Journal Pre-proof

Leishmania infection in cats and feline leishmaniosis: an updated review with a proposal of a diagnosis algorithm and prevention guidelines

André Pereira, Carla Maia

PII: S2667-114X(21)00029-7

DOI: https://doi.org/10.1016/j.crpvbd.2021.100035

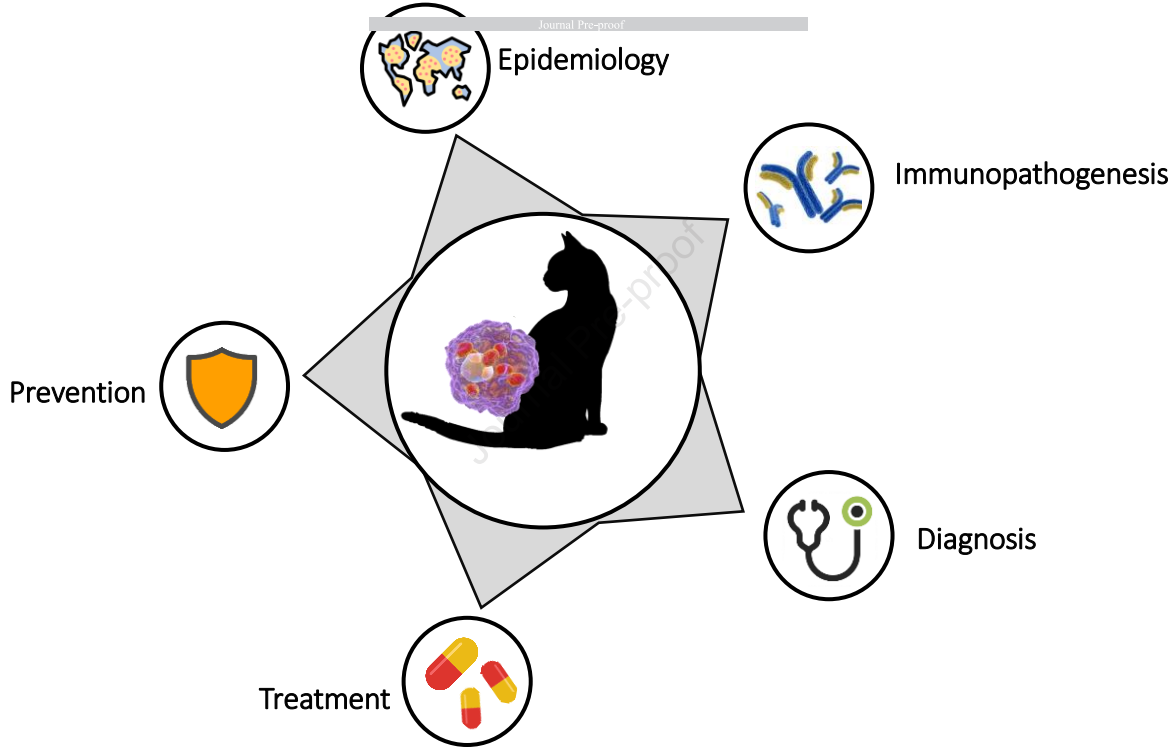
Reference: CRPVBD 100035

To appear in: *Current Research in Parasitology and Vector-Borne Diseases*

Received Date: 22 April 2021

Accepted Date: 27 May 2021

Please cite this article as: Pereira, A., Maia, C., *Leishmania* infection in cats and feline leishmaniosis: an updated review with a proposal of a diagnosis algorithm and prevention guidelines, *Current Research in Parasitology and Vector-Borne Diseases*, https://doi.org/10.1016/j.crpvbd.2021.100035.



