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What do we know about feline leishmaniosis?

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Abstract

According to the World Health Organization (WHO), endemic areas of leishmaniosis have spread and the number of reported cases has increased. Europe is one of the continents with greatest risk of the re-emergence of this zoonosis. The significance of the cat as a reservoir of *Leishmania* species and not simply an accidental host seems to be gaining ground, mainly because: (i) cats can present increased seropositivity between serological analyses, but the pattern of seropositivity is not consistent between cats; (ii) cats can be infected for some months and thus are available for sandflies; and (iii) cats transmit the *Leishmania* species agent in a competent form. Furthermore, cats have behavioural characteristics that contribute to infection by *Leishmania infantum* and, as such, feline leishmaniosis (FeL) has been reported worldwide. When clinical signs of FeL are present, they are non-specific and frequently occur in other feline diseases. If they go undiagnosed, they can contribute to an underestimation of the actual occurrence of the disease in cats. The low seroprevalence titres, along with the commonly asymptomatic infection in cats, can further contribute to the underestimation of FeL occurrence. This work aims to raise awareness about FeL among veterinarians by providing a review of the current status of FeL infection caused by *L infantum* worldwide, the major clinicopathological features of infection, along with recent developments on FeL diagnosis, treatment and prevention.

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Epidemiology of feline leishmaniosis

Leishmaniosis is a parasitic disease caused by an obligate intracellular protozoan of the genus *Leishmania* (Kinetoplastida, Trypanosomatidae).[1] Species from the genus *Leishmania* are subdivided in two subgenera: *Leishmania* (includes species from the Old World, namely *L major, L infantum, L donovani* and *L tropica*, and those found in the New World, namely *L chagasi* [syn *L infantum*], *L mexicana, L amazonensis* and *L venezuelensis*) and *Viannia* (only occurring in Central and South America; eg, species *L* [*Viannia*] *braziliensis*).[1–3] Species from genus *Leishmania* identified as infective for felids include *L infantum*,[1,2] *L mexicana*,[3] *L venezuelensis*,[4] and *L* (*Viannia*) *braziliensis*.[5,6] Most *L infantum* strains belong to zymodeme MON-1, assumed to be responsible for zoonotic leishmaniosis, affecting humans, canids, felids and other hosts.[7–11]

The vectors of *Leishmania* species belong to the genus *Phlebotomus* (Diptera, Psychodidae) in the Old World and *Lutzomya* in the New World.[12,13]

Leishmaniosis in domestic cats (*Felis catus*) was described for the first time in 1912, in Algeria, in a cat that lived with a dog and a child, both infected with leishmaniosis.[14] Since then, as summarised in Table 1, feline leishmaniosis (FeL) has been globally reported, but it is found more frequently in countries bordering the Mediterranean Sea.[26] On the American continent, FeL has been reported particularly in Central America,[3] Brazil[16,19] and Paraguay.[18]

Recent cases and studies involving the occurrence of *L infantum* in cats suggest that these animals act as a reservoir.[11,26–28] The classification of cats as accidental hosts or primary or secondary reservoirs remains an ongoing discussion.[11,26–28] The classification of a host as primary,

