by 64.7% and was lower than baseline in 83.3% (5/6) dogs. On the other hand, half of the dogs had higher clinical scores at 7 months compared to 3 months, indicative of possible relapse after treatment discontinuation. A similar trend was observed for the parasitic density that was measured in bone marrow, lymph nodes and blood by qPCR: at 7 months it was significantly lower compared to Day 0 (bone marrow, lymph nodes), and qPCR was negative in 1 of 6 (bone marrow) or 2 of 6 (blood) dogs. Between 3 and 7 months, it increased in 2 of 6 (bone marrow, blood) or 5 of 6 (lymph nodes) dogs. Total *Leishmania*-specific IgG IFA titres remained stable, and the only adverse effect was a decreased neutrophil count at 3 months. Possible changes in cell-mediated immune responses were evaluated with a non-validated approach (measurement of parasite-specific IgG subclasses and of INF-γ and IL-4 mRNA in blood), and there are no data on the evolution of clinicopathologic abnormalities and the infectivity of treated dogs to sand flies.

Conclusion: *O*-alkyl-hydroxamate (MTC-305) cannot be recommended for the treatment of CanL because of the incomplete clinical response, the tendency for relapse after treatment discontinuation (SORT: moderate) and the limited information on some critical features of this treatment, such as the evolution of clinicopathologic abnormalities, infectivity to sand flies and *Leishmania*-specific cell-mediated immunity.

8.3.8 | (−)-α-Bisabolol

This molecule is a sesquiterpene causing mitochondrial damage and perhaps apoptosis to *Leishmania*. It has been administered at 30 mg/kg orally once daily in two cycles, each with a 4-week duration, that were separated by 1 month without treatment and has been evaluated in a single RCT (Table S9) [188]. In that study, it was given to six dogs with CanL of clinical severity that is hard to determine, the diagnosis of which was confirmed by serology and blood qPCR, and was compared to meglumine antimoniate. The quality of the study is intermediate.

Only half of the dogs (3/6) completed the trial, and ITT statistical analysis was not performed, making interpretation of the results difficult. At 7 months (4 months after the end of the 3-month treatment period) none of the dogs was clinically cured. However, clinical improvement occurred between 2 and 4 months, and by 7 months the clinical score had decreased by 33.3% and was lower than baseline in all three dogs. On the other hand, 1 of 3 dogs had higher clinical score at 7 compared to 4 months, indicative of possible relapse. By 7 months, haematocrit was increased by 7.1% but platelet count was decreased by 50.8%, total proteins and globulins were increased by 13% and 24.3%, respectively, albumins and albumin/globulin ratio were decreased by 3% and 30%, respectively, and BUN and creatinine were increased by 36.9% and 70.5%, respectively. Parasitic density was measured in bone marrow, lymph nodes and blood by qPCR but their changes are not reported; nevertheless, at 7 months each one of the three tissue samples was qPCR negative in 1 of 3 dogs, but bone marrow (1/3 dogs) and blood (2/3 dogs) parasitic density was higher at 7 compared to 4 months. Parasite-specific IgG IFA titres remained stable, and no adverse effect was recorded. Possible changes in cell-mediated immune responses were evaluated with a non-validated approach (measurement of INF-γ and IL-4 mRNA in blood) and there are no data on the infectivity of treated dogs to sand flies.

Conclusion: (−)-α-bisabolol cannot be recommended for the treatment of CanL because of the incomplete clinical response, the deterioration of important clinicopathologic abnormalities (SORT: moderate) and the lack of information on some critical features of this treatment such as the evolution of infectivity to sand flies and *Leishmania*-specific cell-mediated immunity.

8.3.9 | Artesunate

This molecule, which causes apoptosis of *Leishmania*, has been isolated from extracts of the *Artemisia annua* plant. In a single RCT (Table S10) [236], it was administered at 25 mg/kg orally once daily for 6 days to 16 dogs with CanL of unknown (most likely mild) severity, that had been confirmed by serology or blood qPCR. In that study, which is of low quality, the efficacy and safety of artesunate were compared to those of meglumine antimoniate-allopurinol combination. After 6 months, 13.3% of the dogs had died of CanL, 46.7% were clinically improved and 26.7% were clinically cured; death occurred between the 2nd and 3rd month, clinical improvement was first noticed at 1 month and clinical cure at 3 months. No data on the evolution of clinicopathologic abnormalities or the infectivity to sand flies are provided, but at the end of the study, 80% of dogs with positive blood qPCR at time 0 became negative, whereas the reverse was not seen. *Leishmania*-specific cell-mediated immunity was not studied but the humoral immune response declined, with a significant reduction of IFA titre starting at 1 month and resulting in 58.3% of the dogs being seronegative at 6 months. No adverse effects were recorded.

Conclusion: Artesunate cannot be recommended for the treatment of CanL because of the moderate rate of clinical response, the risk of death from CanL (SORT: moderate) and the lack of information on some critical features of this treatment such as the evolution of clinicopathologic abnormalities, infectivity to sand flies and *Leishmania*-specific cell-mediated immunity.

8.3.10 | Combinations of Drugs With Direct Anti-Leishmania Activity

As none of the above drugs is effective for both short-term and long-term treatment of CanL, their combinations have been explored. At least in theory, combining drugs with different mechanism of anti-*Leishmania* activity can increase efficacy and prevent relapses of the disease.

8.3.10.1 | Meglumine Antimoniate–Allopurinol Combination. The efficacy and safety of meglumine antimoniate–allopurinol combination have been evaluated in 11 RCTs (Table S11) [182, 223, 235–243]. In these studies, this combination was compared to monotherapies with allopurinol [223], metronidazole (plus spiramycin) [235], artesunate [236] and a nutritional supplement with antioxidant properties (DiLsh; Dynamopet, Italy) [237]. It was also compared to meglumine antimoniate combinations with allopurinol plus domperidone [243] or plus deslorelin [242], with metronidazole (plus spiramycin) [238], and with a dietary supplement containing nucleotides and an AHCC (Impromune; Bioiberica S.A.U., Spain) [241], and to the miltefosine–allopurinol [240] and allopurinol–aminosidine [182, 239] combinations. In eight studies the daily dose of meglumine antimoniate was 100 mg/kg and the duration of treatment varied from 20 to 28 days [182, 223, 236, 239–243], whereas in two studies it was administered at a higher daily dose (110–200 mg/kg) and for a longer period (30–90 days) [235, 238], and in one study it was under-dosed (40 mg/kg/day) for 1 month [237]. The daily dose of allopurinol varied from 20 to 40 mg/kg, starting simultaneously with meglumine antimoniate and continuing for a total treatment period of 3 weeks to 7 months [182, 223, 235–243]. The number of dogs varied from six [223] to 38 [241], the confirmation of CanL diagnosis was based on serology (11/11) [182, 223, 235–243] and on demonstration of parasite or parasitic DNA by microscopy (8/11) [182, 223, 235, 238, 239, 241–243] and/or molecular methods (8/11) [235–238, 240–243]. The severity of CanL is reported only in two studies that included dogs at LeishVet stages II (6/12) and III (6/12) [242] or at Canine Leishmaniosis Working Group (CLWG) classification system stage C [243], whereas in another study serum creatinine concentration < 1.2 mg/dL and absence of 'liver disease' was an inclusion criterion [223], and in three studies dogs with CKD IRIS stage III–IV were excluded [182, 238, 239]. The quality of these studies is high [182], intermediate [223, 235, 237, 239–243] or low [236, 238].

According to one of these studies where a very stringent clinical scoring system was used, 52.6% (10/19) dogs had absolutely no clinical signs at the end of the trial (Day 180) [182], whereas in another RCT clinical cure, first recorded at 1 month, was present in only 18.2% (4/22) dogs at 6 months [236]. This difference is easily explained because in the latter study allopurinol was administered only for 1 month, whereas in the former it was given for the entire 6-month period. Clinical improvement, starting between 14 and 30 days [223, 235, 236, 238, 243], was seen in 80% (8/10) of the dogs at 3 months [235], and in 54.5% (12/22, in addition to the 4/22 clinically cured) dogs at 6 months despite short-term (1 month) allopurinol administration [236]. All studies reporting total clinical score and/or number of clinical signs per dog and/or severity of clinical signs found them to be significantly lower at the end compared to Day 0 [182, 235, 237, 240–242]. Death due to CanL, treatment adverse effects or irrelevant reasons was uncommon [0% (0/6, 0/10, 0/12, 0/14, 0/15 or 0/18) [223, 235, 237, 238, 242, 243], 5% (1/20) [182, 239], 5.3% (2/38) [241] or 8.3% (3/36) [240]] except in the RCT where allopurinol was administered for only 1 month: 5 months later, 23.1% (6/26) of the dogs had died (2/6 due to CanL) [236]. Relapses after treatment discontinuation were studied in only one RCT where treatment was administered for 3 months. In this study, the clinical score started to increase after 30–60 days and after 4 months 25% (2/8) of the dogs had a relapse, with their clinical score being higher than on Day 0 [235]. Relapses are also reported in long-term, open label trials despite continued administration of allopurinol for years [244].

Starting from the first 10–30 days of treatment, improvement or amelioration of clinically important clinicopathologic abnormalities, including anaemia [182, 223], hyperproteinaemia [182, 223, 241], hypoalbuminaemia [182, 237, 241], hyperglobulinaemia [182, 238], increased gamma-globulin concentration [237, 240, 241], decreased albumin/globulin ratio [182, 240] and

increased concentration of positive acute phase proteins like CRP [182, 223, 241], ferritin [241] and ceruloplasmin [223] was an almost uniform finding. The only exceptions were lack of change in protein electrophoresis abnormalities and CRP concentration after 3 weeks of treatment [243], and lack of change in total protein, albumin, alpha2-globulin and gamma2-globulin concentrations recorded in the RCT where meglumine antimoniate was under-dosed [237], and in two RCTs where allopurinol was administered for only 50 days [223] or 3 months [238]. There was no evidence of deterioration in kidney function: BUN [182, 237, 239, 241] and creatinine [237, 240, 241] concentrations remained stable, inorganic phosphorus concentration decreased [182], prevalence of proteinuria did not change [239] or was decreased [240], and UPC values decreased [239, 241]. In two RCTs there was increased creatinine concentration at 2 or 6 months, but it was attributed to increased muscle mass, because it was not accompanied by parallel changes in BUN or inorganic phosphorous concentrations, the prevalence of proteinuria or UPC values [182, 239]. The lack of nephrotoxicity is further supported by the results of an open trial that included dogs at LeishVet stage II or III of CanL with CKD IRIS stage I or II, where, in addition to the classical markers of kidney function, a stable glomerular filtration rate was shown during meglumine antimoniate-allopurinol plus symptomatic treatment for CKD [245].

An early (starting from the first month) and sustained reduction of parasitic load, based on bone marrow and/or lymph node microscopy [182] and qPCR [182, 240, 241] was found. Of the initially positive dogs, at the end of 6-month treatment period, 36.8% became negative on bone marrow and lymph node microscopy and bone marrow qPCR [182]. Four months after the end of 90-day treatment period 25% became bone marrow, lymph node and blood PCR-negative [235], and 5 months after the end of 30-day treatment period 57.1% became blood qPCR-negative [236]. Infectiousness to sand flies was not evaluated in these 10 RCTs; however, an observational study showed that all eight initially positive dogs became negative on xenodiagnosis after 6 months of treatment [166].

Meglumine antimoniate and allopurinol combination treatment induced *Leishmania*-specific cell-mediated immunity, exemplified by the increased prevalence of positive leishmanin skin test at the end of 6-month treatment period (73.3%) compared to Day 0 (31.6%) [182], whereas the significant increase of CD4+, along with the non-significant increase of CD8+ lymphocytes, at 6 months may denote restoration of the non-specific cell-mediated immune defects of CanL [241]. With the exception of one short-term (90 day) RCT [235], an early (starting at 1–3 months) reduction of *Leishmania*-specific antibody concentrations in serum, that was significant at the end of treatment, was a uniform finding [182, 236, 237, 240–242]. Also, 57.9% of initially seropositive dogs became seronegative at 6 months [182], and 0%–29.4% remained seronegative 4–9 months after treatment discontinuation [235, 236, 238]. However, in one short-term, RCT [235] antibody concentration increased after allopurinol withdrawal in 50% of the dogs.

Adverse effects were seen in 0%–60% of the dogs [182, 237, 238, 243], and included injection site reactions [182, 235, 236] sometimes necessitating meglumine antimoniate discontinuation [235], 'asthenia' [240], vomiting [240], acute pancreatitis causing death (5%) [182, 239], possible cutaneous drug eruption [235], biochemical evidence of hepatotoxicity [235] and xanthinuria [241]. It is also logical to anticipate some additional adverse effects that have already been reported in the RCTs on meglumine antimoniate (depression, lethargy, anorexia, weight loss, diarrhoea) and on allopurinol (renal mineralisation) monotherapies, as well as those reported in non-RCTs evaluating their combination (xanthine lithiasis) [246].

Finally, although not examined in these RCTs, it is logical to assume that the repeated administration of meglumine antimoniate and the long-term administration of allopurinol promote drug resistance in *L. infantum*, like when these drugs are used as monotherapies.

Conclusion: Meglumine antimoniate–allopurinol combination is indicated for the treatment of CanL due to the consistent, albeit of limited quality, LoE showing that it results in clinical improvement or cure and in amelioration of clinically important clinicopathologic abnormalities. It also reduces parasitic load and infectivity to sand flies, upregulates parasite-specific cell mediated immunity and downregulates humoral immunity in most dogs, and is reasonably safe and non-nephrotoxic (SORT: strong). The daily recommended dose for meglumine antimoniate is 100 mg/kg SC, administered either once daily or divided every 12 h, for 28–30 days. Lower doses may be less effective and higher doses (or longer treatment periods) may increase toxicity without offering obvious therapeutic benefits (SORT: moderate). The allopurinol dose should be 10 mg/kg orally twice daily for at least 6 months. Higher doses may increase the frequency of adverse effects (SORT: weak) and shortened treatment periods are associated with clinical relapses (SORT: strong). Close monitoring for adverse effects, especially during the first month, is necessary (SORT: strong). Repeated administration of meglumine antimoniate and unnecessary extension of the allopurinol administration period should be avoided due to the risk of induction of resistant strains of *L. infantum* (SORT: moderate).

The use of *liposomal* formulations of *meglumine antimoniate* in combination with *allopurinol* has been evaluated in two RCTs (Table S12) [208, 224]. Conventional [208, 224] and a combination of conventional and polyethylene glycol (PEG)-containing (PEGylated) [224] liposomes were used as carrier of meglumine antimoniate that was administered at the dose of 23 mg/kg (corresponding to 6.5 mg antimony/kg) IV every 4 days, for six dose [208, 224]. In one study a second treatment 'cycle' was given after a 40-day discontinuation period [224]. In the first RCT allopurinol was administered at a daily dose of 20 mg/kg for 140 days [208], whereas in the second study a much higher than the usual daily dose (60 mg/kg) was given for 130 days [224]. In these studies, the two types of liposomes (conventional or conventional/PEGylated combination) were compared to each other [224], and the liposomal meglumine antimoniate-allopurinol combination was compared to liposomal meglumine antimoniate monotherapy [208], allopurinol monotherapy [208, 224] and to placebo or no treatment [208, 224]. The number of treated dogs was eight [208] or nine [224], the diagnosis of CanL was confirmed by serology and bone marrow PCR, and

all dogs had Stage II or III CanL based on a modified LeishVet staging algorithm [208] or an unspecified staging system [224]. The quality of both studies is low.

Of the eight dogs treated with meglumine antimoniate in conventional liposomes plus allopurinol at the usually recommended dose, 25% (2/8) died of unrelated causes. All remaining dogs responded with significantly improved clinical signs at the end of treatment and maintained for 2 months, compared to baseline. Two months after allopurinol discontinuation, 50% of dogs (3/6) were considered clinically cured. None of the remaining three dogs showed evidence of relapse [208]. The only useful data on clinical response that can be extracted from the second RCT are that at the end of allopurinol administration period (Day 130) and 4 months later, dogs treated with the combination of conventional and PEGylated liposome-encapsulated meglumine antimoniate plus allopurinol had significantly lower clinical scores in comparison to the no treatment group, and that 4 months after allopurinol discontinuation they had a significantly lower score compared to the group treated with conventional liposome-encapsulated meglumine antimoniate plus allopurinol [224]. Also, although not clearly stated in the manuscript, it seems that the percentage of dogs with clinical relapse within 4 months after treatment discontinuation was 25% and 50% for the conventional plus PEGylated liposome and the conventional liposome groups, respectively [224]. The evolution of clinicopathologic abnormalities is not reported, except that there were no changes in BUN or creatinine concentrations suggesting lack of overt nephrotoxicity [224].