Leishmania infection in cats and feline leishmaniosis: an updated review with a proposal of a diagnosis algorithm and prevention guidelines

André Pereira [a], Carla Maia [a], *

[a] *Global Health and Tropical Medicine (GHMT), Instituto de Higiene e Medicina Tropical (IHMT), Universidade NOVA de Lisboa, 1349-008 Lisboa, Portugal*

[*]Corresponding author
E-mail address: carlamaia@ihmt.unl.pt (C. Maia)

ABSTRACT

Leishmaniosis is a vector-borne disease caused by protozoans of the genus *Leishmania*, which are transmitted to vertebrates, including cats, through the bites of female phlebotomine sandflies. An increasing number of epidemiological and experimental studies concerning *Leishmania* infection in cats, as well as case reports of clinical leishmaniosis in these felids, have been published in recent years. In the present study, a comprehensive review was made by sourcing the National Library of Medicine resources to provide updated data on epidemiology, immunopathogenesis, diagnosis, treatment, and prevention of feline leishmaniosis. Cats were found infected with *Leishmania* parasites worldwide, and feline leishmaniosis appears as an emergent disease mostly reported in countries surrounding the Mediterranean Sea and Brazil. Cats with impaired immunocompetence seem to have a higher risk to develop clinical disease. The main clinical and clinicopathological findings are dermatological lesions and hypergammaglobulinemia, respectively. Diagnosis of feline leishmaniosis remains a challenge for veterinarians, in part, due to the lack of diagnosis support systems. For this reason, a diagnostic algorithm for clinical decision support is proposed for the first time here. No evidence-based treatment protocols are currently available, and these remain empirically based. Control measures are limited and scarce. Thus, a set of prevention guidelines are herein suggested.

Keywords: Cats; Diagnosis algorithm; Feline leishmaniosis; *Leishmania*; Prevention guidelines; Treatment.

1. Introduction

Leishmaniosis is a disease that affects humans and both domestic and wild animals worldwide and is caused by protozoans of the genus *Leishmania.* The infection typically occurs through the bites of female phlebotomine sand flies of the genera *Phlebotomus* in the Old World and *Lutzomyia* in the New World (WHO, 2010).

In contrast to dogs, cats have been considered for several years as accidental hosts resistant to leishmaniosis. Nevertheless, this felid now appears as a relevant piece within the ecological system in which *Leishmania* parasites are maintained indefinitely (Asfaram et al., 2019). Feline *Leishmania* infection has frequently been reported in endemic areas of South America, Southern Europe and Western Asia, and the number of reported cases of feline leishmaniosis has been increasing in recent years (Pereira et al., 2019b; Baneth et al., 2020; da Costa-Val et al., 2020; Fernandez-Gallego et al., 2020).

The present review aimed to provide updated information concerning the epidemiology of *Leishmania* infection in cats and clinical management of feline leishmaniosis (FeL) with emphasis on immunopathogenesis, diagnosis, treatment, prognosis, and prevention, as well as the development of an algorithm to assist diagnosis and delineate strategic guidelines to prevent feline infection.

2. Search strategy, eligibility, and review

A comprehensive literature search was performed on 10 March 2021 by sourcing National Library of Medicine (NLM) resources through PubMed (https://pubmed.ncbi.nlm.nih.gov/) using the following Boolean string: (“leishmania” [MeSH Terms] OR “leishmania”[All Fields] OR “leishmanias” [All Fields] OR “leishmaniae” [All Fields] OR (“leishmaniasis” [MeSH Terms] OR “leishmaniasis” [All Fields] OR “leishmaniosis” [All Fields] OR “leishmaniases” [All Fields])) AND (“cat” [All Fields] OR (“felis” [MeSH Terms] OR “felis” [All Fields]) OR (“felidae” [MeSH Terms] OR “felidae” [All Fields] OR “felid” [All Fields] OR “felids” [All Fields]) OR (“cats” [MeSH Terms] OR “cats” [All Fields] OR “felines” [All Fields] OR “felidae” [MeSH Terms] OR “felidae” [All Fields] OR “feline” [All Fields])). Search results were saved as a comma-separated value (CSV) file, subsequently imported into Microsoft® Excel®. Study eligibility was manually assessed by two independent investigators in a blinded manner. Only available original research articles concerning *Leishmania* infection in cats were retained, including those published in languages other than English (Fig. 1). Except for the epidemiological section (which included data from all

Leishmania spp. in felids belonging to the genus *Felis*), the present review refers exclusively to infection of domestic cats (*Felis catus*) by *L. donovani* (*sensu lato*). Although this complex is formally comprised of *L. donovani* (*sensu stricto*), *L. chagasi* and *L. infantum*, for the remainder of this review, *L. infantum* will be used to refer strictly to feline infection by *L. donovani* (*s.l.*).