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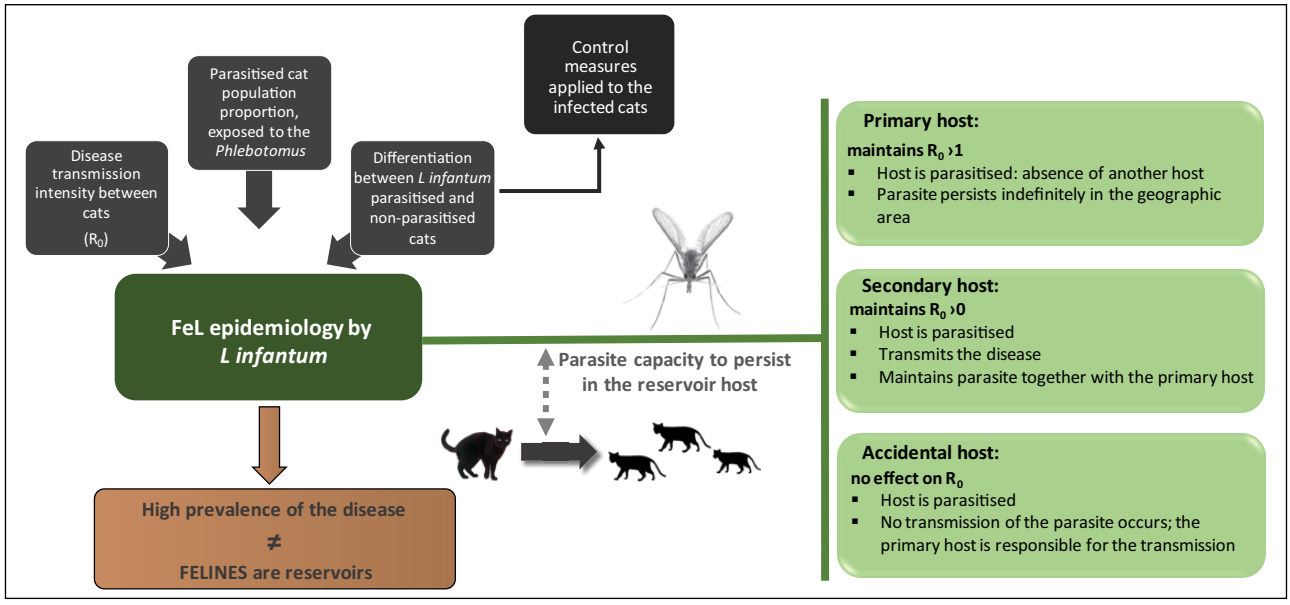

Figure 1 Proposed flow chart for the study of the role of the cat in the epidemiology of *Leishmania infantum* infection (modified from Quinnell and Courtenay[29]). R_0 = basic reproduction number

Table 1 Compilation of worldwide epidemiological surveys of feline leishmaniosis due to *Leishmania infantum*

Country (region)	Seroprevalence (total number of samples)	Diagnostic assay (cut-off titre)	Confirmatory assay: results
Italy (Milan)[15]	25.3% (233)	IFI (1:80)	qPCR: 0%
Brazil (Araçatuba)[16]	4.64% (302)	IFI (1:40)	ELISA: 12.91% Direct parasitological exam: 9.93%
Mexico (Yucatan Peninsula)[17]	22.1% (95)	ELISA (Fe-SOD)	–
Paraguay (Asuncion)[18]	0.94% (317)	IFI	–
Brazil (Araçatuba)[19]	25.4% (55)	ELISA	
	10.9% (55)	IFI (1:40)	–
Iran[20]	9.23% (195)	Immunochromatography	–
Portugal (Lisbon)[10]	1.3% (76)	IFI	PCR: 20.3% (28/138)
Portugal (north region)[9]	2.8% (316)	DAT	
		ELISA	–
Greece (north region)[21]	3.87% (284)	ELISA	–
Israel (Jerusalem)[22]	6.7% (104)	ELISA	–
Portugal (Lisbon)[23]	20% (20)	IFI	PCR: 30.4% (7/23)
Spain (south region)[2]	60% (183) with titre ⩾10 28.3% (183) with titre ⩾40	IFI	PCR: ELISA: 25.7% Direct parasitological exam: 3/7 tested positive
Italy[24]	16.3% (203)	IFI	–
Italy[25]	0.9% (110)	IFI	–

DAT = direct agglutination test; Fe-SOD = iron superoxide dismutase; IFI = indirect immunofluorescence; qPCR = quantitative polymerase chain reaction

secondary (synonymous with minor) or accidental is based on the capacity of *Leishmania* species to persist, indefinitely or temporarily, in a population that is a reservoir of the disease, characterised by a basic reproduction number (R_0),[29] as schematically depicted in Figure 1.

Available data from epidemiological surveys (Table 1) and case reports (Table 2) suggest that the cat can act as a reservoir host of *L infantum* but not as an accidental host.[36] Possible justifications include[8,10,28,37]: (1) cats can be infected and do not develop illness – even if they present clinical signs, a chronic presentation will ensue; (2) in the peripheral blood of cats, the protozoan is in an infective form to the vector; (3) cats cohabit with human beings, namely in endemic areas of canine leishmaniosis (CaL) and (4) sick cats infected with *Leishmania* species do not recover without anti-leishmanial therapy.