Bone marrow, spleen, liver, and/or skin qPCR showed reduced parasitic load at the end of treatment [224] and 2 [208] or 4 [224] months later compared to time 0. The results regarding the parasitological-negative dogs after allopurinol discontinuation are inconsistent. In one RCT, 50% (3/6) were negative (bone marrow, spleen, liver and skin qPCR, plus bone marrow culture) at 2 months [208], whereas in the other RCT none of the 18 dogs was negative (bone marrow, spleen and liver qPCR, plus skin IHC) at 4 months [224]. A reduction of infectivity to *L. longipalpis* was recorded in one study: 50% (3/6) dogs were positive on xenodiagnosis on Day 0 and none of them at the end of the treatment or 2 months later [208].

There are no data reported on the effects of treatment on cell-mediated immunity, and results of *Leishmania*-specific IgG responses are discordant across treatment groups. Two months after the end of treatment with conventional liposome-encapsulated meglumine antimoniate plus allopurinol at the usual dose there was a significant reduction of IFA titres and 33.3% (2/6) of the dogs became seronegative. At the end of treatment with conventional liposome-encapsulated meglumine antimoniate plus allopurinol at the high dose, there were no significant changes in IFA titres or ELISA ODs compared to time 0, and this was maintained at a 4-month follow-up visit [224]. At the end of treatment with conventional/PEGylated liposome-encapsulated meglumine antimoniate plus allopurinol at the high dose, there were no significant changes in IFA titres but ELISA ODs were significantly lower than at baseline, and this was maintained at a 4-month follow-up visit [224].

In one RCT, temporary IV infusion-related adverse effects (salivation, vomiting, defecation) occurred in all dogs and xanthine nephrolithiasis in half of them, probably due to the high daily dose of allopurinol [224].

Conclusion: Despite some evidence (limited quality) for efficacy, liposomal meglumine antimoniate-allopurinol combination cannot be recommended for the treatment of CanL, due to the inconsistency of the results, the lack of information on some critical features of this treatment such as the evolution of clinicopathologic abnormalities and *Leishmania*-specific cell-mediated immunity (SORT: moderate), and the lack of head-to-head comparison with conventional meglumine antimoniate plus allopurinol combination treatment (SORT: weak).

8.3.10.2 | Meglumine Antimoniate–Aminosidine Combination. In addition to direct anti-*Leishmania* activity, co-administration of aminosidine with meglumine antimoniate may modify the pharmacokinetics of the latter by increasing its persistence in blood [247]. The efficacy and safety of meglumine antimoniate-aminosidine combination were evaluated in a single RCT (Table S13) [186], where it was compared to meglumine antimoniate and to aminosidine monotherapies. Eleven dogs with CanL of unknown severity, confirmed by serology and microscopy or culture, were treated with a typical dose of meglumine antimoniate (106 mg/kg, once daily, SC) and a low dose of aminosidine (3.5 mg/kg, twice daily, SC) for 21 days; the quality of the study is intermediate.

Ten of the 11 dogs (90.9%) responded to the treatment, and their response was considered complete (3/10), good (6/10) or moderate (1/10). However, 5/10 (50%) relapsed within approximately 40 (1/5) or 160 (4/5) days after treatment discontinuation. Bone marrow and lymph node microscopy showed a significant reduction of parasitic load at the end of treatment and after 40, 100 and (by lymph node microscopy only) 160 days. In total, both bone marrow and lymph node microscopy were negative for *Leishmania* amastigotes in 72.7% (8/11) of the dogs at the end of treatment and maintained after 40 days. These parameters remained negative in 63.6% (7/11) at 100 days post- treatment and in 45.5% (5/11) 160 days post-treatment. The IFA titres were significantly lower compared to baseline at the end of treatment and after 40 and 100 days, but not after 160 days and none of the dogs became seronegative. There were no serious adverse effects and only one dog had a transient increase in BUN and creatinine concentrations [186].

No information on the evolution of important clinicopathologic abnormalities, the infectivity to sand flies, the possible changes in cell-mediated immune responses and the induction of resistant strains of the parasite is provided by this RCT. Theoretically, the combination of these drugs may prevent the development of parasite resistance [248]. However, if resistance will develop against one of the two drugs, it may also involve the other one due to cross-resistance [199]. Also, being an aminoglycoside, aminosidine may promote bacterial resistance.

Conclusion: Meglumine antimoniate-aminosidine combination cannot be recommended for the treatment of CanL because of the relapses after treatment discontinuation (SORT: moderate).

8.3.10.3 | Meglumine Antimoniate–Metronidazole Combination. One RCT (Table S14) [238] compared the efficacy and safety of meglumine antimoniate-metronidazole (with spiramycin) combination with meglumine antimoniate-allopurinol combination in 14 dogs with CanL of unknown severity (a creatinine serum concentration > 2 mg/mL was an exclusion criterion). Meglumine antimoniate was administered at 55–100 mg/kg SC twice daily for 30 or 60 days, and metronidazole (plus spiramycin) at 25 mg/kg (plus 150,000 IU/kg) orally once daily for 90 days. The diagnosis of CanL was confirmed by serology, microscopy and/or PCR and the quality of the study is low.

There was a significant clinical improvement, first witnessed at 30 days, and none of the dogs had died at the conclusion of the study, but the rate of clinical cure, clinical improvement without cure and clinical relapse after treatment discontinuation is not reported. The only available information on the evolution of clinicopathologic abnormalities is that albumin and globulin concentrations increased and decreased, respectively, in dogs treated with meglumine antimoniate for 60 dogs but not in those treated for 30 days. The evolution of parasitic load and possible changes in cell-mediated immunity are unknown, whereas 9 months after treatment discontinuation 50% (7/14) dogs were seronegative and no adverse effects are reported [238]. No data are available on the infectivity of treated dogs to sand flies [238].

Conclusion: Meglumine antimoniate-metronidazole combination cannot be recommended for the treatment of CanL because of the limited information on some critical features of this treatment such as the evolution of parasitic load, infectivity to sand flies and *Leishmania*-specific cell-mediated immunity, the lack of any obvious benefit compared to the standard meglumine antimoniate–allopurinol combination (SORT: moderate) and the long-term administration of an antibacterial agent (SORT: weak).

8.3.10.4 | Meglumine Antimoniate–O-Alkyl-Hydroxamate (MTC-305) Combination. This combination has been evaluated in a single RCT (Table S15) [187], where meglumine antimoniate and *O*-alkyl-hydroxamate were administered at 104 mg/kg and at 1.5 mg/kg, respectively, SC once daily in two 4-week cycles separated by 1 month without treatment. In that study, the combination treatment was compared to meglumine antimoniate and to *O*-alkyl-hydroxamate monotherapies (six dogs per group) and administered to dogs with CanL (LeishVet stages I–III), the diagnosis of which was confirmed by serology and qPCR. The quality of the study is intermediate.

At 7 months (4 months after the end of the 3-month treatment period), 1 of 6 (16.7%) dogs had zero clinical score (clinically cured) and then remaining 5 of 6 (83.3%) had lower clinical scores compared to Day 0. Clinical improvement occurred between 2 and 4 months, and by 7 months the clinical score had decrease by 62.5%. On the other hand, 2 of 6 (33.3%) dogs had higher clinical scores at 7 months compared to 3 months, indicative of possible relapse after treatment discontinuation. A similar trend was observed for the parasitic density that was measured in bone marrow, lymph nodes and blood by qPCR: at 7 months, blood qPCR was negative in 2 of 6 (33.3%) dogs, and between 3 and 7 months, it increased in 3 of 6 (bone marrow, lymph nodes) or 1 of 6 (blood) dogs. Total *Leishmania*-specific IgG IFA titres were lower at 7 months compared to time 0 in 5 of 6 (83.3%) dogs, with 2 of 6 (33.3%) being seronegative, but 1 of 6 (16.7%) had higher titre than at 3 months. Weight loss during the first 2 months of treatment was recorded in 2 of 6 (33.3%) dogs. Possible changes in cell-mediated immune responses were evaluated with a non-validated approach (measurement of parasite-specific IgG subclasses and INF-γ and IL-4 mRNA in blood), and there are no data on the evolution of clinicopathologic abnormalities or the infectivity of treated dogs to sand flies.

Conclusion: Meglumine antimoniate plus *O*-alkyl-hydroxamate (MTC-305) combination cannot be recommended for the treatment of CanL because of the tendency for relapse after treatment discontinuation (SORT: moderate) and the limited information on some critical features of this treatment such as the evolution of clinicopathologic abnormalities, infectivity to sand flies and *Leishmania*-specific cell-mediated immunity.

8.3.10.5 | Miltefosine–Allopurinol Combination. The efficacy and safety of this combination in dogs with CanL have been evaluated in two RCTs (Table S16) of 6 [249] or 7 [240] months duration. In one study, the registered dose of miltefosine (2 mg/kg orally once daily for 28 days) plus the usual dose of allopurinol (10 mg/kg orally twice daily for 7 months) was compared to meglumine antimoniate–allopurinol combination [240], or two dosage regimens of miltefosine (the registered one for 30 days and a modified one, starting at 1.2 mg/kg once daily for the first 5 days and followed by 2.5 mg/kg once daily for 25 days). Both protocols included combination with allopurinol (10 mg/kg orally twice daily for 6 months), and were compared to each other [249]. The number of treated dogs varied from 16 [249] to 37 [240], the diagnosis of CanL was confirmed by serology and either lymph node microscopy [249] or bone marrow PCR [240], and the severity of the disease is specified in one study, where dogs at LeishVet stages II or III were included [249]. The quality of both studies is intermediate.

Neither study specifies the percentage of clinically cured and/or improved dogs; however, in both RCTs, starting from 2 to 3 months, there was a significant improvement of total clinical score that, at the end of treatment period was lower than on baseline by 61.7% [249], 71.6% [249] or 89.9% [240]. None of the dogs died or was euthanised due to CanL, but an unspecified number of clinical relapses, despite continued allopurinol administration, is reported in one RCT in dogs that were treated with the registered miltefosine dosage regimen [249]; the same observation is found in open long-term trials [244].

Of the clinically important laboratory parameters, PCV was significantly increased at 1 month [249], γ-globulin concentrations at the end of the study were within reference interval in 47.8% of the dogs [240] and albumin/globulin ratio started to increase at 3 months and by 7 months had normalised in 25.9% of the dogs [240]. There were no changes in creatinine concentrations throughout the study [240] or in UPC at 1 and 2 months [249], and the latter parameter normalised by the end of the study in 27.8% of the dogs with initially abnormal values [240].

Based on qPCR, the results on bone marrow parasitic load are discordant: in one RCT there was no difference between Day 0 and Day 60 [249], whereas in the second study there was a

significant reduction of the amount of *Leishmania* DNA, starting on day 28 [240]. In the first study, the prevalence of dogs that converted from positive (Day 0) to negative (Day 60) bone marrow qPCR was 14.3% (2/14) for those treated with the registered miltefosine dosage regimen and 50% (7/14) for those treated with the modified regimen, whereas the relevant figures for lymph node microscopy were 94.4% (17/18) and 93.8% (13/16), respectively [249]. In general, the results of non-controlled trials are in favour of a significant reduction of parasitic load in lymph nodes and/or blood during long-term miltefosine-allopurinol treatment [244, 250, 251]. The effect of treatment on parasite transmission to sand flies or on *Leishmania*-specific cell-mediated immunity was not evaluated in either RCT or in any other study. There was a significant reduction in IFA titres starting at 28 days of treatment and continuing over the next 6 months [240].

Adverse effects were recorded in 0% [240] or 12.5%–16.7% [249] of the dogs; they were not severe, and included vomiting, soft stools and diarrhoea [249]. Induction of miltefosine-resistant strains of *L. infantum* was confirmed in a single dog [218].

Conclusion: Miltefosine–allopurinol combination is indicated for the treatment of CanL due to the generally consistent (apart from the evolution of parasitic load), albeit of limited quality, LoE showing that it results in clinical improvement, in amelioration of clinically important clinicopathologic abnormalities, in downregulation of humoral immunity and that it is safe and non-nephrotoxic (SORT: moderate). To increase the SORT, additional RCTs are necessary and some features of this treatment (evolution of infectiousness to sand flies and of cell-mediated immunity) should be studied. The daily recommended dose of miltefosine is 2 mg/kg for 4 weeks, because the modified dosage regimen (1.2 mg/kg for the first 5 days followed by 2.5 mg/kg for 25 days), does not offer any appreciable clinical benefit and its safety has not been evaluated in toxicologic studies (SORT: weak). Repeated administration of miltefosine, like unnecessarily extending the treatment period on allopurinol, should be avoided due to the risk of induction of resistant strains of *L. infantum* (SORT: moderate).

8.3.10.6 | Allopurinol–Aminosidine Combination. The efficacy and safety of allopurinol–aminosidine combination have been evaluated in two RCTs (Table S17) [182, 239], of 2- [239] or 6- [182] month duration and including 20 dogs with CanL. Allopurinol was administered at the usual dose (10 mg/kg twice daily for 2 or 6 months) and aminosidine at the recommended dose of 15 mg/kg SC once daily for 28 days. The diagnosis of CanL was confirmed by serology and lymph node and/or bone marrow microscopy, and CKD IRIS stage III or IV was an exclusion criterion. In both studies, the comparator was the meglumine antimoniate–allopurinol combination, and their quality is intermediate [239] or high [182].

One dog died of unknown reasons during the first week of treatment. Of the remaining dogs, 21.4% (4/19) were clinically cured at 6 months, using a very stringent clinical scoring system, and there was a significant reduction of the number of clinical signs per dog, in the prevalence of 8 of 16 different clinical signs that were present on Day 0 and in the severity of 12 of 16 clinical signs. The prevalence and/or severity of most clinicopathologic abnormalities (anaemia, hyperproteinsemia, hyperglobulinsemia, low albumin-globulin ratio, CRP) was significantly lower at 6 months compared to time 0, and there was no evidence of nephrotoxicity based on BUN, creatinine, inorganic phosphorus and UPC at 2 and 6 months.

Lymph node and bone marrow microscopy showed a reduction of parasitic load, starting at 4 weeks. Bone marrow parasitic load, based on qPCR, was significantly lower at 6 months than on Day 0; moreover, at 6 months, 26.3% (5/19) dogs were negative on bone marrow qPCR and microscopy and lymph node microscopy. No data are available on the infectivity of treated dogs to sand flies.

Allopurinol-aminosidine combination treatment induced *Leishmania*-specific cell-mediated immunity, exemplified by the higher number of dogs with positive leishmanin skin test at 6 months (47.4%) compared to baseline (10.5%). In parallel, IFA titres were significantly lower from the first month, and at the end of the trial 21.1% (4/19) dogs were seronegative.

In addition to the single dog that died suddenly, aminosidine injection site reactions were common (55%) [182]. However, there was no evidence of nephrotoxicity and ototoxicity was excluded based on brainstem auditory evoked responses and normal neurological examination [239].

A direct comparison between allopurinol–aminosidine and meglumine antimoniate–allopurinol combination showed some advantages of the latter, such as increased prevalence of dogs with no clinical signs, lower number of clinical signs and clinicopathologic abnormalities per dog, and higher chance for reduction of hyperglobulinaemia and IFA titres at 6 months [182]. Also, being an aminoglycoside, aminosidine may promote bacterial resistance.

Conclusion: Allopurinol–aminosidine is indicated for the treatment of CanL due to the good quality LoE showing that it results in clinical improvement or cure, amelioration of clinically important clinicopathologic abnormalities, reduction of parasitic load, upregulation of parasite-specific cell-mediated immunity with downregulation of humoral immunity. The combination is reasonably safe and non-nephrotoxic or ototoxic (SORT: strong). Allopurinol dose should be 10 mg/kg orally twice daily for at least 6 months, and aminosidine should be administered at 15 mg/kg SC once daily for 28 days (SORT: weak). The case of sudden death during aminosidine administration indicates that close monitoring is necessary (SORT: strong). As meglumine antimoniate–allopurinol combination seems to be more effective and aminosidine may promote bacterial resistance, allopurinol–aminosidine should be considered a second-line treatment of CanL (SORT: strong) that may be particularly valuable in dogs that relapse despite multiple courses of meglumine antimoniate and/or miltefosine and may be at increased risk to harbour-resistant parasites (SORT: weak).