3. Aetiology, distribution, and risk factors

To date, six species belonging to the subgenus *Leishmania* and one to the subgenus *Viannia* have been identified in domestic cats (*F. catus*) through DNA or isoenzyme-based typing methods (Fig. 2):

(i) *L.* (*L.*) *amazonensis* in Brazil (De Souza et al., 2005; Carneiro et al., 2020);

(ii) *L.* (*L.*) *infantum* in Brazil (Schubach et al., 2004; De Souza et al., 2005; da Silva et al., 2008; Vides et al., 2011; Sobrinho et al., 2012; de Morais et al., 2013; Benassi et al., 2017; Metzdorf et al., 2017; Marcondes et al., 2018; Rocha et al., 2019; Berenguer et al., 2020; da Costa-Val et al., 2020;), southern Europe (Ayllón et al., 2008, 2012; Maia et al., 2008; Tabar et al., 2008; Maia et al., 2010; Millán et al., 2011; Chatzis et al., 2014a; Maia et al., 2014, 2015b; Persichetti et al., 2016, 2018; Attipa et al., 2017a; Diakou et al., 2017; Otranto et al., 2017; Colella et al., 2019; Pereira et al., 2019c, 2020; Ebani et al., 2020), western Europe (Ozon et al., 1998; Pratlong et al., 2004; Pocholle et al., 2012; Richter et al., 2014) and western Asia (Hatam et al., 2010; Dincer et al., 2015; Akhtardanesh et al., 2017; Attipa et al., 2017b; Mohebali et al., 2017; Karakuş et al., 2019; Asgari et al., 2020; Baneth et al., 2020);

(iii) *L.* (*L.*) *major* in Portugal (Pereira et al., 2020) and Turkey (Paşa et al., 2015);

(iv) *L.* (*L.*) *mexicana* in the USA (Craig et al., 1986; Trainor et al., 2010; Minard et al., 2017) and Venezuela (Rivas et al., 2018);

(v) *L.* (*L.*) *tropica* in western Asia (Paşa et al., 2015; Can et al., 2016; Akhtardanesh et al., 2017);

(vi) *L.* (*L.*) *venezuelensis* in Venezuela (Bonfante-Garrido et al., 1991);

(vii) and *L.* (*V.*) *braziliensis* in Brazil (Schubach et al., 2004; da Costa-Val et al., 2020) and French Guiana (Rougeron et al., 2011).

Besides, DNA of *L. infantum* and putative *L. major*/*L. donovani* (*s.l.*) hybrid parasites were detected in wild cats (*Felis silvestris*) in Spain (Del Río et al., 2014) and in a domestic cat in mainland Portugal (Pereira et al., 2020), respectively.

The proportion of cats infected with or exposed to *Leishmania* has been assessed in several epidemiological studies through parasitological, serological, or molecular methods (Table 1 and Table 2). However, reported values vary greatly (from 0 to > 70%) and appear to be

influenced by local endemicity, sampling bias and heterogeneity/performance of diagnostic methodologies (manly cut-off, target gene and sample used for testing).

Specific antibodies or *Leishmania* DNA have been mostly detected in domestic cats living in endemic areas of South America, the Mediterranean Region and western Asia. Some studies also suggest that wild cats from Spain (Del Río et al., 2014; Risueño et al., 2018) and sand cats (*Felis margarita*) from Saudi Arabia (Morsy et al., 1999) are frequently exposed to *Leishmania* infection.

In non-endemic countries, as seen in dogs, feline *Leishmania* infection has been particularly associated with cats travelling to or rehomed from southern Europe and Brazil (Rüfenacht et al., 2005; Richter et al., 2014; Maia & Cardoso, 2015; Schäfer et al., 2021). Also, antibodies to *Leishmania* were detected in three domestic cats living in the UK, but in all cases, the travel and clinical history were unknown (Persichetti et al., 2017).