Table 2 Compilation of worldwide case reports of feline leishmaniosis due to *Leishmania infantum*

Country (region)	Cat identification	Clinical signs	Diagnostic assay
France (south, St André Roche)[30]	14-year-old male cat	Papular and ulcerative dermatitis at the base of the ear, head and interscapular region; weight loss; history of recurrent pododermatitis	Histopathology Western blotting qPCR Blood culture
Portugal (Porto)[31]	4-year-old female cat	Depression and reduced appetite; severe non-regenerative anaemia; pancytopenia Mild increase in γ-globulin concentration	Bone marrow cytology Buffy coat cytology PCR Indirect haemagglutination (1:100)
France (south, Biot)[7]	6-year-old female cat	Whole-body cutaneous lesions with depilation and ulcero-crusted seborrhoeic dermatitis; emaciation	Histopathology Bone marrow cytology PCR Western blotting Direct agglutination (1/10240)
France (south, Grasse)[8]	13-year-old neutered male cat	Ulcerative lesion in the left temporal region, initially reported as discrete crusts, with concurrent diagnosis of squamous cell carcinoma; splenomegaly	IFI ELISA Histopathology Western blotting Blood culture
Spain (Barcelona)[32]	8-year-old female cat	Slightly depressed, thin and poor-quality coat; moderate diffuse gingivitis and marked faucitis; bilateral panuveitis and secondary glaucoma; mild azotaemia, hyperglobulinaemia and moderate polyclonal gammopathy; diabetes mellitus	ELISA Ocular histopathology Bone marrow cytology PCR
Brazil (São Paulo)[33]	2-year-old male cat	Nodular lesion on the nose; weight and muscle loss; lymphadenomegaly	IFI (1:80) PCR
Italy[34]	14-year-old female cat	Anorexia and respiratory distress; emaciated and dehydrated; small crusty ulcer (0.5 cm), haematic cyst	IFI (1:640) Lesions cytology
	6-year-old male cat	History of bite abscess and aural itchiness; acute upper respiratory tract infection; popliteal lymphadenomegaly	Lymph node cytology PCR IFI (1:1280)
	10-year-old female cat	Anorexia, weight loss, depression; uveitis; severe non-regenerative anaemia, leukopenia and thrombocytopenia	Lymph node cytology PCR IFI (1:640)
	Adult male cat	Persistent submandibular lymphadenomegaly, stomatitis and severe periodontitis; history of generalised alopecia and deep ulcers around the neck and weight loss	Lymph node cytology PCR IFI (1:640)
Italy (Imperia)[25]	6-year-old female cat	Lethargy and an ulcerated nodule on the eyelid; weight loss, dysorexia; severe ulcerative stomatitis, generalised lymphadenopathy and splenomegaly	IFI (1:80) Histopathology Lesion and lymph node cytology PCR Electron microscopy
Spain[35]	3-year-old female cat	History of abortion; recurrent alopecia of abdomen and neck; desquamation and erythema of the edge of the ears	IFI (1:160) Popliteal lymph node cytology
	5-year-old female cat	Severe jaundice and vomiting	Histopathology Electron microscopy

IFI = indirect immunofluorescence; qPCR = quantitative polymerase chain reaction

Additionally, cats have behavioural characteristics that can contribute to exposure. They are nocturnal predators, operating in a 1.5 km radius of their residence, using forests as hunting territory. These are ideal elements to link the sylvatic and domestic cycles, favouring the dissemination of parasites.[38] Cats are thus considered as disease amplifiers.[15]

Pathogenesis and lesions

The classification as accidental host is further challenged by evidence that felids are usually asymptomatic.[10,22,26] In a study comprising 200 cats, only two animals revealed clinical signs, specifically crusty lesions of the dorsal cervical region along with hepatosplenomegaly.[39] Clinical manifestations of the disease comprise visceral, cutaneous and mucosal signs. Visceral signs are associated with high mortality and systemic involvement of the organism. Cutaneous or mucocutaneous signs are frequently associated with dissemination of parasites through other tissues, causing significant morbidity.[11,35,38]