8.4 | Immunomodulators

8.4.1 | Domperidone

Domperidone is a prokinetic and antiemetic drug acting through antagonism of dopamine D2 receptors. By the same mechanism, in the central nervous system domperidone induces serotonin

and, subsequently, prolactin release. The latter stimulates innate (e.g., increased neutrophil oxidative activity) and cell-mediated (e.g., increased *Leishmania* antigen-stimulated INF-γ production by PBMCs) immunity [252]. Onset of efficacy is quite fast (from the 2nd day of administration), and the registered dose is 0.5 mg/kg orally once daily for 1 month, that is repeated in cycles separated by a 3-month off-drug period.

The efficacy and safety of domperidone for the treatment of CanL have been evaluated in one RCT (Table S18) [253]. In that study, 30 seropositive dogs without clinical signs of CanL but with CKD IRIS stage I or II (that was assumed to be due to CanL) were fed a renal diet for 11 months with (treatment group) or without (control group) domperidone. In the treatment group, domperidone was administered twice, starting on Day 90 and on Day 210. The quality of the study is intermediate.

No information about the evolution of clinical signs is provided, but 1 of 15 (6.7%) dogs from each group died of CanL. In the domperidone-treated dogs, serum creatinine and SDMA concentrations did not differ between the beginning and the end of the study, whereas both biochemical markers deteriorated significantly in the control group. No treatment-related adverse effects were recorded.

In an open trial, domperidone did not prevent the appearance of new clinical signs and/or clinicopathologic abnormalities of CanL in 25% (3/12) seropositive dogs with CKD. The remaining 9 of 12 dogs remained stable and, by the end of the 6-month study period, they had significantly decreased globulins, gamma-globulins and CRP compared to time 0, no change in their UPC and decreased ELISA ODs [254]. In another open trial, 7.1% (2/28) dogs died of CanL, but 85.7% (24/28) showed clinical improvement, associated with increased diameter of leishmanin skin test reaction and increased PBMC proliferation in response to parasite antigen [255]. The impact of domperidone administration on the infectivity of treated dogs to sand flies has not been studied.

Conclusion: Domperidone cannot be recommended for the treatment of CanL because it has been tested only in a few dogs from a specific subgroup that presented early-stage CKD, without additional clinical signs or clinicopathologic abnormalities of the disease, making impossible to determine the overall efficacy of the treatment (SORT: weak).

8.4.2 | Nutritional Supplements

A nutritional supplement with antioxidant properties, of mixed marine and plant origin, marketed under the trade name DiLsh was tested in one RCT (Table S19), where it was compared with meglumine antimoniate-allopurinol combination [237]. In this study, the nutritional supplement was administered at a daily dose of 0.5 g/kg orally (in the food) for 3 months to 15 dogs with CanL of unclear severity, that was confirmed by serology and bone marrow PCR. The quality of the study is intermediate.

By the end of the trial, there was significant reduction of total clinical score and none of the dogs had died of CanL. Of the clinicopathologic abnormalities, there was only a significant decrease of gamma-globulins and no significant changes of total protein, albumin, BUN and creatinine concentrations. The evolution of parasitic load and infectivity to sand flies was not examined, cell-mediated immunity was evaluated with a non-validated approach (measurement of IL-6, IL-10, TNF-α and leptin concentrations in serum) and there was a significant reduction of ELISA ODs. Adverse effects were not recorded.

Conclusion: The nutritional supplement DiLsh cannot be recommended for the treatment of CanL because of the limited information on critical features of this treatment such as the evolution of parasitic load, infectivity to sand flies and *Leishmania*-specific cell-mediated immunity.

8.4.3 | Monoclonal Antibody Against Canine IL-10 Receptor

In a single RCT (Table S20) of 6-month duration and intermediate quality, anti-canine IL-10 receptor monoclonal antibodies were compared to liposomal meglumine antimoniate [207]. Two IM injections (4 mg/kg) of the monoclonal antibody were administered, 21-days apart, to 11 dogs with CanL of unknown severity, confirmed by bone marrow culture and serology.

There was an initial (Days 30 and 90) non-significant improvement, followed by deterioration of total clinical score that, at the end of the trial, was almost the same as on Day 0. No changes were found in haematocrit, platelet count, total protein, globulin, BUN and creatinine concentrations or in bone marrow parasitic density, whereas infectivity to sand flies and humoral immunity were not tested. At different time points there were multiple, often temporary, changes of PBMCs immunophenotype. On Day 180 there was a significant decrease of T-cell proliferation in response to *Leishmania* antigen, and of CD5-CD16+ natural killer cell absolute numbers, compared to Day 0. No adverse effects were observed.

Conclusion: Monoclonal antibodies against canine IL-10 receptor cannot be recommended for the treatment of CanL due to lack of efficacy (SORT: moderate).

8.4.4 | Vaccines

A vaccine containing a partially purified fraction derived from *L. infantum* promastigotes with a molecular weight of 67–94 KDa, called LiF2, was administered to eight dogs with CanL in one RCT (Table S21) of intermediate quality [190]. The dose of the vaccine was 50 μg/dog administered intramuscularly (IM) for three times at weekly intervals. The diagnosis of CanL was confirmed by bone marrow microscopy but the severity of the disease was unclear. In this study, the vaccine was compared to meglumine antimoniate and to the meglumine antimoniate–LiF2 vaccine combination. After 3 months, 25% (2/8) of the dogs were clinically cured and 75% (6/8) improved and at 6 months the cure rate was 100% (8/8). At both time points, bone marrow parasitic density decreased, 25% (2/8) dogs were negative on both microscopy and culture and the parasiticidal activity of macrophages increased (at least in some dogs). No information on the

evolution of clinicopathologic abnormalities, infectivity to sand flies, humoral immunity and adverse effects was provided.

Conclusion: The LiF2 vaccine cannot be recommended for the treatment of CanL because of the limited information on critical features of this treatment such as the evolution of clinicopathologic abnormalities, infectivity to sand flies, *Leishmania*-specific humoral immunity and adverse effects.

In one RCT (Table S21), the vaccine Leish-110f, containing a polyprotein composed of three recombinant *Leishmania* proteins (TSA, LmSI1 and LeIF) at 25 μg/dose, and the adjuvant monophosphoryl lipid A (MPL) plus squalene in a stable emulsion (MPL-SE) at 25 μg/dose was administered SC three times at 3-week intervals, to six dogs with CanL. All dogs were considered 'symptomatic' (i.e., not 'oligosymptomatic'), the diagnosis of CanL was confirmed by microscopy and/or culture, the duration of the RCT was 6 months. The efficacy of the vaccine was compared to meglumine antimoniate, meglumine antimoniate–vaccine combination, MPL-SE adjuvant and placebo [189]. At the end of the study, clinical improvement was seen in none of the dogs, the evolution of their major clinicopathologic abnormalities is not clearly reported and all dogs were still positive on bone marrow and/or skin microscopy and/or culture. There was no evidence of stimulation of cell-mediated immunity based on PBMC proliferation in response to parasite antigen, the anti-*Leishmania* IgG titre was higher compared to Day 0, and none of the dogs became seronegative. No adverse effects were reported [189].

In another publication describing the results of two RCTs (Table S21) of intermediate quality [191], the same vaccine with the same adjuvant, now called Leish-111f, was administered at a lower dose (20 μg vaccine plus 20 μg adjuvant), SC four or six times at 1-week intervals to 18 (study #1) or 10 (study #2) dogs with CanL of unknown severity, confirmed by microscopy, culture or serology. The vaccine was compared to meglumine antimoniate, meglumine antimoniate–vaccine combination, the adjuvant and placebo (saline) or no treatment. In the first study, clinical improvement was seen in all 18 dogs 6 months after the first vaccination, but 2.5 years later, of the 12 dogs that had not been lost to follow-up or died for unrelated reasons, 75% (9/12) were considered clinically healthy, 25% (3/12) had a relapse and two of them had died of CanL. In the second RCT, the clinical efficacy was much lower. At 6 months 50% (5/10 dogs) had improved, whereas the remaining 5 of 10 dogs deteriorated between 1 and 4 months and they either received rescue treatment or died. Interestingly, the responders were mainly dogs with low severity of CanL on Day 0. No additional information is provided, except that in study #2, 6/10 (60%) dogs were negative on microscopy or culture at 6 months [191].

Conclusion: The vaccine Leish-110f with the adjuvant MPL-SE cannot be recommended for the treatment of CanL due to low efficacy in two out of three published RCTs (SORT: moderate).

The efficacy and safety of a vaccine containing recombinant cysteine proteinase of *Leishmania* (rLdccys1) as antigen and *Propionibacterium acnes* as adjuvant were tested in one RCT (Table S21) [256]. The vaccine was administered at a dose of 500 μg rLdccys1 plus 500 μg adjuvant SC three times at 1-month intervals to 10 dogs with CanL of undetermined severity (without pancytopenia or creatinine concentration > 2 mg/dL) confirmed by bone marrow culture and serology. The control groups received only the adjuvant or placebo, and the quality of the study is intermediate. The clinical score of vaccinated dogs did not improve after 1, 2, 3 or 4 months, and they died after 12–14 months. Vaccine antigen-specific cell-mediated and humoral response was apparent during treatment, and, by the time of death, their spleen parasitic burden was 7-log lower compared to the controls. Local adverse effects of transient nature were recorded in most dogs.

Conclusion: The vaccine containing rLdccys1 antigen with the adjuvant *P. acnes* cannot be recommended for the treatment of CanL due to lack of efficacy (SORT: moderate).

A vaccine containing *L. braziliensis* antigen and the adjuvant MPL was tested in one RCT (Table S21) of 90-day duration [257]. The vaccine was administered SC once daily to 10 dogs, at gradually increased doses for the first 5 days and then at the maximal dose (600 μg vaccine plus 25 μg adjuvant) until Day 10, followed by a 10-day discontinuation period. This was followed by 10-day re-administration at the maximal dose, a 10-day discontinuation and a 10-day re-administration. The control dogs received adjuvant or no treatment, the diagnosis of CanL was confirmed by serology and PCR. The severity of CanL was unknown. The quality of the study is intermediate. Dogs were euthanised after 3 months and the vaccinated group was found to have 96% lower spleen parasitic burden, based on qPCR, compared to controls. The expression of IL-12, INF-γ, TNF-α and inducible nitric oxide synthetase (iNOS) mRNA in the spleen was significantly higher, and that of IL-10 and transforming growth factor-b1 (TGF-β1) was significantly lower compared to controls. No clinical information is provided in the RCT. However, in a non-randomised adjuvant-controlled trial of 5-month duration, there was clinical improvement in 70% of vaccinated dogs, which was accompanied by normalisation of red blood cell parameters, platelet count, BUN and creatinine, reduction of bone marrow parasitic load and infectivity to sand flies, activation of *Leishmania*-specific cell-mediated immunity, increased transcription of INF-γ and TNF-α and decreased transcription of IL-4 and IL-10 [258].

Conclusion: The vaccine containing *L. braziliensis* antigen with the adjuvant MPL cannot be recommended for the treatment of CanL due to the limited information on most critical features of this treatment such as the evolution of clinical signs and clinicopathologic abnormalities, infectivity to sand flies and *Leishmania*-specific humoral immunity, and the relatively low clinical response rate in a non-randomised trial (SORT: moderate).

8.5 | Combinations of Drugs With Direct Anti-Leishmania Activity and Immunomodulators

8.5.1 | Meglumine Antimoniate-Nutritional Supplement Combination

A nutritional supplement containing nucleotides and an AHCC compound (Impromune) may modulate immune responses through non-specific stimulation of cell-mediated immunity

and Th1 cytokine production [241, 259]. The efficacy and safety of the combination of this supplement with meglumine antimoniate were tested in a single RCT (Table S22) [241]. In this study, 32 dogs with CanL were treated with meglumine antimoniate at the recommended dose (100 mg/kg SC daily) for 4 weeks, and with the dietary supplement at 32 mg/kg of nucleotides plus 17 mg/kg AHCC daily for 6 months. These dogs were compared to 38 dogs with CanL randomised to standard treatment with meglumine antimoniate-allopurinol combination. The confirmation of CanL diagnosis was based on positive serology and bone marrow or lymph node microscopy and/or PCR. The severity of the clinical picture of CanL, evaluated by a clinical scoring system with a maximum value of 55, was probably mild (clinical score 7.67 ± 3.84). The quality of the study is intermediate [241].

The number of dogs achieving complete or partial clinical cure by the end of the study is not reported, but there was a significant reduction in the clinical score, that was first witnessed at the end of meglumine antimoniate administration. At 6 months, none of the dogs had died of CanL and the clinical score was significantly lower compared to dogs treated with meglumine antimoniate-allopurinol combination. There was a significant decrease of total protein, gamma-globulin, CRP and ferritin concentrations, a significant increase of albumin on Days 30 and 180 compared to baseline, and there were no changes in the biomarkers of kidney function (BUN, creatinine, UPC).

At the end of the study, the parasitic load (bone marrow or lymph node qPCR) was reduced compared to time 0; the effect of treatment on the infectivity to sand flies was not tested.

The effect of treatment on *Leishmania*-specific cell-mediated immunity was examined using a non-validated approach (measurement of CD4+ and CD8+ cells in unstimulated blood samples) and a significant reduction of ELISA ODs at the end of the study compared to baseline was demonstrated.

No treatment-related adverse effects were reported [241].

Conclusion: Despite the improvement of clinical signs and clinicopathologic abnormalities and the reduced parasitic load and humoral response (SORT: moderate), the meglumine antimoniate-dietary nucleotide plus active hexose correlated compound combination cannot be recommended as a routine alternative to the standard meglumine antimoniate–allopurinol combination because the efficacy was tested mainly in dogs with CanL of mild severity (SORT: weak) and due to lack of information on some critical features of this treatment (percentage of dogs achieving clinical cure or improvement, percentage of dogs with amelioration of clinicopathologic abnormalities, infectivity to sand flies, effect on *Leishmania*-specific cell-mediated immunity).

8.5.2 | Meglumine Antimoniate-Vaccines Combination

The combination of meglumine antimoniate at a high dose (300 mg/kg IM every other day for 20 administrations) with the LiF2 vaccine (50 μg/dog IM three times at weekly intervals) was compared to meglumine antimoniate monotherapy and to LiF2 monotherapy in one RCT (Table S23), that included eight dogs in each treatment group [190]. The diagnosis of CanL was confirmed by microscopy of bone marrow aspirates, the severity of CanL is unclear, and the quality of the study is intermediate [190].

At 3 months all dogs were considered clinically cured but no information on the evolution of clinicopathologic abnormalities is available. At 3 and 6 months, bone marrow aspirates by microscopy and culture were negative in 8 of 8 (100%) dogs but their infectivity to sand flies was not studied. The leishmanicidal activity of macrophages increased after treatment but there is no information about *Leishmania*-specific humoral immune response. No adverse effects were encountered [190].

Conclusion: The meglumine antimoniate-LiF2 vaccine combination cannot be recommended for the treatment of CanL because of the limited information on critical features of this treatment such as the evolution of clinicopathologic abnormalities, infectivity to sand flies and *Leishmania*-specific humoral immunity.

The safety and efficacy of meglumine antimoniate and vaccine Leish-110f/Leish-111f combination were examined in two RCTs (Table S23) [189, 191]. The number of treated dogs was six [189] and 13 [191], the dosage regimen of meglumine antimoniate varied (100 mg/kg IM, daily for 10 days followed by 10-day discontinuation and then by administration for 10 more days [189] or 20 mg/kg IV daily for 30 days) [191] and the vaccine was administered at 20 μg (plus 20 or 25 μg of MPL-SE) per dog SC once weekly for four times [191] or every 3 weeks for three times [189]. The combination treatment was compared to meglumine antimoniate [189, 191] and vaccine [189, 191] monotherapies, to vaccine adjuvant [189] and to placebo [189] or no treatment [191]. The diagnosis of CanL was confirmed by microscopy (bone marrow, lymph node, spleen, skin) [189, 191], culture (bone marrow, spleen) [189, 191] and/or serology [191], and the severity of CanL is not reported. The quality of these studies is low [189] or intermediate [191].