Although blood transfusion is regarded as a probable non-vector-borne transmission pathway of *Leishmania* in cats, no feline infection cases by this parasite (screened by PCR) were identified among eligible blood donors (Marenzoni et al., 2018; Mesa-Sanchez et al., 2020).

Several factors have been highlighted as possibly associated with *Leishmania* infection in cats based on univariate analysis, including old age (Akhtardanesh et al., 2017; Junsiri et al., 2017; Morganti et al., 2019; Asgari et al., 2020), male sex (Cardoso et al., 2010; Sobrinho et al., 2012; Montoya et al., 2018a; Asgari et al., 2020; Latrofa et al., 2020), non-neutered status (Otranto et al., 2017; Latrofa et al., 2020), presence of clinical or clinicopathological abnormalities (such as crusting skin lesions, leukopaenia, increase in alanine aminotransferase (ALT) levels, lymphadenomegaly, lymphocytosis and neutrophilia) (Ayllón et al., 2008; Sherry et al., 2011; Sobrinho et al., 2012; Spada et al., 2013; Akhtardanesh et al., 2017; Otranto et al., 2017; Latrofa et al., 2020), concomitant infections (such as feline coronavirus (FCoV), feline immunodeficiency virus (FIV), feline leukaemia virus and *Toxoplasma gondii*) (Sherry et al., 2011; Sobrinho et al., 2012; Spada et al., 2013, 2016; Montoya et al., 2018a), geographical area/local environment (such as altitude and rural areas) (Nasereddin et al., 2008; Cardoso et al., 2010; Asgari et al., 2020), lifestyle (such as access to the outdoors) (Rocha et al., 2019) and cohabitation with dogs (Rocha et al., 2019; Morelli et al., 2020). Epidemiological studies using logistic regression models (a powerful analytic research tool that avoids confounding effects) have evidenced that adult cats (Iatta et al., 2019; Akhtardanesh et al., 2020), males (Iatta et al., 2019; Akhtardanesh et al., 2020), non-neutered (Iatta et al., 2019), or with concomitant infections by FeLV (Martín-Sánchez et al., 2007; Sherry et al., 2011; Spada et al., 2013; Akhtardanesh et al., 2020), FIV (Iatta et al., 2019; Akhtardanesh et al., 2020), "*Candidatus*

Mycoplasma turicensis" or *Hepatozoon* spp. (Attipa et al., 2017b) have an increased risk for *Leishmania* infection.

4. Immunopathogenesis

In dogs, several studies have provided evidence demonstrating that the course of *L. infantum* infection is directly linked to the immune response. Development of progressive disease in susceptible dogs is typically characterised by high antibody levels and an impaired ability to mount a strong and effective cell-mediated response characterised by the expression of interferon-gamma (IFN-γ), tumour necrosis factor-alpha (TNF-α), and interleukin (IL)-2 (reviewed by Maia & Campino, 2018). However, very limited data are available on the pathogenesis of leishmaniosis in cats. Experimental studies involving intravenous/intraperitoneal inoculation of axenic promastigotes suggest that cats are hypothetically less susceptible to developing disease by *L. infantum* when compared to dogs, despite also presenting a long-lasting parasitaemia (Kirkpatrick et al., 1984; Akhtardanesh et al., 2018). Recently, Priolo et al. (2019) demonstrated that cats naturally exposed to *L. infantum* infection produce IFN-γ following *ex vivo* blood stimulation with parasite antigens, as reported in dogs (Solano-Gallego et al., 2016). This finding is important to highlight that *Leishmania* parasites can elicit a protective cell-mediated immune response in cats. The only study assessing the role of the complement system in feline *L. infantum* infection showed that, contrary to humans and dogs, cat's proteins are consumed by parasites in the lectin pathway, which hypothetically may justify their low predisposition to develop clinical disease (Tirado et al., 2021).