The first reported cases of FeL were characterised by cutaneous manifestations, without visceral involvement,[4,7,34,35,40] with dry local lesions in the form of papules and nodules, and exudative lesions in the form of crusts and ulcers.[11,28] The importance of screening cats presenting dermatitis, nodular or ulcerative, was further shown by Navarro et al[38] who described 15 cats infected with leishmaniosis presenting cutaneous expression of the illness, namely skin lesions in the mucocutaneous junction (nose, lips and ears) as well as ocular lesions. Granulomatous perifoliculitis, lichenoid dermatitis and pododermatitis were also described.[41] Similarly, a clinical case of a 14-year-old cat positive for feline immunodeficiency virus (FIV), with a 3 year history of recurrent pododermatitis, unresponsive to antibiotics and characterised by exudative and erythematosus lesions, was reported. Besides a 20% weight loss, the cat presented three circumscribed cutaneous injuries (at the base of the ear, head and interscapular region), all with ulcerated or haemorrhagic papules. The histopathological examination of these cutaneous lesions revealed the presence of macrophages, with cytoplasmic inclusion bodies, consistent with *Leishmania* species forms. A complete parasitological examination of the skin biopsy further confirmed *Leishmania* species. A fourth lesion in the auricular pavilion was consistent with squamous cell carcinoma.[30]

Lymphadenomegaly was also frequently reported, accompanied by fever, scaling and alopecia of the head and abdomen, ulcers on bony prominences, history of abortion,[35] mild periodontitis,[28] onychogryphosis, cachexia with muscular atrophy and weakness.[42]

Moreover, ocular leishmaniosis was described as featuring ocular lesions such as corneal exudative ulcers, panuveitis and panophthalmitis.[32,38] Although seldom found, cats with visceral leishmaniosis but without cutaneous signs have also been reported, presenting fever, jaundice, vomiting, lymphadenomegaly, lesions of the oral mucosa with gingivitis, anaemia and leukopenia.[28,31,32] Renal failure associated with FeL has also been described,[38] although it is less evident than in dogs. Indeed, in dogs renal failure is a well-recognised syndrome and a cause of death.

A synergism between squamous cell carcinoma and FeL has been proposed, given that while the carcinoma could take advantage of the proliferation of the protozoan, the parasite could initiate the development of the neoplasia, or both. Lesions compatible with squamous cell carcinoma were described in the left temporal region[8] and in the auricular pavilion[30] of two FIV-positive cats. It is noticeable that FIV and/or feline leukaemia virus (FeLV) infections were referred to as FeL predisposing factors based on the ensuing immunosuppression.[34,39,43] Supporting studies found a strong association (~70%) for cats between leishmaniosis and FIV,[34] and even a statistically significant correlation with FeL and FIV[16] and FeLV.[44] However, other studies contradict this positive correlation between FIV and/or FeLV and FeL infection.[7,10,24,26,28,31,33,40–42]

Other agents with a significant prevalence among feline populations and with a possibility of serological cross-reaction with *Leishmania* species can be mentioned. Regarding *Toxoplasma gondii*, of which cats are considered reservoirs, the majority of the studies did not observe a positive correlation between both infections.[9,22,41,44] Coinfection with feline infectious peritonitis[33] or *Trypanosoma cruzi*[17] were considered of minor significance.

Immunological features of leishmaniosis

Based on CaL, we can assume that, in cats, leishmaniosis involves cell-mediated immunity (CMI), with activation of macrophages for the destruction of the amastigote forms. The high antibody titres (Table 2), present in some symptomatic cats, do not confer immunity against the disease.[45] Nevertheless, some investigations have shown that animals with increased titres of anti-*Leishmania* antibodies presented decreased positivity in PCR, whereas the biggest positivity through PCR occurred more frequently in cats with reduced antibody titres.[2,39] This suggests that the immune response in felids differs from the response observed in dogs, explaining the high number of asymptomatic infected cats, and the variable clinical manifestation of the disease, showing that lesions occur before the production of antibodies. When these lesions are in a resolution phase, seroconversion occurs, suggesting that the humoral immune response is protective in FeL.[2] Ultimately, it shows that conventional serological methods to detect active infection in cats are not always reliable.[2] Figure 2 illustrates the possible immune response of felids to *L infantum* infection.