At 6 months after treatment initiation 0%–16.7% dogs had died, 50% were clinically cured and 33.3%–92.3% improved. Evolution of clinicopathologic abnormalities is reported in one of the studies [189], where there was a significant increase in haematocrit and albumin concentration after 1–2 months and normalisation of gamma-globulins after 6 months, accompanied by negative bone marrow microscopy and culture, negative skin microscopy, and negative xenodiagnosis in 40% (2/5) of the surviving dogs. The effect of treatment on *Leishmania*-specific cell-mediated immunity was not different from placebo, but there was a decline in IgG responses after 6 months with 40% (2/5) dogs becoming seronegative [189]. No adverse effects were reported.

Conclusion: The meglumine antimoniate–Leish-110f/Leish-111f vaccine combination cannot be recommended for the treatment of CanL because of the lack of an obvious benefit compared to meglumine antimoniate monotherapy (SORT: moderate).

8.5.3 | Meglumine Antimoniate–Allopurinol–Domperidone Combination

The efficacy and safety of the addition of domperidone to meglumine antimoniate-allopurinol combination treatment was

evaluated in a single RCT (Table S24) of very short (3 weeks) duration [243]. During the study, 36 dogs with CanL stage C (CLWG classification system) were treated with meglumine antimoniate and allopurinol at the recommended doses (100 mg/kg SC once daily and 10 mg/kg orally twice daily, respectively), whereas half of them (18/36) were randomly assigned to also receive domperidone at the registered dose (0.5 mg/kg orally once daily). The diagnosis of CanL was confirmed by skin, lymph node and/or bone marrow microscopy and/or PCR and by positive serology. The quality of the study is intermediate.

Clinical signs of CanL improved in all dogs by the end of the 2nd week of treatment (however, clinical improvement by that time was an inclusion criterion) and none of them died or was euthanised. The only information on the evolution of clinicopathologic abnormalities is that serum electrophoresis profile was not restored by the end of the study, and that CRP, after a transient increase on Day 3, gradually decreased, being significantly lower on Days 14 and 21 compared to baseline. No information on the evolution of parasitic density, infectivity to sand flies, parasite-specific cell-mediated or humoral immunity was provided, and no adverse effects were reported. There were no important difference between the two groups [243].

Conclusion: The addition of domperidone to the meglumine antimoniate-allopurinol combination treatment cannot be recommended for the treatment of CanL because of the lack of an obvious benefit (SORT: moderate) and the limited information on some critical features of this treatment such as the evolution of parasitic density, infectivity to sand flies and *Leishmania*-specific cell-mediated and humoral immunity.

8.5.4 | Meglumine Antimoniate–Allopurinol–Deslorelin Combination

Deslorelin is a gonadotropin-releasing hormone agonist that is used for chemical sterilisation of male and female dogs and cats [260]. Under the assumption that long-term blockage of testosterone production in intact male dogs may have a beneficial effect on the immune response against the parasite, the efficacy and safety of the addition of deslorelin to meglumine antimoniate–allopurinol combination were tested in a single RCT (Table S25) [242]. In this study, meglumine antimoniate and allopurinol were administered at the recommended therapeutic regimen (100 mg/kg SC daily for 4 weeks, and 10 mg/kg orally twice daily for 6 months, respectively) and a single 4.7 mg deslorelin implant was injected SC. Eleven intact male dogs received deslorelin (and 12 intact male dogs were treated only with meglumine antimoniate and allopurinol), CanL diagnosis was confirmed by positive serology and positive microscopy or PCR of bone marrow and/or lymph nodes, and all dogs were at CanL LeishVet stages IIa, IIb or III. The quality of the study is intermediate [242].

Clinical score at 3 and 6 months was significantly lower than on day 0 and compared to the control (meglumine antimoniate-allopurinol) group. Similarly, IFA titres were significantly lower at 3 and 6 months than on Day 0, and significantly lower than the control group at 6 months. No treatment-related adverse effects were recorded although 1 of 11 (9.1%) dog died due to a seemingly unrelated cause (congestive heart failure) [242].

There are no data on relapses after treatment discontinuation, the evolution of clinically important clinicopathologic abnormalities, parasitic load and infectivity to sand flies, or possible effects to *Leishmania*-specific cell-mediated immune response.

Conclusion: In intact male dogs, the addition of deslorelin to meglumine antimoniate-allopurinol treatment may offer some clinical benefits and seems to be safe (SORT: moderate). However, more information on critical features of this therapeutic strategy (evolution of clinicopathologic abnormalities, parasitic load, infectivity to sand flies and *Leishmania*-specific cell-mediated immunity) is needed to be considered as standard-of-care (SORT: weak).

8.5.5 | Allopurinol–Metronidazole–Ketoconazole-n-3 Fatty Acid–B Vitamin Combination

In addition to allopurinol and metronidazole, ketoconazole has direct anti-*Leishmania* activity, due to the inhibition of parasite cytochrome P450 enzymes, leading to accumulation of 14-methyl sterols that may affect cell membrane fluidity and permeability [219]. Ketoconazole has been tested as monotherapy for CanL in an open trial including 14 dogs with promising results [261]. N-3 fatty acids were hypothesised to be helpful for the treatment of CanL due to their anti-inflammatory and antioxidant properties, whereas the role, if any, of B-complex vitamins is obscure [262]. The efficacy and safety of allopurinol–metronidazole–ketoconazole–n-3 fatty acid–B vitamins combination were tested on one RCT (Table S26) [262]. In that study allopurinol was administered at the recommended dose (10 mg/kg twice daily) for either 270 or 320 days, metronidazole at 25 mg/kg twice daily for 30 days, ketoconazole at 10 mg/kg once daily for 40 days, the n-3 (eicosapentaenoic and docosahexaenoic) fatty acids at 1000 mg/kg once daily for 300 or 360 days and the B complex vitamin liquid formulation at 2 drops/kg once daily for 300 or 360 days. All 30 dogs were treated will all the above but in a different order: one group (group A; $n = 15$) was treated during the first 30 days with n-3 fatty acids and B vitamins only, then metronidazole (for 30 days) and ketoconazole (for 40 days) were added and were replaced by allopurinol from Day 90 until Day 360. In the second group (group B; $n = 15$), metronidazole and ketoconazole were the only interventions during the first 30 and 40 days, respectively, allopurinol was started on Day 41 and continued Until day 360, and the n-3 fatty acids and B vitamins started on Day 60 and continued until Day 360. The diagnosis of CanL was confirmed by bone marrow and/or lymph node microscopy and PCR and all dogs were classified as LeishVet stage I or II. The quality of the study is low [262].

Irrespective of the treatment group, on Day 360 all dogs that were not lost to follow up (6/15 and 12/15 for groups A and B, respectively), presented at least one clinical sign of CanL, but their clinical scores improved, starting at 3 (group A) or 6–12 (group B) months. At the end of the study, 20%–27.3% of them were classified at a lower stage of CanL (LeishVet stage I) compared to day 0 (LeishVet stage II). At the same time point, and considering only those dogs that presented each of the following clinicopathologic abnormalities at baseline, haematocrit normalised in 50% (1/2) group A and in 57.1% (4/7) group B dogs, platelet count normalised in 66.7% (2/3) group B dogs, total protein concentration

normalised in 25% (1/4) group A and in 10% (1/10) group B dogs, albumin concentration normalised in 0% (0/1) group A and in 71.4% (5/7) group B dogs, globulin concentration normalised in 25% (1/4) group A and in 30% (3/10) group B dogs, and albumin/globulin ratio normalised in 66.7% (2/3) group A and in 14.3% (1/7) group B dogs. BUN concentration normalised in 50% (1/2) group A and in 100% (3/3) group B dogs, creatinine concentration normalised in one group A and one group B dog and UPC normalised in one group A and in 60% (3/5) group B dogs. In addition, some dogs showed improvement without normalisation of their haematocrit (1/2 in group A), platelet count (1/3 in group B), total protein (1/4 in group A and 5/10 in group B), albumin (1/1 in group A and 1/7 in group B) and globulin (2/4 in group A and 3/10 in group B) concentrations, albumin/globulin ratio (3/7 in group B), BUN concentration (1/2 in group A) and UPC (1/5 in group B). These improvements of clinicopathologic abnormalities became evident between Day 60 and Day 360 but they were not accompanied by a reduction of bone marrow parasitic load based on qPCR or by a reduction of ELISA OD. At the end of the study, 20% (1/5) and 18.2% (2/11) group A and group B dogs, respectively, were bone marrow qPCR negative. The evolution of infectivity to sand flies and cell-mediated immunity were not examined and no adverse effects were recorded [262].

Conclusion: Allopurinol–metronidazole–ketoconazole–n-3 fatty acid–B vitamin combination cannot be recommended for the treatment of CanL due to the moderate efficacy (SORT: moderate) and the lack of information on critical features of this treatment such as the evolution of infectivity to sand flies and *Leishmania*-specific cell-mediated immunity. Due to the design of the study, it is not possible to draw conclusions about the efficacy of ketoconazole. Due to lack of a standardised diet, it is unclear if the addition of n-3 fatty acids and B complex vitamins offers some benefit (SORT: weak). Also, it is questionable if it is prudent to delay the administration of anti-*Leishmania* drugs by 1 month during which only n-3 fatty acids and B complex vitamins are administered (SORT: weak). However, n-3 fatty acids may have some beneficial effects on proteinuria (SORT: weak).

8.5.6 | Allopurinol–Vaccine Combination

The efficacy and safety of the combination of allopurinol and LeishF2 vaccine was tested in a single RCT (Table S27) [222]. Allopurinol was administered for 3 months at the recommended daily dose (20 mg/kg) and the vaccine was injected six times at 3-week intervals. The combination was administered to 8 dogs and was compared to allopurinol monotherapy and no treatment. The diagnosis of CanL was confirmed by bone marrow qPCR, but the severity of the disease is not reported. The quality of the study is intermediate [222].

After 1 year (9 months after treatment discontinuation), none of the dogs had died of CanL, and their total clinical score was significantly lower than the no treatment group, but not compared to allopurinol monotherapy. No further information about the clinical signs (e.g., percentage of dogs with clinical cure or improvement) and about the evolution of clinicopathologic parameters is provided. Bone marrow qPCR was negative in 7/8 dogs at 2 months and in all dogs at 6 and 12 months. Moreover, at the latter time point, liver and kidney qPCR was negative in all dogs and lymph node and spleen PCR was positive in 1 of 8 dogs. At 2, 6 and 12 months, bone marrow parasitic density was significantly lower compared to the no treatment group, at 13 months it was also lower compared to allopurinol monotherapy, and there was no tendency for increased parasitic density between 3 (treatment end) and 12 months (end of the study). No information on infectivity to sand flies, parasite-specific cell-mediated or humoral immunity were provided, and no adverse effects were reported [222].

Conclusion: The allopurinol–LeishF2 vaccine combination treatment cannot be recommended for the treatment of CanL because of the lack of an obvious benefit over allopurinol monotherapy, except for the lower parasitic density after 1 year (SORT: moderate), and due to the limited information on some critical features of this treatment such as the evolution of clinicopathologic abnormalities, infectivity to sand flies and *Leishmania*-specific cell-mediated and humoral immunity.

8.6 | Symptomatic Treatment

Some manifestations of CanL may need additional therapeutic interventions. The most common problems are proteinuria, arterial hypertension, uremic syndrome and epistaxis. Unfortunately, there is a shortage of clinical trials on the optimal management of these manifestations in the setting of CanL.

Proteinuria may have multiple negative consequences, including deterioration of kidney excretory function and CKD, arterial hypertension, hypoalbuminaemia, nephrotic syndrome and pulmonary thromboembolism [233, 245, 263]. As recommended treatments for CanL can decrease or ameliorate proteinuria [239–241], and depending on UPC values, creatinine and iP concentrations, a waiting period, varying between 3 days and 8 weeks, before adding symptomatic treatment for proteinuria has been proposed [172, 264]. Possible interventions include a diet low in phosphorous, angiotensin converting enzyme inhibitors (ACEIs, like benazepril or enalapril), angiotensin receptor blockers (e.g., telmisartan), aldosterone receptor blockers (e.g., spironolactone), n-3 fatty acid supplements, and, as a last resort, immunosuppressive and/or cytotoxic drugs like prednisolone, mycophenolate mofetil, cyclophosphamide, chlorambucil, ciclosporin and/or azathioprine [172, 265]. Usually ACEIs are the first line of medical treatment for proteinuria, and in an open clinical trial on 12 dogs with CanL at LeishVet stage II or III with CKD IRIS stage I or II, feeding a kidney-protective diet (12/12 dogs) and administration of benazepril (6/12 dogs) along with meglumine antimoniate-allopurinol combination resulted in significant reduction of UPC at 3 months, although proteinuria was still present in 3 of 5 initially proteinuric dogs [245]. Caution is advised if immunosuppressive and/or cytotoxic drugs need to be used because they may lead to initial treatment failure or relapse [266, 267]. The decision to start immunosuppressive treatment necessitates confirmation of immune-mediated glomerulonephritis through histopathology and/or direct immunofluorescence and/or electron microscopy [172]. Also, concurrent administration of azathioprine and allopurinol is contraindicated due to the risk of myelosuppression [94].

Conclusion: The addition of benazepril in the treatment of dogs with CanL and proteinuria may be beneficial (SORT: weak). In unresponsive cases, additional therapeutic interventions may be considered, but the benefit/harm ratio should be addressed on a case-by-case basis, especially if immunosuppressive and/or cytotoxic medication is prescribed (SORT: weak).

Hypertension is very common in dogs with CanL [233], but there are no reports on the efficacy of antihypertensive treatment. Therefore, the typical therapeutic strategy for the symptomatic management of canine hypertension can be followed, starting with ACEIs and, if they are not effective enough, adding angiotensin receptor blockers and then calcium channel blockers (e.g., amlodipine) [172, 265, 268].

Apart from the case report of a dog with acute kidney injury that responded to haemodialysis and later to standard treatment against CanL (initially allopurinol and later addition of miltefosine) [59], there are no data on treatment of advanced CKD/uremic syndrome in this disease. Due to the poor prognosis, euthanasia is often considered. Otherwise, standard symptomatic treatment should start as soon as possible, and, depending on the case, it may include haemodialysis, fluid/electrolyte parenteral administration, kidney diet, phosphate binders (e.g., aluminium hydroxide, calcium acetate or carbonate), calcitriol, antiemetics (e.g., maropitant, ondansetron) and stimulators of erythropoiesis (e.g., darbepoetin-a) [268, 269]. After stabilisation, treatment for CanL should start but there are scarce data on the safety of recommended drugs in these patients, except for allopurinol and marbofloxacin [232, 270]. Due to the low efficacy of allopurinol monotherapy and the relapses after marbofloxacin discontinuation, combination treatment should be considered after a certain period decided on a case-by-case basis. The potential of the aminoglycoside aminosidine to cause additional kidney damage and some evidence of histologic changes in the kidneys of healthy dogs after meglumine antimoniate but not after miltefosine administration [271] should be taken into consideration.

Conclusion: Dogs with CanL and uremic syndrome should first be stabilised with the symptomatic treatment for CKD, and then allopurinol or marbofloxacin can be administered followed by combination treatment (SORT: weak).

Epistaxis can cause blood-loss anaemia and even death. In the absence of studies, symptomatic treatment should be adjusted to the severity of bleeding and may include cold packs, nasal cavity tamponade, temporal ligation of carotid artery, blood transfusion and oxygen supplementation. As ulcerative rhinitis and thrombocytopathy are major causes of nasal bleeding in CanL [66], a short course of glucocorticoids at anti-inflammatory dose (e.g., prednisolone or prednisone, 0.5–1 mg/kg orally once daily for 7–21 days) may be beneficial by reducing nasal inflammation and restoring platelet function [272].

Conclusion: Epistaxis in dogs with CanL should be treated symptomatically and perhaps with a short course of glucocorticoids at anti-inflammatory dose (SORT: weak).