5. Clinical presentation and clinicopathological findings

Feline leishmaniosis caused by *L. infantum* is mostly reported in adult (median age: 7 years; range: 2–21 years) domestic short-hair cats living in or travelling to endemic countries of southern Europe and Brazil. The disease has a chronic course and may be manifested by a plethora of clinical signs and/or clinicopathological abnormalities, which are summarised in Table 3 and Table 4, respectively. About one-third of cats with leishmaniosis showed concomitant infections/diseases including FIV (Hervás et al., 2001; Poli et al., 2002; Pennisi et al., 2004; Grevot et al., 2005; Pocholle et al., 2012; Pimenta et al., 2015; Fernandez-Gallego et al., 2020), FeLV (Poli et al., 2002; Grevot et al., 2005; Pereira et al., 2019c), FCoV (Pennisi et al., 2004; Savani et al., 2004), *T. gondii* (Pennisi et al., 2004), *Bartonella henselae* (Pennisi et al., 2004), *diabetes mellitus* (Leiva et al., 2005), *pemphigus foliaceus* (Rüfenacht et al., 2005),

neoplasia (Grevot et al., 2005; Pocholle et al., 2012; Maia et al., 2015b) and/or were under immunosuppressive therapies at the time of diagnosis (Fernandez-Gallego et al., 2020).

Dermatological disorders were found in about 75% of reported clinical cases. Although uncommon, they may occur in the apparent absence of other obvious signs of disease (Fernandez-Gallego et al., 2020). Nodular dermatitis seems to be the main cutaneous lesion associated with FeL and is typically found on the eyelids (Hervás et al., 2001; Richter et al., 2014; Pimenta et al., 2015; Leal et al., 2018; Pereira et al., 2019c; Fernandez-Gallego et al., 2020; Silva et al., 2020). Erosive/ulcerative dermatitis is another clinical finding suggestive of FeL and has been identified on the head (Hervás et al., 2001; Grevot et al., 2005; Coelho et al., 2010; Pocholle et al., 2012; Maia et al., 2015b; Basso et al., 2016; Brianti et al., 2019; Headley et al., 2019; Fernandez-Gallego et al., 2020), extremities (Rüfenacht et al., 2005; Coelho et al., 2010; Basso et al., 2016; Fernandez-Gallego et al., 2020; Silva et al., 2020), trunk (Pocholle et al., 2012; Fernandez-Gallego et al., 2020), and over bony prominences (Hervás et al., 1999). Although less frequent, some cats with clinical leishmaniosis showed onychogryphosis (da Silva et al., 2010; Headley et al., 2019), a rather specific sign of canine leishmaniosis (CanL) (Maia & Campino, 2018). Generalised or focal lymphadenopathy appears as a common finding in FeL (Hervás et al., 1999, 2001; Poli et al., 2002; Savani et al., 2004; Pennisi et al., 2004; Maroli et al., 2007; da Silva et al., 2010; Brianti et al., 2019; Fernandez-Gallego et al., 2020; Silva et al., 2020) as well as non-specific signs including lethargy/depression (Poli et al., 2002; Pennisi et al., 2004; Leiva et al., 2005; Rüfenacht et al., 2005; Marcos et al., 2009; Pocholle et al., 2012; Richter et al., 2014; Fernandez-Gallego et al., 2020), anorexia/inappetence (Pennisi et al., 2004; Rüfenacht et al., 2005; Marcos et al., 2009; da Silva et al., 2010; Fernandez-Gallego et al., 2020), and weight loss (Ozon et al., 1998; Hervás et al., 1999; Pennisi et al., 2004; Savani et al., 2004; da Silva et al., 2010; Fernandez-Gallego et al., 2020; Silva et al., 2020).

Approximately one-fourth of cats with clinical leishmaniosis showed uveitis (Hervás et al., 2001; Pennisi et al., 2004; Verneuil, 2013; Richter et al., 2014; Pimenta et al., 2015; Leal et al., 2018; Pereira et al., 2019c; Fernandez-Gallego et al., 2020); stomatitis (Hervás et al., 2001; Leiva et al., 2005; Maroli et al., 2007; Verneuil, 2013; Maia et al., 2015b; Migliazzo et al., 2015; Fernandez-Gallego et al., 2020) and/or cardiorespiratory signs such as dyspnoea/tachypnoea, pallor, abnormal respiratory sounds, nasal discharge and sneezing (Hervás et al., 2001; Pennisi et al., 2004; Marcos et al., 2009; da Silva et al., 2010; Richter et al., 2014; Migliazzo et al., 2015; Maia et al., 2015b; Basso et al., 2016; Leal et al., 2018; Headley et al., 2019; Altuzarra et al., 2020; Silva et al., 2020). Musculoskeletal (i.e. muscle atrophy; da Silva et al., 2010), neurological (i.e. ataxia; Fernandez-Gallego et al., 2020), and urogenital (i.e. vaginal bleeding; Maia et al., 2015b) signs were also occasionally described, but in some cases, they appear to be