The natural resistance of cats to leishmaniosis is widely suggested by the spontaneous healing of the lesion, which is often characterised by minimal or limited pathological changes.[38,43]

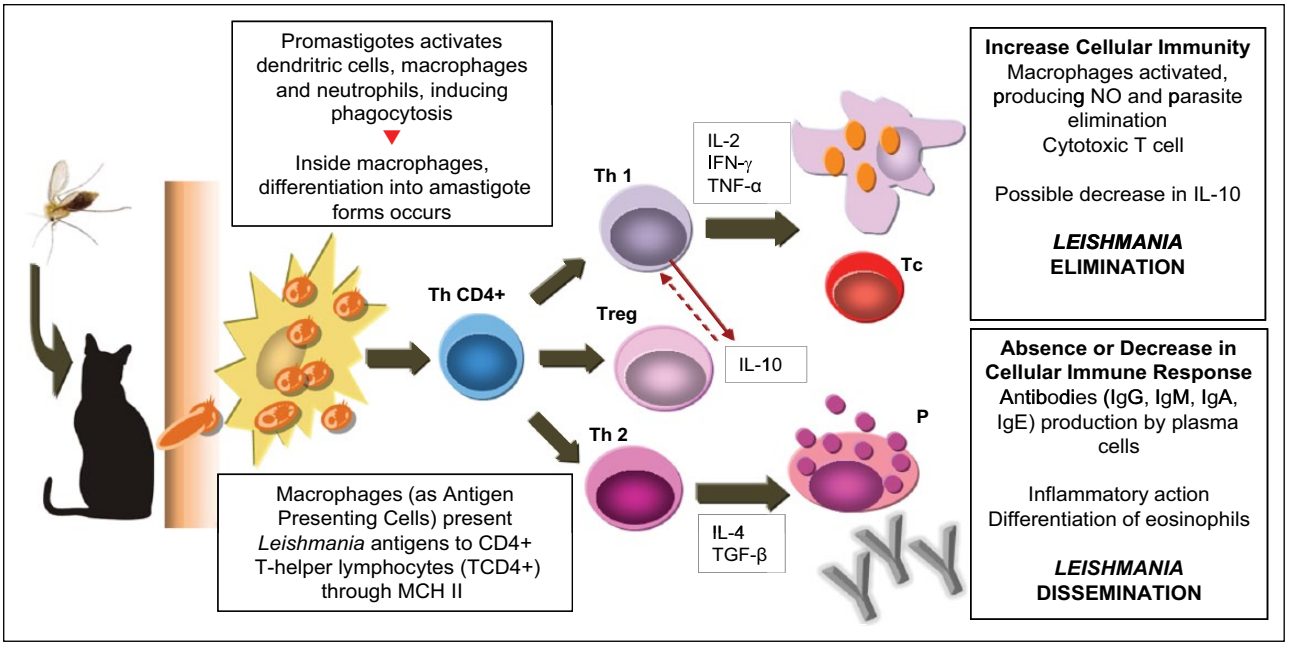

Figure 2 Suggested immune response of felids to *Leishmania* species infection, in accordance with the immune mechanism in canine leishmaniosis (modified from Barbiéri[45]). IFN-γ = interferon gamma; IL = interleukin; MCH II = major histocompatibility complex; NO = nitric oxide; P = plasma cell; Tc = cyotoxic cell; TCD4+ = T-helper cell; Th1 = type-1 T-helper cell; Th2 = type-2 T-helper cell; TGF-β = transformation growth factor beta; TNF-α = tumor necrosis factor alpha; Treg = regulatory T cell

Diagnosis

Laboratory methods are essential for the diagnosis of *Leishmania* species infection (Figure 3).

In clinically manifested visceral leishmaniosis, haemogram and biochemical analysis frequently show leukocytosis with neutrophilia, as well as urea and aspartate aminotransferase above the reference intervals. Creatinine, alanine aminotransferase and alkaline phosphatase can present normal values.[42] Neutrophilia with monocytosis and hyperglobulinaemia with polyclonal gammopathy was also reported.[32]

Direct observation of the parasite might be undertaken through cytology and/or skin biopsy, namely from cutaneous lesions, lymph node or bone marrow.[7,39] Cytology by aspiration or impression can be carried out in affected organs, such as the liver, spleen and kidney.[8,31,46] Direct parasitological examination of the popliteal lymph node by aspiration cytology was recently demonstrated to be more sensitive compared with cytology from other organs, such as bone marrow, the spleen or liver.[39] *L infantum* amastigotes were also found in the cytoplasm of neutrophils in both blood and buffy coat smears (4% of the neutrophils), as well as in the splenic parenchyma and the follicular centres of lymph nodes.[31]