8.7 | Treatment of Concurrent Diseases

A wide variety of comorbidities, mainly of hormonal, neoplastic, infectious and parasitic aetiology, have been reported in dogs with CanL, and have been attributed to dual breed predisposition, co-endemicity, inadequate protection from insect/vector bites, CanL-induced immunosuppression and/or the effect of comorbidities to the immune system that may render a resistant, subclinically infected dog to develop CanL [38, 273–275]. It has been proposed that comorbidities should be actively searched in dogs with CanL, especially those with atypical manifestations and poor response to treatment. Their simultaneous (or sequential) treatment may improve the final outcome [276–278].

Conclusion: Treatment of comorbidities may increase the efficacy of treatment for CanL (SORT: weak).

An uncommon comorbidity involving the skin is a pustular dermatitis, different from the pustular form of CanL, resembling pemphigus foliaceus clinically, cytologically, histopathologically [94] and immunologically (S. Colombo, P. Bizikova: personal communication 2024). Reportedly, these lesions do not regress during anti-*Leishmania* treatment and necessitate administration of anti-inflammatory or immunosuppressive agents (systemic and topical glucocorticoids, ciclosporin, azathioprine) sometimes, but not always, at low doses and for a short period [94, 95]. However, some of the authors have seen many dogs with CanL and pemphigus foliaceus that necessitated intense and extended immunosuppressive treatment, which greatly interfered with the efficacy of anti-*Leishmania* treatment and the prognosis. Again, concurrent administration of azathioprine and allopurinol is contraindicated.

Conclusion: Pustular skin disease that does not respond to the standard treatment of CanL should be treated with glucocorticoids and perhaps other immunosuppressive drugs (SORT: weak).

8.8 | Measures Against Sand Fly Bites

Despite the lack of relevant studies, it is reasonable to protect dogs under treatment from further sand fly bites that may inject new parasites and that will also inject insect saliva, that may have local immunosuppressive effects [279]. This is also important to reduce the risk of transmission to other dogs and humans. Therefore, it is prudent to use, for life, effective insect repellents in all treated dogs living in endemic areas. Unfortunately, the efficacy of insect repellents is not absolute and not all effectively treated dogs are necessarily unable to transfer parasites to vectors; furthermore, if this happens, the transmitted parasites would have been exposed to drugs and may be more likely to be drug-resistant. Under laboratory conditions, some sand fly species (*Ph. perniciosus*, *L. longipalpis*) fed on healthy dogs receiving isoxazolines (afoxolaner, fluralaner) at registered dosage regimens show increased lethality within a time frame shorter than the time required for *Leishmania* spp. to evolve to the metacyclic promastigote stage and be able to infect new hosts

[280–282]. Therefore, administration of isoxazolines for life may reduce the spread of drug-resistant strains of *L. infantum*.

Conclusion: Effective topical insect repellents and oral isoxazolines (afoxolaner, fluralaner) at the registered dosage regimens are recommended for all dogs treated for CanL that live in endemic areas (SORT: weak).

8.9 | Treatment Monitoring

The aim of close patient monitoring during the whole treatment period is to ensure the efficacy of the selected therapeutic intervention(s), detect possible relapses during long-term allopurinol administration and avoid or treat medication adverse effects. The moderate-to-strong SORT in favour of allopurinol in combination with meglumine antimoniate or with miltefosine as first-line treatment, and the strong SORT in favour of allopurinol–aminosidine as a second-line treatment, were based on RCTs, reflecting the efficacy and safety of these interventions for the average dog with CanL, the severity of the latter depending on the inclusion criteria of each study. Therefore, efficacy cannot be guaranteed for every treated dog, especially if we consider that drug-resistant strains of *L. infantum* do exist, may become more common in the future and their prevalence may differ among geographical areas [169, 198, 199, 218]. Also, due to the low number of dogs enrolled in these RCTs and in non-controlled trials that were used to obtain safety information, published studies may not have been enough to capture uncommon adverse effects.

In addition to a detailed history, thorough physical examination and measurement of blood pressure, the minimum laboratory examinations should evaluate those parameters found to be associated with the response to treatment, lack of response or relapses during treatment, in the RCTs and in open clinical trials. These include complete blood count, serum biochemistry (including at minimum total proteins, albumins, globulins, albumin/globulin ratio, BUN, creatinine, iP and ALT), serum protein electrophoresis, complete urinalysis including measurement of UPC, quantitative serology and evaluation of parasitic burden (e.g., lymph node and/or bone marrow semi-quantitative microscopy [283] and/or qPCR) [102, 107, 128, 169, 172, 223, 240, 244, 246, 250, 283–288]. Blood qPCR is not considered reliable, probably due to daily variations in circulating number of parasites, but it has the advantage of easier sampling [102, 128, 169]. Also, monitoring of acute phase proteins (e.g., CRP, ceruloplasmin, ferritin), especially if their concentration was abnormal at baseline, can provide valuable information on treatment efficacy and may predict future relapses [223, 285, 289, 290]. Furthermore, kidney and urinary bladder ultrasonography (U/S) should be considered in allopurinol treated dogs with xanthinuria and/or clinical signs of urolithiasis [225] and close monitoring for acute pancreatitis (e.g., canine specific pancreatic lipase, abdominal U/S) is advised during meglumine antimoniate administration [239].

All of the aforementioned examinations are complementary and not interchangeable; there are multiple examples of relapsing dogs where clinical signs (most commonly skin lesions or peripheral lymphadenomegaly) were the first abnormalities [169, 172, 270] and of dogs developing end-stage CKD before presenting clinical signs of CanL. Also, the frequent practice of heavily relying on the evolution of antibody titres is strongly discouraged; persistence of antibody titres in responders and lack of a substantial increase of these titres in relapsing dogs are not uncommon [169, 181, 240, 246, 287].

Timing of re-examinations should be tailored to the needs of each patient: from daily in critically ill hospitalised dogs, to after 1–2 weeks, 4 weeks (end of meglumine antimoniate or miltefosine or aminosidine administration), 3 months, 6 months and every 6 months thereafter for the whole treatment duration in dogs showing complete response [100, 102, 107, 172]. The selection of laboratory examinations performed each time will also depend on the patient, but also on the expected time to show meaningful improvement, the invasiveness of sampling and cost. Although quantitative serology is typically considered the last examination showing significant changes during effective treatment and usually recommended after 3–6 months, an end-point sera dilution ELISA can show significant reduction of antibody concentrations already from the end of the first month of meglumine antimoniate–allopurinol combination treatment [102].

If there is no response to initial treatment or a fast relapse despite continuous allopurinol administration, an alternative drug should be considered (e.g., meglumine antimoniate instead of miltefosine and *vice versa*) because, at least in theory, there is an increased probability of parasites being resistant to the initially selected medication. If the relapse occurs later, the same or an alternative treatment can be considered, and the dog should be scrutinised for concurrent diseases causing immunosuppression. Finally, in dogs needing repeated treatment cycles, it may be prudent to avoid administering meglumine antimoniate, miltefosine or aminosidine for more than 2–3 cycles each, but to switch among them due to the possibility of drug resistance.

Conclusion: Close monitoring of all dogs under treatment of CanL is necessary (SORT: strong) at time intervals adjusted to each patient (SORT: weak). Minimum laboratory examinations should include complete blood count, serum biochemistry, serum protein electrophoresis, complete urinalysis including UPC, quantitative serology and evaluation of parasitic burden by microscopy and/or qPCR ideally in tissues with high parasitic density during CanL (SORT: strong). The results of all these examinations should be considered along with the history and physical examination findings, before taking any medical decisions (SORT: moderate).

8.10 | Treatment Discontinuation and Follow-Up

Unfortunately, there is a lack of properly designed longitudinal studies aiming to detect surrogate markers predicting whether a well-controlled dog will relapse or not after allopurinol discontinuation. On the other hand, long-term allopurinol administration carries the risk of induction of resistant strains of *L. infantum* that may jeopardise currently advised treatment protocols [169]. Moreover, long-term allopurinol treatment is associated with adverse effects mainly related to xanthinuria (e.g., kidney mineralisation, urolithiasis) [225]. For this reason, it has

been proposed to discontinue allopurinol if all the following conditions are met: (i) it has been administered continuously for at least 6–12 months; (ii) all clinical signs and laboratory abnormalities of CanL have resolved, except those that may be irreversible (e.g., posterior segment ocular lesions, kidney fibrosis) or persist without being provoked by the parasite or the immune response (e.g., glomerulonephritis); (iii) baseline antibody concentrations are reduced and do not show tendency to increase in successive quantitative serologic examinations (without the need for the dog to become seronegative); (iv) baseline parasitic burden has been reduced to the point that *Leishmania* amastigotes cannot be found on microscopy and only a low amount of parasite DNA is present in lymph nodes, bone marrow, spleen or skin [99, 107, 172].

After allopurinol discontinuation lifelong monitoring is mandatory, with re-examinations every 6–12 months or at any time point there is suspicion of relapse. If the latter is confirmed, treatment should be restarted with the same therapeutic protocol or an alternative one. Although there are no relevant studies, it is logical to continue insect repellents and isoxazolines for life. Immunomodulators, such as domperidone, nutritional supplements or deslorelin implants (in intact male dogs), may also be considered because typically they are safe, not very expensive and due to their mode of action they do not induce drug resistance.

Secondary prophylaxis with the periodic administration of drugs with direct anti-*Leishmania* efficacy has been effectively practiced in immunosuppressed (e.g., HIV-positive) humans with VL because of the high risk of relapse [291, 292]. A similar strategy was shown to be effective in an open trial where after discontinuation allopurinol was re-administered, at the recommended dose, 1 week every month on long-term [293]. However, the initial course of allopurinol was shorter than the minimum recommended 6-month period and intermittent allopurinol administration will expose the parasite to fluctuating drug concentrations that can induce resistance. For this reason, and considering the high importance of preserving allopurinol efficacy, this practice is strongly discouraged.

Conclusion: Allopurinol administration should be discontinued after a minimum period of 6–12 months if clinical and laboratory abnormalities have resolved, and antibody concentrations and parasitic load have decreased (SORT: weak). Following discontinuation, lifelong monitoring for possible relapses is necessary (SORT: weak), continuous use of insect repellent and isoxazolines is advised (SORT: weak) and administration of immunomodulators may be considered (SORT: weak). Periodic allopurinol administration as secondary prophylaxis is strongly discouraged (SORT: weak).

8.10.1 | Summary of Recommendations for the Treatment of CanL due to *L. infantum*

- Euthanasia of dogs with CanL for public health purposes cannot be recommended. Euthanasia of individual dogs can be considered if proper treatment cannot be administered and if prognosis is poor.
- Administration of drugs with direct anti-*Leishmania* activity should be avoided in subclinically infected dogs.
- The aim of treatment is not parasitological cure, but induction of *Leishmania*-specific cell-mediated immunity.
- Recommended treatments for CanL include meglumine antimoniate–allopurinol combination (first-line treatment), miltefosine–allopurinol combination (first-line treatment) and allopurinol–aminosidine combination (second-line treatment). Marbofloxacin may be considered for the initial management of dogs with advanced CKD or bacterial infections sensitive to this fluoroquinolone.
- Non-recommended treatments for CanL include monotherapy with meglumine antimoniate, liposomal formulations of meglumine antimoniate, miltefosine, allopurinol, aminosidine, metronidazole, *O*-alkyl-hydroxamate (MTC-305), (−)-α-bisabolol, artesunate, domperidone, nutritional supplement DiLsh, monoclonal antibodies against canine IL-10 receptor, LiF2 vaccine, Leish-110f vaccine with MPL-SE and rLdccys1 antigen with *P. acnes* or *L. braziliensis* antigen with MPL. Non-recommended combination treatments include liposomal meglumine antimoniate–allopurinol, meglumine antimoniate–aminosidine, meglumine antimoniate–metronidazole, meglumine antimoniate–*O*-alkyl-hydroxamate (MTC-305), meglumine antimoniate–dietary nucleotide/active hexose correlated compound, meglumine antimoniate–Leish-110f/Leish-111f vaccine, meglumine antimoniate–allopurinol–domperidone, meglumine antimoniate–allopurinol–deslorelin, allopurinol–metronidazole–ketoconazole–n-3 fatty acid–B vitamin and allopurinol–LeishF2 vaccine.
- Benazepril may be beneficial in dogs with proteinuria.
- Dogs with uraemic syndrome should first be stabilised with symptomatic treatment, and then allopurinol or marbofloxacin can be administered followed by combination treatment.
- Epistaxis should be treated symptomatically and with a short anti-inflammatory course of glucocorticoids if necessary.
- Pustular skin disease that does not respond to the standard treatment of CanL should be treated with glucocorticoids and other immunosuppressive drugs if necessary.
- Early diagnosis and treatment of comorbidities may increase the overall efficacy of treatment.
- Insect repellents (to restrict further exposure to sandflies) and isoxazolines like afoxolaner or fluralaner (to restrict spread of drug-resistant parasites) are recommended for all dogs under treatment living in endemic areas.
- Close monitoring, at time intervals adjusted to each patient, is necessary. Minimum laboratory examinations should include complete blood count, serum biochemistry, serum protein electrophoresis, complete urinalysis including UPC, quantitative serology and evaluation of parasitic burden by microscopy and/or qPCR from target organs.
- Allopurinol should be discontinued after a minimum period of 6–12 months if clinical and laboratory abnormalities

have resolved, and antibody concentrations and parasitic density have substantially decreased.

- Following allopurinol discontinuation, lifelong monitoring for relapses is necessary, continuous use of insect repellent and isoxazolines is advised and administration of immunomodulators may be considered. Periodic allopurinol administration is strongly discouraged.

9 | Prevention of Canine Leishmaniosis

In endemic areas, prevention of CanL is based on the reduction of exposure of dogs to sand fly bites (e.g., insect repellents, environmental insecticides, not spending the night outdoors, use of fine mesh nets), avoidance of using infected dogs as blood donors or breeding animals and boosting parasite-specific cell-mediated immune responses of subclinically infected dogs to avoid development of the disease (e.g., vaccines, immunomodulators). These approaches should be adjusted to the needs of each dog and the epidemiological situation in each endemic area, and, since they contribute to the prevention of CanL through different mechanisms, they should be considered additional to each other, and when feasible, they can/should be combined.

Temporal use of insect repellents has been recommended for dogs travelling from non-endemic countries to endemic areas during the period of sand fly activity. Subclinically infected dogs living in non-endemic areas should not be used as blood donors or breeding animals.

9.1 | Insect Repellents

As *Leishmania* spp. transmission in endemic areas occurs mainly when sand fly vectors bite infected dogs, especially those with CanL, prevention of vector bites is crucial to reduce the risk of transmission to other dogs and animal species, including humans [294]. By virtue of the irritating and killing effect exerted by pyrethroids (e.g., deltamethrin, flumethrin, permethrin) against phlebotomine sand flies, these molecules have been used in different formulations (i.e., impregnated collars or spot-on formulations) to reduce the rate of sand fly bites. Insect repellents have been recommended year-round or during defined period of sand fly activity for all dogs living or visiting endemic areas, and for infected dogs living in non-endemic areas where sand fly vectors are endemic [295]. While there is a range of products in the market with repellent and insecticidal efficacy against sand flies, results from laboratory studies do not prove field efficacy for prevention of CanL. The latter should be confirmed by randomised clinical trials under field conditions.

9.1.1 | Deltamethrin 4% Collar

Four RCTs (Table S28) evaluated the efficacy of collars impregnated with deltamethrin 4% (Scalibor Protector Band; MSD Animal Health) in preventing *L. infantum* transmission by sand flies [296–299]. The efficacy was compared to 'no collar' [296–299], to flumethrin 4.5% plus imidacloprid 10% collar [298] and to vaccination with excreted-secreted proteins from amastigotes of *L. infantum* with saponin QA-21 as adjuvant (CaniLeish; Virbac) [298]. In two studies, conducted in shelter dogs [297, 298], collars were renewed after about 4 months and dogs were followed for a total of 8 months [298] or 24 months [297]. In the other two RCTs, conducted in privately-owned dogs, collars were applied at the beginning of the transmission season, were not renewed, and dogs were re-evaluated after 6–12 months [296, 299]. The number of dogs treated with deltamethrin 4% collars varied from 60 [297, 298], to 354 [296] and 454 [299]. All these dogs were seronegative at the beginning of each trial and efficacy was determined by the absence of seroconversion by the end of the study. Presence of clinical signs of CanL was also evaluated in two studies [297, 298], and bone marrow PCR and microscopy at the final follow-up were conducted in one [298]. The quality of these studies is intermediate [296, 298, 299] or low [297].