secondary to concomitant diseases (Maia et al., 2015b; Fernandez-Gallego et al., 2020). Other clinical manifestations rarely found and which may represent a further diagnostic challenge to veterinarians include: depigmentation (Rüfenacht et al., 2005; Pocholle et al., 2012), cutaneous bloody cyst (Pennisi et al., 2004), pruritus (Rüfenacht et al., 2005; Pocholle et al., 2012), footpad hyperkeratosis (Fernandez-Gallego et al., 2020), hepatomegaly (Pennisi et al., 2004; Leiva et al., 2005), splenomegaly (Poli et al., 2002; Leal et al., 2018), bruising (Maia et al., 2015b), mastitis (Pereira et al., 2019c), chorioretinitis (Pennisi et al., 2004; Fernandez-Gallego et al., 2020), corneal opacification (Hervás et al., 2001; Pimenta et al., 2015), glaucoma (Leiva et al., 2005; Richter et al., 2014), blepharitis (Brianti et al., 2019), chemosis (Fernandez-Gallego et al., 2020), ocular masses (Hervás et al., 2001), glossitis (Fernandez-Gallego et al., 2020), jaundice (Hervás et al., 1999; Fernandez-Gallego et al., 2020), abdominal distension (Leiva et al., 2005), and vomiting/diarrhoea (Hervás et al., 1999; Fernandez-Gallego et al., 2020).

Most consistent laboratory abnormalities found in FeL cases include anaemia (generally of the normochromic, normocytic type) (Hervás et al., 1999; Pennisi et al., 2004; Marcos et al., 2009; Richter et al., 2014; Pimenta et al., 2015; Pereira et al., 2019c; Fernandez-Gallego et al., 2020) and hyperproteinaemia with hypergammaglobulinaemia (Ozon et al., 1998; Hervás et al., 1999; Poli et al., 2002; Pennisi et al., 2004; Leiva et al., 2005; Marcos et al., 2009; Richter et al., 2014; Basso et al., 2016; Leal et al., 2018; Brianti et al., 2019; Pereira et al., 2019c; Altuzarra et al., 2020; Fernandez-Gallego et al., 2020). The latter was detected in more than 80% of sick cats and should be investigated as a possible biomarker of FeL. Leukocytosis (Ozon et al., 1998; da Silva et al., 2010; Fernandez-Gallego et al., 2020) and leukopaenia (Pennisi et al., 2004; Rüfenacht et al., 2005; Richter et al., 2014) are inconsistent findings, whereas thrombocytopenia (Pennisi et al., 2004; Marcos et al., 2009; Richter et al., 2014; Pimenta et al., 2015; Basso et al., 2016; Pereira et al., 2019c) and azotaemia (Pennisi et al., 2004; Leiva et al., 2005; Marcos et al., 2009; da Silva et al., 2010; Leal et al., 2018; Fernandez-Gallego et al., 2020) have been frequently reported. About a quarter of the sick cats presented proteinuria (Marcos et al., 2009; Leal et al., 2018; Fernandez-Gallego et al., 2020), suggesting a possible association between FeL and kidney disease as described in dogs. Recently, Chatzis et al. (2020) observed that cats infected with *Leishmania* parasites had higher concentrations of inorganic phosphorus than non-infected cats, reinforcing this assumption. Mild increases of liver enzyme activities are also described (Fernandez-Gallego et al., 2020), but less frequently than in cases of CanL (Maia & Campino, 2018).