Histopathology has an acceptable sensitivity and specificity, especially for the diagnosis of cats with cutaneous lesions.[30] Immunohistochemistry can be used as a confirmatory method[38] or as first-line diagnosis.[19]

The culture of *Leishmania* species promastigotes is an additional direct method, but it has some disadvantages, as it features low sensitivity and is time-consuming, taking too long to obtain results.[30] Although blood, bone marrow or lymph node samples can be used, some authors consider that blood is not a suitably sensitive specimen for culture in cats because of the low parasitaemia and small amount collected, resulting in lower sensitivity of the culture method.[2]

The established higher sensitivity of molecular techniques such as PCR, which further allows the confirmation of *L infantum*,[1,11,47] makes this a good option to confirm the diagnosis and for detection in asymptomatic animals.[37] However, the detection of DNA of *L infantum* may not necessarily mean the existence of an active infection. In addition, it has been shown that afterwards, kinetoplast and nuclear parasite DNA degradation occurs very rapidly.[48] In dogs, the most suitable method to detect the DNA of *Leishmania* species is a lymph node biopsy.[10]

One of the most important serological techniques is indirect immunofluorescence (IFI) assay, also known as the indirect fluorescence antibody test (IFAT). The IFI cut-off titre can be set at 1:80 for cats, as in dogs, following the work of Pennisi et al[49] carried out with positive and negative controls. Nevertheless, further studies are needed to confirm the best cut-off value for this technique to discriminate between positive and negative samples. Although IFI is considered the reference technique for CaL and human leishmaniosis (HuL) diagnosis,[23] for FeL diagnosis results should be interpreted together with other diagnostic methods and clinical signs.[17,50] Low antibody titres or even seronegativity could be the result of

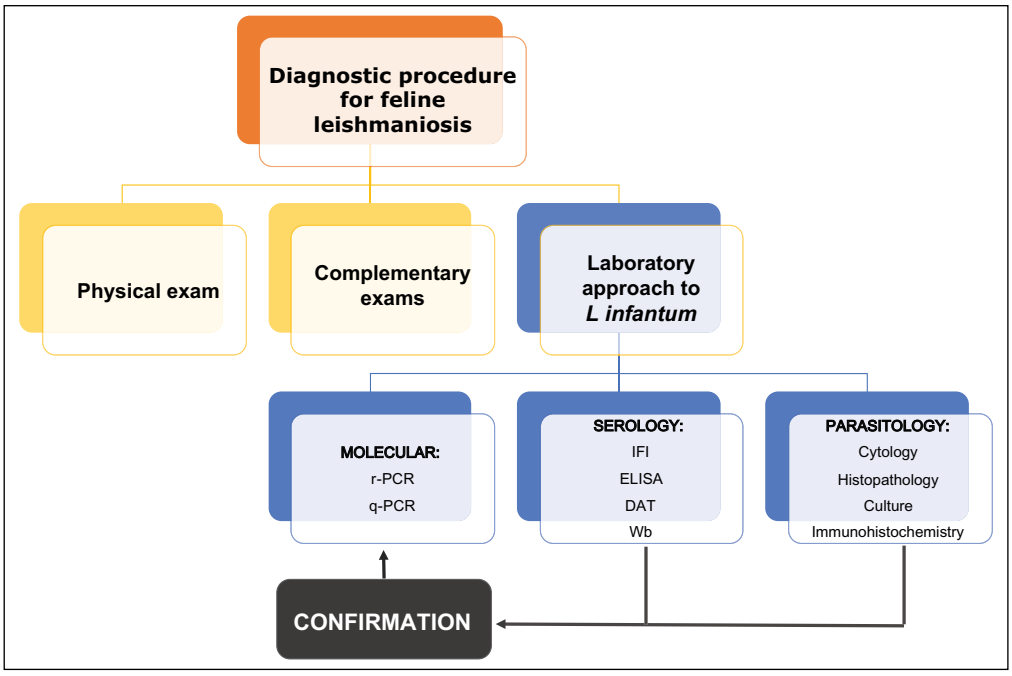

Figure 3 Feline leishmaniosis diagnostic methodologies. DAT = direct agglutination test; IFI = indirect immunofluorescence; Wb = Western blotting

the potentially predominant cellular immune response in cats, ultimately revealed as low seroprevalence in FeL surveys.[10,31] The use of cut-off antibody titres derived from CaL can further explain such low seroprevalence.[17,28] Antigenic changes after multiple in vitro culture passages of promastigotes used in the IFAT technique were also described, which could explain some variability in antibody titres reported.[51,52]