According to the results, the percentage of seronegative dogs at the end of each study varied from 88.6% to 98.7%, being always significantly higher than that of untreated dogs (58.8%–93.3%). In one study, collared dogs that seroconverted presented significantly less clinical signs of CanL compared to the controls, suggesting that less *Leishmania* parasites may have been transmitted and/or less exposure to sand fly saliva [297]. However, this was confuted in a similar higher quality study [298], where no difference was found in the prevalence of clinical signs, bone marrow PCR and microscopy positivity between treated and untreated dogs that developed seropositivity. In the latter study, local skin irritation was reported as the only adverse effect in 5% of treated dogs [298].

A recent meta-analysis of 12 randomised and non-randomised controlled trials of at least 5-month duration, concluded that use of deltamethrin 4% collar decreased seroconversion and/or positive results of parasitological tests (mainly microscopy) and/or positive results of molecular tests by 54%. The relative risk of collared compared to uncollared dogs of becoming positive in one of these tests was 0.461 [300]. However, there was heterogeneity of results among studies and a risk of publication bias was found [300].

Conclusion: In endemic areas, deltamethrin 4% impregnated collar is recommended as a first-line measure to prevent exposure to *L. infantum* during the transmission period (SORT: moderate).

9.1.2 | Flumethrin 4.5% Plus Imidacloprid 10% Collar

Four RCTs (Table S29) evaluated the efficacy of polymer matrix collars containing flumethrin 4.5% and imidacloprid 10% (Seresto; Elanco) in preventing *Leishmania* transmission by sand flies [298, 301–303]. The efficacy was compared to 'no collar' in all studies [298, 301–303], and also to deltamethrin 4% collar [298] and vaccination with excreted-secreted proteins from amastigotes of *L. infantum*, with saponin QA-21 as adjuvant (CaniLeish; Virbac) [298]. In three studies, conducted in shelter dogs [298, 301, 302], collars were applied at the beginning of the transmission season and were left in place for 7–8 months, and the dogs were followed for 10–12 months. In the study conducted in privately owned animals, collars were applied at the beginning of the transmission season and replaced after 8 months, and the dogs were examined at 16 months [303]. The number of dogs treated with flumethrin

4.5% plus imidacloprid 10% collars varied from 55 to 102. All dogs were seronegative at the beginning of each trial and efficacy was determined by the absence of clinical signs and of seroconversion by the end of the study. In addition, PCR and microscopic examination of different organs (bone marrow, lymph node, skin, conjunctiva) were conducted in all RCTs [298, 301–303]. The quality of all four RCTs is intermediate.

According to the results, the percentage of seronegative dogs at the end of each study varied from 96.1% to 100%, being always higher than untreated dogs (60.6%–86%), but this difference was significant in only one study [302]. On the other hand, in two of the studies conducted in shelters, 100% of collared dogs were seronegative at the end of the trial, and the lack of significant difference from controls may have been due to their close vicinity with the collared ones, resulting in a 'blanket' effect [298, 301]. In the other two studies, the majority (95%–100%) of collared dog did not present clinical signs of CanL, whereas the relative percentage was lower for the controls (around 70%, although raw data do not always permit precise calculations) [302, 303]. The percentage of collared dogs with negative skin [298, 301, 302] and/or bone marrow [298, 301, 302] and/or lymph node [303] and/or conjunctiva [301, 303] PCR and/or negative bone marrow [298, 301, 302] and/or lymph node [303] microscopy at the end of the trials was always numerically higher than that of uncollared controls (100% vs. 31.4% [301], 97.7% vs. 82.7% [302], 96.4% vs. 76% [298] and ≤95.7% vs. ≤68.8%) [303].

A recent meta-analysis of three randomised and non-randomised controlled trials of at least 5-month duration concluded that use of flumethrin 4.5% plus imidacloprid 10% collar decreased seroconversion and/or positive results of parasitological tests (mainly microscopy) and/or positive results of molecular tests by 90%, and thus the relative risk of collared compared to uncollared dogs to become positive in one of these tests was 0.098 [300]. Also, there was no evidence of heterogenicity of the results among studies [300].

Conclusion: In endemic areas, flumethrin 4.5% plus imidacloprid 10% collar is recommended as a first-line measure to prevent exposure to *L. infantum* during the transmission period (SORT: moderate).

9.1.3 | Permethrin 50% Plus Imidacloprid 10% Spot-On

Two RCTs (Table S30) evaluated the efficacy of a spot-on containing permethrin 50% and imidacloprid 10% (Advantix, Elanco), for the prevention of *Leishmania* transmission by sand flies [304, 305]. Both studies were conducted in sheltered dogs and the efficacy of this product was compared between treated and untreated control dogs. The product was applied at the registered dose every 21 days for 12 months [305], or for a transmission season (8 months) every 28 days [304]. In the latter study a second group of treated dogs was also included and in these dogs the product was applied every 14 days for the same 8-month period [304]. The number of dogs treated as per label (i.e., every 21–28 days) was 71 [305] and 209 [304], whereas 218 dogs were treated every 2 weeks [304]. All dogs were seronegative at the beginning of the study and efficacy was determined by absence of seroconversion and negativity of lymph node [304] or bone marrow [305] microscopy and skin [304, 305] and/or bone marrow [305] PCR at 12 months. The quality of both RCTs is high.

According to the results, the percentage of treated dogs that remained seronegative varied from 98.9% to 100% and was higher the untreated dogs (≥52.4%–94.2%). Most (98.9%) [304] or all (100%) [305] treated dogs were microscopy- and PCR-negative, compared to ≥52.4% (bone marrow microscopy, bone marrow PCR, and skin PCR) [305], 94.2% (skin PCR) [304] and 98.4% (lymph node microscopy) [304] untreated controls. More frequent (i.e., every 2 weeks) than the registered interval for successive applications of the product did not offer any benefit [304]. No adverse effects were reported.

Conclusion: In endemic areas, permethrin 50% plus imidacloprid 10% spot-on, applied every 3–4 weeks during the transmission period, is recommended as a first-line measure to prevent exposure to *L. infantum* (SORT: strong).

9.2 | Environmental Insecticides

Mass control of sand flies has been attempted, mainly in areas where human VL is endemic, by eradicating their breeding places (a non-practical approach), and applying environmental insecticides (e.g., cyhalothrin, cypermethrin, deltamethrin, DDT), sometimes in combination with insect growth regulators (e.g., diflubenzuron), usually in spray forms or in impregnated nets for animal shelters and sometimes in combination with lures containing synthetic pheromones that attract sand flies to the insecticide-treated surfaces [306–308].

In a RCT that was conducted in Brazil but not analysed herein because it did not fulfil the eligibility criteria, spraying close to chicken houses every 3 months, along with the use of lures containing *L. longipalpis*-attracting pheromone, was more effective than placebo for the reduction of the risk of seroconversion of seronegative dogs living in the household and risk of the same dogs to become blood PCR positive. However, deltamethrin 4% collars were more effective than this strategy in reducing the incidence of seroconversion, equally effective in reducing blood PCR positivity and less effective in reducing the number of male (but not of female) sand flies [307]. Moreover, in an old systematic review, insecticide spraying was not found to decrease the prevalence of seropositivity [309] and in a recent open study environmental insecticide use was more common in dogs with CanL compared to subclinically infected dogs [273]. Finally, development of insecticide resistant sand flies is a major concern [310], like environmental pollution and, depending on the insecticide, toxicity for humans and animals.

Conclusion: In endemic areas, use of environmental insecticides for the prevention of CanL cannot be recommended because of lack of efficacy superior to insect repellents, and due to concerns about environmental pollution and toxicity to humans and animals (SORT: weak).

9.3 | Indoor Confinement and Use of Fine Mesh Nets

Although many veterinarians, at least in southern Europe [311], recommend indoor confinement of dogs during the night (the period of maximum sand fly activity) as a preventive measure against CanL, there are no published data on the efficacy of this practice. The same is true for dog confinement in cages covered by fine mesh nets, that is also commonly recommended by practicing veterinarians [311, 312], especially when insect repellents cannot be used or are contraindicated [313].

Conclusion: In endemic areas, indoor confinement and use of fine mesh nets cannot be recommended for the prevention of CanL due to lack of data on efficacy (SORT: weak). However, when these measures are feasible, easy to implement, and not stressful for dogs, there is no concern against their implementation (SORT: weak).

9.4 | Not Using Infected Dogs as Blood Donors

The IV injection of *L. infantum* amastigotes, despite bypassing the natural route of parasite inoculation (dermis) and the immunomodulatory effects of sand fly saliva, has been used effectively for experimental infection of dogs and, in susceptible animals or with high inoculums, for induction of CanL [314]. Similarly, in areas where sand fly transmission of the parasite does not occur, transfusion of infected blood or blood products has been shown to cause infection and perhaps CanL to the recipients [315, 316]. It is reasonable to assume that the same can happen in endemic areas, although the epidemiologic significance is probably much lower, considering the relatively lower proportion of dogs that will receive blood transfusions during their life compared to dogs naturally exposed to *L. infantum*. On the other hand, blood recipients are by default immunosuppressed, and this may increase the chances to develop CanL if they become infected.

For these reasons, it has been proposed to regularly (e.g., twice per year) examine blood donors using serology, blood PCR and, due to the intermittent nature of parasitemia, PCR in another sample, like lymph node, bone marrow or spleen aspirate [99, 295]. An alternative approach could be to perform PCR in every blood products or to remove white blood cells (leukodepletion) [317]. Obviously, seropositive or subclinically infected dogs should be excluded from blood donors and PCR-positive blood products should be discarded.

Conclusion: Blood donors should be examined periodically (serology, PCR), or all blood products should be examined by PCR or leukodepleted (SORT: weak).

9.5 | Not Using Infected Dogs as Breeding Animals

Vertical transmission of *L. infantum* is well-documented and adequate to sustain the persistence of infection and CanL over decades, in areas where vectorial transmission does not occur [318]. As for blood transfusion, the epidemiological importance of vertical transmission in endemic areas is obscure but probably not negligible, considering that 3.6%–4.2% of puppies had evidence of infection (positive PCR and/or microscopy) and/or were seropositive before the beginning of the first sand fly season of their life [305, 319]. Although removal from the reproduction pool and spaying all infected females seems straightforward in non-endemic areas, the high prevalence of subclinical infection in endemic areas renders this approach impractical. Since seropositive dogs and, even more, dogs with CanL tend to have the highest parasitic burdens, they may be more likely to infect their offsprings [320, 321], and their removal from reproduction seems feasible in endemic areas [295].

Conclusion: In areas where the main route of parasite transmission may be vertical (i.e., absence of sand flies), removal of all infected bitches dogs from reproduction is the mainstem preventive measure (SORT: weak). In endemic areas, seropositive bitches or bitches with CanL should not be bred (SORT: weak).

9.6 | Vaccines

Development of vaccines effective in preventing the appearance of CanL in subclinically infected dogs is very difficult, because protozoa are, in general, much more complex organisms compared to viruses or bacteria and induction of humoral responses is not protective against CanL [322]. This is further exemplified by the fact that, worldwide, there is no licensed vaccine against human leishmaniases and especially against human VL due to *L. donovani* or *L. infantum*. At the time of this writing, the only commercially available vaccines registered for the prevention of CanL are the protein Q vaccine (LetiFend; LETI Pharma) and the plasmid vector pPAL encoding *L. infantum* activated protein kinase C receptor analogue (LACK) vaccine (Neoleish; CZ Vaccines S.A.U.) but there are no published RCTs on the latter.

9.6.1 | Autoclaved *L. major* Promastigote Vaccine

The vaccine is produced in a research institute using cultured *L. major* promastigotes, first mixed with aluminium hydroxide, then they were autoclaved, and subsequently bacillus Calmette–Guerin (BCG) was added as a second adjuvant. Under experimental conditions, the vaccine induces long-term parasite-specific proliferation of peripheral blood lymphocytes, low antibody titres, and partial protection after experimental infection [323, 324]. The efficacy and safety of the vaccine was tested in two RCTs (Table S31) [325, 326]. Each vaccine dose contained 200 μg of parasite protein, 2×10^6 colony forming units (CFU) of BCG and a variable amount of aluminium hydroxide (61.7 μg [325] or 1400 μg) [326]. It was administered intradermally (ID) either once [325] or twice at 1-month interval [326]. In an effort to increase efficacy the second RCT [326], utilised an increased dose of aluminium hydroxide, a booster vaccination, and imiquimod (125 mg) was applied 20 min before on the site of ID injection [326]. Enrolment criteria included lack of clinical signs, negative serology and negative leishmanin skin test. Dogs were allocated to receive either the vaccine (121–182 dogs) or normal saline (113–165 dogs). The quality of both RCTs is low [325, 326].

The efficacy of the vaccine to prevent CanL was not tested in either of these RCTs; instead, negative serology after two transmission seasons (16–18 months) [325, 326] and positive leishmanin skin test at 6 months [326] were used as surrogate markers of efficacy (prevention of seroconversion and induction of *Leishmania*-specific cell-mediated immunity, respectively). In one study, the incidence of seroconversion was significantly lower in the vaccinated dogs compared to controls [325], but in the other study the difference was not significant [326]. However, the prevalence of positive leishmanin skin test as 6 months was significantly higher among vaccinated dogs [326]. Ulceration at the vaccination site developed in 64.5% of the dogs in the first RCT [325], but only in one dog in the second study, despite the higher dose of aluminium hydroxide and the previous application of imiquimod [326]. Additionally, mild topical reactions (but no systemic adverse effects) were reported in both RCTs [325, 326].

Conclusion: The autoclaved *L. major* promastigote vaccine cannot be recommended for the prevention of CanL due to the lack of information on efficacy to prevent the development of CanL (SORT: weak) and potentially severe (ulcers) local adverse effects (SORT: weak).

9.6.2 | Excreted–Secreted Proteins (Antigens) From Amastigotes of *L. infantum*

The antigen for this vaccine is produced from the supernatant of axenic (i.e., without addition of host cells) cultures of *L. infantum* amastigotes, from which the excreted-secreted proteins (*Li*ESP) of the amastigotes are purified. It contains 50–100 proteins/glycoproteins, in their natural conformation and glycosylation status, most of them belonging to the parasite surface antigen (PSA) family. An excreted protein with molecular weight of 54 kDa seems to be the most immunogenic one [327]. Initially it was tested as an experimental vaccine with the addition of the adjuvant muramyl-dipeptide (MDP). Under laboratory conditions, it was shown to induce parasite-specific cell-mediated immune responses and a Th1-polarised cytokine milieu with increased production of INF-γ, increase the leishmanicidal activity of macrophages and found effective against experimental infection [327–329]. The efficacy and safety of this vaccine, containing 100 μg antigen and 200 μg adjuvant, were examined in two RCTs (Table S31) [329, 330]. The duration of these studies was 8 months [329] or 2 years [330], the number of vaccinated and control dogs was 9 and 9 [329] or 205 and 209 [330], and all dogs were most likely non-infected [329] or clinically healthy and seronegative [330]. In both studies, dogs received two SC vaccine doses at 3- to 4-week intervals and in the long-term study a booster was administered after 1 year, whereas the controls received either the adjuvant MDP [329] or placebo [330]. The quality of these studies is intermediate [329] or low [330].

None of the seven subclinically infected vaccinated (6/7) or control (1/7) dogs developed CanL at 4 months [330], and, at 2 years, none of the 168 vaccinated seronegative dogs that remained in the study presented CanL, in contrast to 2.7% (5/180) of the placebo controls [330]. The authors of the present consensus document tested statistically this difference and it was found to be significant ($p = 0.036$) by one-tailed Fischer's exact test but non-significant ($p = 0.061$) by two-tailed Fischer's exact test. Multiple positive immunological effects of vaccination were found: in vitro, lymphocytes isolated from the blood of vaccinated dogs produced INF-γ and nitric oxide production and leishmanicidal activity of heterologous macrophages was increased [330]. Homologous monocyte-derived macrophages showed increased INF-γ production and leishmanicidal activity [329]. The serum of vaccinated dogs had direct activity against *L. infantum* promastigote and amastigote survival, proliferation, differentiation and infectivity to heterologous macrophages [329] and vaccinated dogs developed positive leishmanin skin test results after 2 and 8 months [329]. The percentage of dogs with negative bone marrow culture and PCR at the end of the 2-year study was significantly lower among vaccinated (99.4%) dogs than controls (93.1%), and all initially infected dogs (vaccinated and controls) were negative [330]. Despite production of IgG against the vaccine antigen [329, 330], at 2 years most vaccinated (95.8%) and control (92.2%) dogs were seronegative using IFA, with cut-off 1/100 [330]. Mild injection site reactions were the only reported adverse effect and were common [330].