6. Diagnosis

Clinical presentation combined with epidemiological context may lead to suspicion of FeL, but for a definitive diagnosis, *Leishmania*-specific laboratory tests are required (Table 5). These include direct tests (cytology, histology, immunohistochemistry, culture, and PCR), demonstrating the presence of the parasite or its components, and indirect tests (serology) assessing the host's response to the parasite infection.

Cytology is strongly advised in cats presenting erosive/ulcerative skin disease, nodular lesions and/or lymphadenomegaly (Hervás et al., 1999; Poli et al., 2002; Savani et al., 2004; Coelho et al., 2010; Richter et al., 2014; Maia et al., 2015b; Pimenta et al., 2015; Basso et al., 2016; Attipa et al., 2017a; Leal et al., 2018; Brianti et al., 2019; Headley et al., 2019; Pereira et al., 2019c; Silva et al., 2020). Material for diagnosis can be obtained by fine-needle biopsy (with or without aspiration), scraping or imprinting. The presence of *Leishmania* parasites has been demonstrated in cytological examinations of feline nodular lesions (Poli et al., 2002; Savani et al., 2004; Richter et al., 2014; Pimenta et al., 2015; Basso et al., 2016; Attipa et al., 2017a; Leal et al., 2018; Brianti et al., 2019; Pereira et al., 2019c; Fernandez-Gallego et al., 2020; Silva et al., 2020), erosive/ulcerative lesions (Maia et al., 2015b; Headley et al., 2019; Fernandez-Gallego et al., 2020; Silva et al., 2020), whole-blood (Marcos et al., 2009; Metzdorf et al., 2017), buffy coat/leucoconcentrate (Martín-Sánchez et al., 2007, Marcos et al., 2009), lymph nodes (Hervás et al., 1999; Poli et al., 2002; Pennisi et al., 2004; Bresciani et al., 2010; Coelho et al., 2010, 2011b; Vides et al., 2011; Sobrinho et al., 2012; Metzdorf et al., 2017; Berenguer et al., 2020; Fernandez-Gallego et al., 2020; Silva et al., 2020), bone marrow (Pennisi et al., 2004; Marcos et al., 2009; Vides et al., 2011; Sobrinho et al., 2012; Metzdorf et al., 2017; Marcondes et al., 2018; Fernandez-Gallego et al., 2020), liver (Vides et al., 2011Mohebali et al., 2017; Fernandez-Gallego et al., 2020), spleen (Vides et al., 2011; Mohebali et al., 2017; Fernandez-Gallego et al., 2020), nasal exudate (Migliazzo et al., 2015), corneal impression (Pimenta et al., 2015), and inflammatory breast fluid (Pereira et al., 2019c). Cytologic preparations consistent with FeL typically have a cell composition characteristic of pyogranulomatous, granulomatous or lymphoplasmacytic inflammation (Poli et al., 2002; Headley et al., 2019; Pereira et al., 2019c). Similar patterns are reported in histological studies on feline paraffin-embedded specimens (Poli et al., 2002; Navarro et al., 2010; Migliazzo et al., 2015; Di Mattia et al., 2018; Leal et al., 2018; Altuzarra et al., 2020). Nevertheless, compared with cytology, histology has the main advantage of providing a more detailed diagnostic information on the tissue architecture, which allows understanding if parasites are indeed associated with lesions (Paltrinieri et al., 2016). Immunohistochemistry may be further performed to confirm the presence of *Leishmania* organisms in biological samples obtained from cats (Poli et al., 2002; Navarro et al., 2010; Migliazzo et al., 2015). Based on histological and immunohistochemical examinations, it has

been observed that this parasite may invade several feline organs/tissues such as skin (Ozon et al., 1998; Poli et al., 2002; Grevot et al., 2005; Rüfenacht et al., 2005; Attipa et al., 2017a; Rivas et al., 2018; Fernandez-Gallego et al., 2020; Silva et al., 2020), nasal and oral mucosa (Pennisi et al., 2004; Migliazzo et al., 2015; Leal et al., 2018), eyes (Hervás et al., 2001; Fernandez-Gallego et al., 2020), nasopharynx (Leal et al., 2018), stomach (Hervás et al., 1999), liver (Hervás et al., 1999; Silva et al., 2020), kidneys (Ozon et al., 1998), spleen (Hervás et al., 1999; Grevot et al., 2005; Marcos et al., 2009; Maia et al., 2015b; Fernandez-Gallego et al., 2020; Silva et al., 2020), bone marrow (Ozon et al., 1998; Pimenta et al., 2015; Silva et al., 2020), and lymph nodes (Hervás et al., 1999), and may also be associated with neoplasia (Grevot et al., 2005; Rüfenacht et al., 2005; Pocholle et al., 2012; Maia et al., 2015b; Altuzarra et al., 2020).