Other serological techniques include ELISA and Western blotting, which have been recently modified by the use of a molecular specific marker that is highly immunogenic, named superoxide dismutase of iron (Fe-SOD).[17]

Therapy and prevention strategies

Information about therapeutic efficacy in FeL cases is scarce, with few investigated cases; the majority of the anti-leishmanial drugs have been studied just for dogs. Even for dogs, some of the studied and licensed treatment options are not considered to be capable of a complete cure.[1] Moreover, the strain of *L infantum* defines the host's clinical manifestation, as it can modulate susceptibility or determine resistance to one drug.[52]

Naturally infected cats do not seem to recover without specific anti-leishmanial therapy.[26] However, a 12 month monitoring study in Spain reported that 11/27 cats (with FeL diagnosed by IFI and/or PCR) had good clinical status without any treatment for *Leishmania* species.[2]

Pentamidine, administrated intramuscularly at the same dose recommended for dogs, allowed a cat to be clinically cured.[34] Nevertheless, allopurinol is the therapy recommended by the European Advisory Board on Cat Diseases (ABCD), at a dosage of 10–20 mg/kg q24h or q12h.[47] A positive clinical response in two cats treated with allopurinol (a 7-year-old with blepharitis and a 14-year-old with conjunctivitis, respectively, both with raised parasitic load) was described.[38] Allopurinol, 100 mg q24h, was also administered to a 14-year-old FIV-positive cat with a 3 year history of recurrent pododermatitis.[30] After 4 months of treatment, this case of disseminated leishmaniosis was considered to be in remission, with healing of the dermal lesions. The treatment further contributed to a reduction in the parasitaemia (11 parasites/ml in contrast with 26 parasites/ml at the point of diagnosis). The cat died 3 months later in a traffic accident. The post-mortem examination revealed development of adipose tissue reservoirs, suggesting improvement of its clinical condition. PCR confirmed the presence of parasites in the circulating blood.

A combination of meglumine antimoniate 5 mg/kg q24h SC with ketoconazole 10 mg/kg q24h PO was successfully administered to a cat with dermatological injuries and visceral involvement. Treatment was followed by three cycles for 4 weeks, with a 10 day interval.[1,39] Treatment of *L mexicana* infection with clotrimazole followed by paromomycin 15% (topically) was not effective. Six months later, the cat developed a new lesion in the nasal mucosa that was managed with levamisole (1 mg/kg q48h), but without clinical success.[34]

Supportive treatment is required in leishmaniosis, especially in animals with visceral compromise, such as hepatic failure and chronic kidney disease, given the hepato- and nephrotoxic potential of some drugs.

Monitoring of hepatic and renal functions in cats receiving anti-leishmanial therapy is thus recommended.[53]

Regression or a positive clinical response in FeL can be determined by the low number of parasites in lymphocytes and macrophages, indicating a cellular response and healing process.[38]

Given the above, prevention should be the main goal. Topical insecticides prevent sandfly bites. Repellents should be used in animals that inhabit or travel to, even if only temporarily, endemic zones.[54] Pyrethrins and pyrethroids exert efficient repellent activity against phlebotomines, and thus are widely accepted and effective in CaL control. The decreased hydrolysis of the esters of pyrethroids makes cats intolerant to pyrethrins and pyrethroids. A recently commercially available pyrethroid class molecule, flumethrin, is reported as safe in cats, being effective against ticks, fleas and arthropods.[55,56] Another option is imidacloprid, which according to a study carried out in Italy, when combined with flumethrin showed efficacy in the prevention of CaL in an area considered hyperendemic.[57] An additional prophylaxis measure recommended in endemic areas is the use of impregnated nets and spraying of shelters and areas occupied by human beings and animals with insecticide solutions.

Gradoni[54] further supports mandatory notification of *Leishmania* species in problematic regions as well as in non-endemic contiguous areas.

Conclusions

This work aims to raise awareness about FeL among veterinarians by providing a review of the current status of FeL infection caused by *L infantum* worldwide, the major clinicopathological features of infection, along with recent developments on FeL diagnosis, treatment and prevention.

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