Conclusion: The *Li*ESP with MDP vaccine can be used for the prevention of CanL, due to the borderline significant protection against development of the disease and the lack of severe adverse effects (SORT: moderate).

The same antigen with a different adjuvant (saponin QA-21) became commercially available as CaniLeish (Virbac) in Europe and some Latin American countries, but at the time of writing production of the vaccine has stopped. It is licensed for clinically healthy, seronegative dogs older than 6 months. Each dose contains at least 100 μg ESP and 60 μg adjuvant, and vaccination schedule includes a prime vaccination of three doses at 3-week intervals, followed by annual boosters. Immunogenicity is similar to the experimental vaccine: priming of lymphocytes that are able to proliferate after exposure to the parasite, produce INF-γ and activate macrophages, with the latter showing increased leishmanicidal activity [331–333]. Protection from experimental infection by *L. infantum* was proven based on clinical presentation and bone marrow qPCR [333].

The efficacy and safety of the commercial vaccine were tested in three RCTs (Table S31) [298, 334, 335]. In all of them, dogs were vaccinated three times at 3-week intervals and in the one RCT that lasted 2 years, an annual booster was administered [334]. All three RCTs enrolled clinically healthy dogs with negative serology [298, 334, 335]; in two of them negative bone marrow PCR [298, 334] and in one of them negative bone marrow microscopy and negative skin PCR [298] were additional inclusion criteria. The number of vaccinated dogs varied from 46 to 71, and were compared to no intervention [298, 334, 335]. The quality of the studies is intermediate [298, 334, 335].

These field trials confirmed the immunogenicity of the vaccine observed under laboratory conditions. Parasite-specific cell-mediated immune responses were examined by PBMC production of INF-γ after stimulation with soluble *Leishmania* antigen (SLA) which was found to be significantly higher 1 and 9 months after the last vaccination of the prime series compared to baseline, and significantly higher compared to the controls only at 1 month [335]. Production of IgG against vaccine antigen (ESP) that also recognise SLA and cause vaccination-induced seroconversion at 8 weeks in 70% of the dogs was shown [334].

The evaluation of vaccine efficacy for prevention of CanL is not possible from the data of the older RCT because dogs found infected (by PCR and/or culture) at the end of the 2-year period without clinical signs but with up to three clinicopathologic abnormalities compatible with CanL or with one clinical sign and up to two clinicopathologic abnormalities, were considered 'clinically healthy' [334]. Regardless, none of the vaccinated dogs died or was euthanised due to CanL, compared to 11.4% (5/44) non-vaccinated controls [334]. In the other two RCTs, both lasting 1 year, the prevalence of CanL at the end of the trial among vaccinated (2/54; 3.7%) and non-vaccinated (1/60; 1.7%) dogs [298], or the detection of ≥2 clinical signs compatible with CanL in vaccinated (9/71; 12.7%) and non-vaccinated (9/74; 12.2%) dogs [335] did not differ. However, the prevalence of ≥2 clinicopathologic abnormalities of CanL among dogs that became seropositive and/or were CanL suspects was significantly higher in the non-vaccinated (85.7%) compared to the vaccinated (47.6%) group [335]. As expected, the number of non-infected dogs did not differ between groups. At the end of the 24-month long RCT, 41.5% (17/41) vaccinated and 28% (11/39) non-vaccinated dogs were bone marrow PCR and culture negative [334]. At the end of a 1-year long trial 72.2% (39/54) vaccinated and 80% (48/60) non-vaccinated dogs were negative on bone marrow and skin PCR plus bone marrow microscopy [298]. Finally, in the third RCT, where only dogs that became seropositive were tested by lymph node PCR at 9 months after the 3rd prime vaccination, 57.1% (12/21) vaccinated and 28.6% (4/14) controls were negative [335]; none of those differences was significant. The same applies to seroconversion: the prevalence of vaccinated dogs that remained seronegative was 88.9% [298] and 70.4% [335], whereas the prevalence of controls that remained seronegative was 88.3% [298] and 81.1% [335], respectively. Again, none of these differences was significant. Adverse reactions are reported in 0% [298], 1.2% (anorexia, apathy) [335] or up to 52.2% (self-limited local reactions) [334] of vaccinated dogs.

Although not examined in a RCT, there is some evidence that vaccinated dogs may become less capable to transmit the parasite to sand flies [336].

Contrary to the above, a recent meta-analysis concluded that the relative risk of infection by *L. infantum* and/or CanL is significantly reduced, and that approximately 3.8 dogs must be vaccinated for one of them to get benefit [i.e., number needed to treat (NNT) = 3.77], which is significantly lower compared to negative controls and protein Q vaccine [337]. The discrepancy with the results of the present systematic review may have been due to the different and variable outcome measures considered in the meta-analysis.

Conclusion: The *Li*ESP with QA-21 vaccine cannot be recommended for the prevention of CanL due to lack of evidence of protection against development of the disease (SORT: moderate).

9.6.3 | Fucose-Mannose Ligand (FML) of *L. donovani*

The fucose-mannose ligand of *L. donovani* with saponin QA-21 was commercially available in Latin America (Leishmune; Fort Dodge Animal Health) but, at the time of writing, its marketing licence has been withdrawn. The efficacy and safety of this vaccine, with added adjuvant, for the prevention of CanL have been tested in one RCT (Table S31) [338]. This study enrolled seropositive dogs without CanL, although six of them presented clinical signs of the disease between enrollment and the start of the interventions. It is unknown if some additional dogs presented relevant clinicopathologic abnormalities at either time point. A total of 31 dogs received the commercial vaccine (1.5 mg FML protein plus 0.5 mg saponin QA-21) with the addition of 1 mg Riedel de Haen saponin, SC three times at 20- to 30-day intervals. Thirty-five dogs were vaccinated in the same way, received allopurinol and some of them amphotericin B, and were compared to 25 untreated controls. The quality of the study is low [338].

After 3 months, all untreated dogs of the control group presented the disease, and 48% of them had died of CanL, compared to 38% of vaccinated dogs (19% died) and 18% of vaccinated dogs that received anti-*Leishmania* treatment (12% died). Death rate between the latter two groups (32% and 20%, respectively) was not different after 4.5 years. All vaccinated dogs that survived at 8 months were leishmanin skin test positive, and lymph node PCR was negative in 33% (vaccination) or 80% (vaccination and chemotherapy) dogs. No adverse effects are reported [338].

Conclusion: The FML and QA-21 vaccine with the addition of Riedel de Haen saponin is recommended for the prevention of CanL in subclinically infected seropositive dogs (SORT: moderate). On the contrary, it is not recommended to also administer either allopurinol or amphotericin B in dogs without CanL (see: treatment).

9.6.4 | LiF2 *L. infantum* Promastigote Fraction

The efficacy and safety of an experimental vaccine containing 20 μg of a fraction (F2) of proteins derived from *L. infantum* promastigotes with molecular weight 67–94 kDa (LiF2) and 100 μg MDP were compared to the adjuvant alone in a RCT (Table S31) of 2-year duration that included 393 seronegative dogs [339]. Vaccination was performed three times at 30 ± 10 day intervals and the quality of the study is intermediate [339]. The prevalence of CanL among vaccinated dogs and controls is not clearly reported, but the prevalence of seroconversion at 12–14 and 24 months was significantly higher in the former. One dog presented anaphylaxis, and some additional but mild adverse effects were witnessed [339].

Conclusion: LiF2 with MDP vaccine cannot be recommended for the prevention of CanL due to the lack of evidence of protection against development of the disease (SORT: moderate).

9.6.5 | Live Gentamycin-Attenuated *L. infantum* (H Line)

A strain of *L. infantum* was exposed in vitro to gentamycin, resulting in attenuation of expression of some important parasite proteins, including tryparedoxin peroxidase, and inability to disseminate from the site of vaccination [340, 341]. Under

laboratory conditions, intradermal or IV administration resulted in strong cell-mediated immunity (enhancement of PBMC proliferation after exposure to SLA with increased production of INF-γ, decreased production of IL-10, and induction of positive leishmanin skin test results), production of parasite-specific antibodies and protection from the development of CanL after experimental infection with wild-type parasites [340, 341]. The efficacy and safety of this live-attenuated vaccine was tested in a RCT (Table S31) of 2-year duration, that enrolled 103 seronegative and PCR-negative dogs (all German shepherd dog crosses), that were either vaccinated (55/103) SC (100 μL of a suspension of stationary stage promastigotes) once or received placebo (48/103) [342]. The quality of the RCT is low.

None of the vaccinated dogs and 29% of the controls developed CanL that was classified as LeishVet stage I (1/9 dogs), II (7/9 dogs) or III (1/9 dogs). Also, none of the vaccinated dogs and 29% of the controls were blood or lymph node PCR-positive at 24 months. Finally, the prevalence of seropositivity was significantly lower (8.3%) in vaccinated dogs compared to the controls (38.7%) and in the former dogs it could be attributed to the production of IgG against the attenuated vaccine strain of *L. infantum*. No adverse effects are reported [342]. However, a safety concern has been expressed: the vaccine strain may regain virulence if sand flies will feed on the vaccination site and become infected [342]. Although this will likely be of minor importance in an endemic area, there is a general concern about live-attenuated parasites regaining virulence. Large and properly designed studies are necessary to prove safety of live vaccines [324].

Conclusion: Live, gentamycin-attenuated *L. infantum* (H strain), although effective for the prevention of CanL (SORT: moderate) cannot be recommended for field use until the inability of this strain to revert to a virulent one is proved (SORT: weak).

9.6.6 | Protein A2 Vaccine

A recombinant amastigote protein called A2, with saponin Quil A as adjuvant, became commercially available as LeishTec (Hertape) in Brazil, but at the time of writing its marketing licence has been withdrawn. It was licensed for clinically healthy, seronegative dogs, older than 4 months. Each dose contains at least 100 μg rA2 and 500 μg adjuvant, and the vaccination schedule includes a prime vaccination with three doses at 3-week intervals, followed by annual boosters. The vaccine was immunogenic with production of anti rA2 antibodies [343, 344] and after experimental infection, the increased production of INF-γ with decreased production of IL-10 [344], were associated with partial protection against development of CanL and decreased parasitic density compared to the controls [344]. The efficacy and safety of the vaccine were tested in three RCTs (Table S31) [345–347] each of them including 274–278 vaccinated (3 prime vaccinations at 2–3 week intervals) and 272–281 non-vaccinated controls. In one RCT, negative serology was an inclusion criterion [345], whereas in the other two RCTs seronegative and blood qPCR-negative dogs as well as "subclinically" infected dogs (defined as presenting less than two clinical signs of CanL, even if it is actually a wrong definition) were enrolled [346, 347]. The quality of all three studies is intermediate [345–347].

The evaluation of vaccine efficacy for the prevention of CanL is possible from the data of one of these RCTs of 9-month duration, where mortality from CanL was diagnosed in 4.4% of vaccinated and in 11.4% of non-vaccinated dogs, but the difference was not significant [346]. Parasitic burden may have been reduced in vaccinated subclinically infected dogs because, at the end of a 18-month long trial, the prevalence of negative microscopy (bone marrow, skin, lymph nodes and skin), culture (bone marrow) and xenodiagnosis was significantly higher (92.6%) than in the controls (82.4%). However, the difference in prevalence of negative xenodiagnosis in a subgroup of these dogs did not differ between vaccinated (32.7%) and non-vaccinated (44.2%) ones [345]. Moreover, in another RCT there was no difference between groups in the prevalence of positive blood qPCR at 9 months [346]. Despite the production of IgG against rA2, most dogs remained seronegative when whole parasite antigen or recombinant antigens other than rA2 were used; approximately 18 months after vaccination, significantly more vaccinated dogs (97.6%) than controls (87.5%) remained seronegative [345]. Severe adverse effects (death) occurred in 3/274 (1.1%) vaccinated dogs [347]; in addition, mild adverse effects occurred in 3.1%–8% (22/274) [346, 347].

Conclusion: Protein A2 vaccine with saponin Quil A cannot be recommended for the prevention of CanL due to lack of evidence of protection against development of the disease (SORT: moderate) and serious adverse effects observed in a minority of dogs (SORT: moderate).

9.6.7 | Protein Leish111f or MML)

Protein Leish111f is a chimeric protein produced by the fusion of recombinant *L. major* thiol-specific antioxidant (TSA or MAPS), recombinant *L. major* stress-inducible protein-1 (LmSTI1 or M15) and recombinant *L. braziliensis* elongation initiation factor (LeIF) [348]. Vaccinated dogs showed lymphocyte proliferation responses after in vitro exposure to either the vaccine protein or to SLA, and they produce IgG against the chimeric protein [348]. The efficacy and safety of a vaccine containing 45 μg/dose of Leish111f and either 50 μg MPL-SE (an agonist of toll-like receptor-4) or 45 μg Adjuprime as adjuvants were tested in a single RCT (Table S31), of 2 years' duration that included 30 vaccinated seronegative dogs and 15 placebo controls. Prime series vaccination included three administrations at 4-week intervals, followed by an annual booster. The quality of the RCT is intermediate [348].

None of the controls developed CanL by the end of the trial, contrary to 20% of vaccinated dogs. There was no difference between groups in the incidence of infection and, at the end of the study period, bone marrow nPCR was negative in 8% of vaccinated dogs and in 0% of controls. The same applies to the results of serology and at the end of the study, using two serological tests, 32%–64% (vaccinated) or 42.8%–71.4% (controls) remained seronegative. No adverse effects were reported [348].

Conclusion: The Leish111f with either MPL-SE or Adjuprime cannot be recommended for the prevention of CanL due to the lack of protection against development of the disease (SORT: moderate).

9.6.8 | Protein Q Vaccine

Protein Q is a chimeric protein produced by the fusion of five recombinant fragments from *L. infantum* proteins, such as the acidic ribosomal proteins LiP0, Lip2a and Lip2b, and the histone H2A. It does not contain adjuvant, and, at the time of writing, it is commercially available in Europe as LetiFend that contains ≥ 36.7 ELISA Units of protein Q per dose. It can be administered to clinically healthy, IFA- or ELISA-negative dogs older than 6 months. A single prime vaccination should be followed by annual boosters. Vaccination results in production of protein Q-specific antibodies that usually are not detectable by the serological tests used for the diagnosis of CanL and do not interfere with the interpretation of serology tests [349]. Under laboratory conditions, vaccinated experimentally infected dogs were significantly less likely to develop CanL compared to placebo controls and did not develop histological lesions in internal target organs. Vaccinated dogs mounted stronger parasite-specific cell-mediated immunity (positive leishmanin skin test) and had increased nitric oxide production in lymph nodes, both resulting in lower parasitic load in various internal organs and developed less parasite-specific IgG and circulating immune complexes compared to placebo controls [349–351]. The efficacy and safety of the vaccine was tested in one RCT (Table S31) of 2 years' duration, where 549 healthy seronegative kennel dogs were randomised (1/1 ratio) to receive either the vaccine (2 doses SC 1 year apart) or placebo [352]. The quality of the RCT is low because the efficacy was calculated in a subset of enrolled dogs that lived in two of the 19 kennels that participated in the study and, specifically, in those two kennels where that incidence of CanL in the controls was the highest. This means that the true efficacy is expected to be lower among dogs at lower risk to develop CanL.

There was a significant difference in the incidence of CanL during the 2-year study period among vaccinated dogs (4.7%) and controls (10.2%), and, interestingly, among the dogs that became seropositive but received the annual booster or placebo, CanL occurred in 30% and 80%, respectively. Moreover, there is some evidence that the severity of CanL was lower in vaccinated dogs. As expected for a vaccine, there was no difference in the prevalence of positive bone marrow or lymph node microscopy or PCR between groups at the end of the study, but there was also no difference in seropositivity rate. Vaccine-induced, protein Q-specific antibodies were considered unlikely to contribute to this lack of difference, because they were greatly reduced 6 months after each vaccination. No adverse effects were reported [352].

A recent meta-analysis concluded that the relative risk of infection by *L. infantum* and/or CanL is non-significantly reduced and the NNT = 10.99 [337]. Furthermore, field trial results were found to be heterogeneous and there is risk of publication bias.

Conclusion: Protein Q vaccine can be considered a second-line preventive measure for CanL only in dogs at high risk to develop the disease (SORT: weak), because it is partially protective and safe (SORT: moderate).

9.7 | Immunomodulators

9.7.1 | Domperidone

In addition to the use for treatment of CanL, the efficacy of domperidone for prevention of the disease has been examined in one RCT (Table S32) of intermediate quality [171]. In that study, domperidone was administered, at the registered dosage regimen (0.5 mg/kg orally once per day for 1 month) and repeated after 3-month discontinuation periods for a total of 21 months in 44 seronegative healthy dogs, compared to 46 untreated controls. At the end of the trial, 88.6% of treated dogs did not develop CanL and remained seronegative compared to 52.2% of untreated animals (significant difference). Adverse effects were observed in 9% (4/44) of treated dogs and they included mild galactorrhea (2/4) and soft stools (2/4).

Conclusion: In endemic areas, domperidone at the registered dosage regimen is recommended for the prevention of CanL in seronegative dogs (SORT moderate). The efficacy in seropositive dogs is unknown.

9.7.2 | Nutritional Supplements

The same nutritional supplement containing nucleotides and an AHCC (Impromune; Bioiberica S.A.U., Spain) that was tested for the treatment of CanL, in combination with meglumine antimoniate, has also been examined for the prevention of the disease in one RCT (Table S33) of intermediate quality [353]. In that study, subclinically infected dogs with positive serology and positive PCR and/or microscopy in bone marrow and/or lymph nodes were administered either the nutritional supplement (32 mg/kg nucleotides plus 17 mg/kg AHCC, daily for one year; $n = 21$) or placebo ($n = 25$). Among treated dogs, 85% did not develop CanL by the end of the trial and this was significantly higher compared to the controls (54.5%); in addition, considering only dogs that developed CanL, the clinical severity was significantly lower among treated dogs at 6 months but not at 12 months. *Leishmania*-specific antibodies decreased significantly in the treated group only, and no changes were observed in either group in the immunological parameters evaluated (immunophenotype of peripheral blood T-lymphocytes and baseline serum concentration of selected cytokines) or in bone marrow and lymph node parasitic load, assessed by PCR and microscopy. No adverse effect was reported [353].

Conclusion: Daily oral supplementation with dietary nucleotides and active hexose correlated compound is recommended for the prevention of CanL development in subclinically infected, seropositive dogs (SORT: moderate).

9.8 | Miscellaneous

The efficacy of intermittent administration of allopurinol (20 mg/kg orally once daily for 1 week every month) to prevent establishment of infection in healthy, seronegative, non-infected dogs and to prevent the progression to CanL in healthy, seronegative, subclinically infected dogs, was examined in one RCT (Table S34) of intermediate quality [354]. The duration of the

study was 8 months (corresponding to one period of sand fly activity) and included 47 non-infected dogs with negative bone marrow and lymph node microscopy and negative bone marrow PCR (26 received allopurinol and 21 placebo) and 48 seronegative subclinically infected dogs (25 received allopurinol and 23 placebo). None of the dogs developed CanL and there was no difference between treated dogs and controls of either subgroup in the incidence of seroconversion. On the other hand, among non-infected dogs, the incidence of positive microscopy and/or PCR at 12 months was significantly higher in the allopurinol-treated animals. No adverse effects were reported.

Conclusion: In endemic areas, periodic administration of allopurinol for the prevention of CanL among healthy, seronegative and either non-infected or subclinically infected dogs cannot be recommended due to lack of efficacy (SORT: moderate) and is strongly discouraged due to the risk of induction of parasite resistance (SORT: weak).

9.8.1 | Summary of Recommendations for the Prevention of CanL due to *L. infantum*

- In endemic areas, recommended measures for the prevention of CanL include: deltamethrin 4% impregnated collar, flumethrin 4.5% plus imidacloprid 10% collar or permethrin 50% plus imidacloprid 10% spot-on, that should be used in all dogs throughout the transmission period; regular administration of afoxolaner or fluralaner throughout the transmission period; not using infected blood products for transfusion; not breeding seropositive bitches or bitches with CanL; administration of domperidone in seronegative dogs and of dietary nucleotides plus active hexose correlated compound in subclinically infected, seropositive dogs; vaccination with *Li*ESP with MDP vaccine (non-commercially available) may be considered; protein Q vaccine is recommended for dogs living in areas with very high rates of seroconversion in the overall canine population; FML vaccine plus QA-21 with the addition of Riedel de Haen saponin (non-commercially available) is recommended for seropositive, subclinically infected dogs.
- In non-endemic areas, recommended measures for the prevention of CanL include not using infected blood products for transfusion and removal of all infected bitches from reproduction.
- Non-recommended measures for the prevention of CanL include: use of environmental insecticides; indoor confinement of dogs and use of fine mesh nets in their dwellings; vaccination with autoclaved *L. major* promastigotes, with *Li*ESP plus QA-21, with LiF2 plus MDP, with live, gentamycin-attenuated *L. infantum* (H strain), with protein A2 plus saponin Quil A, or with Leish111f vaccine plus either MPL-SE or Adjuprime; periodic administration of allopurinol.

10 | Canine Leishmaniosis due to Species Other Than *L. infantum*

Besides *L. infantum*, dogs living in the New World can be infected by several other species, including *L. amazonensis*, *L. braziliensis*, *L. guyanensis*, *L. mexicana*, *L. panamensis*, *L. peruviana* and *L. naiffi* [355]. Additionally, *L. colombiensis* has been isolated from the bone marrow of a dog from Venezuela [356], but this parasite has recently been transferred to the genus *Endotrypanum* [357]. Co-infections with multiple *Leishmania* species have been reported in different countries [104, 358–360].

Excluding *L. infantum*, which was introduced in the New World by the Conquistadores [5], all above-mentioned species are native to this region and are maintained in nature by several wildlife hosts [361]. Some of these species have a narrow range of vectors, whereas others are more generalists [362]. For instance, *L. mexicana* is transmitted by *Bichromomyia olmeca olmeca*, whereas *L. braziliensis* is transmitted by numerous vectors (e.g., *Nyssomyia intermedia*, *N. neivai*, *N. whitmani*, *Migonemyia migonei*, *Psychodopygus wellcomei* and *P. complexus*) [362]. This may partly explain why *L. braziliensis* is, along with *L. infantum*, the most widespread species infecting dogs in the New World, from Argentina up to Mexico [355, 363].

Except for *L. amazonensis* which can cause disseminated visceral infection in dogs [364], all other New World species cause only cutaneous disease. As an example, dogs infected with *L. braziliensis* or *L. panamensis* usually present nodules and ulcers on the nose, ears, scrotum and hind limbs [365–368]. These lesions may heal spontaneously, but the primary lesions, or even secondary mucosal ones, may reappear months later [369–373]. In experimental studies, dogs infected with *L. braziliensis* developed skin lesions at the inoculation site 4–8 months post-infection [370, 371]. Histopathological findings in ulcers of dogs infected with *L. braziliensis* include chronic inflammation with lymphocytes, plasma cells, and macrophages, along with granulation tissue [367].

In the Old World, two species, *L. major* and *L. tropica*, which typically cause CL in humans, have also been shown to cause disease in dogs. *L. major* infection is usually restricted to the skin and may cause ulceration and exfoliative dermatitis, whereas *L. tropica* may cause cutaneous, mucocutaneous or visceral disease [374–376]. The latter has been described as similar to CanL due to *L. infantum* [376, 377]. Canine infections with *L. major* have been described in Egypt, Saudi Arabia, and Israel, while infections with *L. tropica* were described in several countries in the Middle East and North Africa, including Saudi Arabia, Iran, Israel and Morocco [374, 375, 377–382]. *Leishmania major* is transmitted by *Ph. papatasi* and *Ph. duboscqi*, and *L. tropica* can be transmitted by several sand fly vectors, including *Ph. sergenti* and *Ph. arabicus* [383].

There are no drugs specifically registered for the treatment of the disease caused by the above species in the Old and New World, and, therefore, treatment is off-label. Several protocols have been empirically used with variable therapeutic success [360, 384–387]. Antimonial therapy (e.g., IM, peri-, sub- or intralesional injections) healed the lesions in >80% of dogs [360, 384, 385], whereas the combination of furazolidone and domperidone cured 7 of 8 dogs infected by *L. braziliensis* [386]. In a more recent study, the use of an ointment containing a mixture of 2% chromane-derived hydrazone plus 2% hederagenin glucoside saponins produced complete long-term clinical cure in 56 dogs with cutaneous leishmaniosis from Colombia [387]. The *Leishmania* species responsible for these cases was not determined, but *L. braziliensis*, *L. panamensis* and *L. guyanensis*

have been reported to cause skin lesions in dogs in Colombia. Treatment of skin lesions due to *L. major* and *L. tropica* has mostly been performed with allopurinol, with a generally favourable response [375, 381]. Further RCTs to investigate the efficacy of various protocols for the treatment of the disease caused by *Leishmania* spp. other than *L. infantum* are needed.

11 | Future Trends

In the last decades, enormous progress has been made in understanding the epidemiology and pathogenesis of CanL, and in the diagnosis, treatment and prevention of this important disease. However, there are still areas that need clarification and research. For example, in recent years the role of other parasite reservoirs, such as cats, horses and hares, has emerged. Considering that *Leishmania* parasitises a wide range of mammals (and even non-mammalian vertebrates, if we consider species of the subgenus *Sauroleishmania*), it is possible that the picture of epidemiology of leishmaniosis that we have, centred on dogs and humans, is only partial.

In the clinical setting, it would be very useful for the scientific community to agree on proper design of RCTs and to validate a clinical scoring system to objectively evaluate the outcome of potential new treatments.

Drug resistance is one of the current problems in the treatment of CanL and may result in treatment failures and relapses. Although some of the molecular mechanisms of resistance to the main drugs (antimonials, miltefosine, allopurinol) have been identified, clinicians do not have a laboratory test to identify if CanL is due to drug-resistant parasites. In connection with this issue, it would be necessary to determine precisely what is the most appropriate duration of treatment. Prolonged treatment with allopurinol reduces the risk of relapses, but undoubtedly increases the risk of the parasite developing resistance.

More knowledge is needed on kidney lesions, which are the main cause of death in CanL. Why do some dogs develop glomerulopathies? How can these animals be identified early on, and how can the development of renal lesions be prevented? Which are the most effective treatments for dogs with CanL and CKD?

Finally, in the field of prevention, we expect that a new generation of more effective vaccines may appear in the future. Vaccines more effective in preventing clinical signs and able to prevent infection ('sterilising vaccines') would be a breakthrough. It is quite possible that these vaccines may also be used in subclinically infected (vaccine immunoprophylaxis) or diseased animals (vaccine immunotherapy) and would undoubtedly be of great value in disease control.

Author Contributions

Manolis N. Saridomichelakis: data curation (lead), formal analysis (lead), investigation (lead), methodology (lead), writing – original draft (lead), writing – review and editing (lead). **Gad Baneth:** writing – original draft (equal), writing – review and editing (equal). **Silvia Colombo:** writing – original draft (equal), writing – review and editing (equal). **Filipe Dantas-Torres:** writing – original draft (equal), writing – review and editing (equal). **Lluís Ferrer:** writing – original draft (equal), writing – review and editing (equal). **Alessandra Fondati:** writing – original draft (equal), writing – review and editing (equal). **Guadalupe Miró:** writing – original draft (equal), writing – review and editing (equal). **Laura Ordeix:** investigation (supporting), writing – original draft (equal), writing – review and editing (equal). **Domenico Otranto:** writing – original draft (equal), writing – review and editing (equal). **Chiara Noli:** conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, supervision, validation, writing – original draft (equal), writing – review and editing (equal).

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Disclosure

Clinician's decisions on treatment and prevention of CanL should follow local legislation.

Conflicts of Interest

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Data Availability Statement

The data that supports the findings of this study are available in the Supporting Information of this article.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Randomised controlled trials on the treatment of canine leishmaniosis with meglumine antimoniate. **Table S2:** Randomised controlled trials on the treatment of canine leishmaniosis with liposomal formulations of meglumine antimoniate. **Table S3:** Randomised controlled trial on the treatment of canine leishmaniosis with miltefosine. **Table S4:** Randomised controlled trials on the treatment of canine leishmaniosis with allopurinol. **Table S5:** Randomised controlled trial on the treatment of canine leishmaniosis with aminosidine. **Table S6:** Randomised controlled trial on the treatment of canine leishmaniosis with marbofloxacin. **Table S7:** Randomised controlled trial on the treatment of canine leishmaniosis with metronidazole. **Table S8:** Randomised controlled trial on the treatment of canine leishmaniosis with *O*-alkyl-hydroxamate (MTC-305). **Table S9:** Randomised controlled trial on the treatment of canine leishmaniosis with (−)-α-bisabolol. **Table S10:** Randomised controlled trial on the treatment of canine leishmaniosis with artesunate. **Table S11:** Randomised controlled trials on the treatment of canine leishmaniosis with meglumine antimoniate–allopurinol combination. **Table S12:** Randomised controlled trials on the treatment of canine leishmaniosis with liposomal formulations of meglumine antimoniate–allopurinol combination. **Table S13:** Randomised controlled trial on the treatment of canine leishmaniosis with meglumine antimoniate–aminosidine combination. **Table S14:** Randomised controlled trial on the treatment of canine leishmaniosis with meglumine antimoniate–metronidazole combination. **Table S15:** Randomised controlled trial on the treatment of canine leishmaniosis with meglumine antimoniate–*O*-alkyl-hydroxamate (MTC-305) combination. **Table S16:** Randomised controlled trials on the treatment of canine leishmaniosis with miltefosine–allopurinol combination. **Table S17:** Randomised controlled trials on the treatment of canine leishmaniosis with allopurinol–aminosidine combination. **Table S18:** Randomised controlled trial on the treatment of canine leishmaniosis with domperidone. **Table S19:** Randomised controlled trial on the treatment of canine leishmaniosis with the nutritional supplement DiLsh. **Table S20:** Randomised controlled trial on the treatment of canine leishmaniosis with monoclonal antibody against canine IL-10 receptor. **Table S21:** Randomised controlled trials on the treatment of canine leishmaniosis with vaccines. **Table S22:** Randomised controlled trial on the treatment of canine leishmaniosis with meglumine antimoniate-nutritional supplement containing nucleotides and an AHCC compound combination. **Table S23:** Randomised controlled trials on the treatment of canine leishmaniosis with meglumine antimoniate–vaccine combination. **Table S24:** Randomised controlled trial on the treatment of canine leishmaniosis with meglumine antimoniate–allopurinol–domperidone combination. **Table S25:** Randomised controlled trial on the treatment of canine leishmaniosis with meglumine antimoniate–allopurinol–deslorelin combination. **Table S26:** Randomised controlled trial on the treatment of canine

leishmaniosis with allopurinol–metrinodazole–ketoconazole–n-3 fatty acid–B vitamin combination. **Table S27:** Randomised controlled trial on the treatment of canine leishmaniosis with allopurinol–LeishF2 vaccine combination. **Table S28:** Randomised controlled trials on the prevention of canine leishmaniosis with deltamethrin 4% collar. **Table S29:** Randomised controlled trials on the prevention of canine leishmaniosis with flumethrin 4.5% plus imidacloprid 10% collar. **Table S30:** Randomised controlled trials on the prevention of canine leishmaniosis with permethrin 50% plus imidacloprid 10% spot-on. **Table S31:** Randomised controlled trials on the prevention of canine leishmaniosis with vaccines. **Table S32:** Randomised controlled trial on the prevention of canine leishmaniosis with domperidone. **Table S33:** Randomised controlled trial on the prevention of canine leishmaniosis with a nutritional supplement containing nucleotides and an AHCC compound. **Table S34:** Randomised controlled trial on the prevention of canine leishmaniosis with allopurinol.