Parasite culture is an accurate test allowing conclusive diagnosis of active infection. However, this test is not suitable for rapid diagnosis and is restricted to specialised laboratories. Parasite culture is a starting point for parasite identification and characterisation by isoenzyme electrophoresis (Pratlong et al., 2004). Viable parasites have been isolated from whole blood (Pocholle et al., 2012), nodular lesions (Poli et al., 2002; Basso et al., 2016), liver (Maia et al., 2015b; Silva et al., 2020), spleen (Maia et al., 2015b; Silva et al., 2020), lymph nodes (Pennisi et al., 2004; Maroli et al., 2007; Maia et al., 2015b; Basso et al., 2016; Silva et al., 2020), and bone marrow (Silva et al., 2020) of cats with leishmaniosis.

Polymerase chain reaction (PCR)-based tests has been allowed the identification of *Leishmania* DNA in several feline samples, including whole blood (Marcos et al., 2009; Pocholle et al., 2012; Pimenta et al., 2015; Basso et al., 2016; Attipa et al., 2017a; Brianti et al., 2019; Fernandez-Gallego et al., 2020; Silva et al., 2020), buffy coat (Pereira et al., 2019c), conjunctival and oral swabs (Migliazzo et al., 2015Brianti et al., 2019; da Costa-Val et al., 2020), hair (Urbani et al., 2020), skin (Rüfenacht et al., 2005; da Silva et al., 2010; Richter et al., 2014; Maia et al., 2015b; Basso et al., 2016; Fernandez-Gallego et al., 2020; Silva et al., 2020), nasal tissue (Leal et al., 2018), liver (Maia et al., 2015b; Silva et al., 2020), spleen (Savani et al., 2004; Coelho et al., 2010; da Silva et al., 2010; Maia et al., 2015b; Pimenta et al., 2015; Fernandez-Gallego et al., 2020; Silva et al., 2020), kidneys (da Silva et al., 2010), lymph nodes (Poli et al., 2002; Pennisi et al., 2004; Coelho et al., 2010; da Silva et al., 2010; Maia et al., 2015b; Migliazzo et al., 2015; Pimenta et al., 2015; Silva et al., 2020), bone marrow (da Silva et al., 2010; Richter et al., 2014; Pimenta et al., 2015; Fernandez-Gallego et al., 2020; Silva et al., 2020), and inflammatory breast fluid (Pereira et al., 2019c). Conventional PCR, nested PCR, and real-time PCR (qPCR) targeting kinetoplast minicircle DNA (kDNA) or the small subunit ribosomal DNA (*SSU* rDNA) multicopy genes have been widely used in routine veterinary practise for FeL diagnosis (Pimenta et al., 2015; Brianti et al., 2019; Pereira et al., 2019c) as well

as in epidemiological studies concerning *Leishmania* infection in cats (Maia et al., 2014; Vilhena et al., 2013; Pereira et al., 2020). Nevertheless, two-step PCR to amplify stretches of multicopy genes has increased sensitivity and should be preferred for suboptimal sample testing (i.e. where the parasite load tends to be low) such as whole blood (Pereira et al., 2020). On the other hand, quantitative PCR (qPCR) may further provide information about the amount of parasite DNA present in the sample (Galluzzi et al., 2018). This aspect is particularly relevant for monitoring the efficacy of anti-*Leishmania* treatments (Pocholle et al., 2012; Basso et al., 2016). However, it is important to highlight that a PCR-positive result may only reflect a transient infection and, for this reason, should be carefully interpreted in a clinical context. PCR products may be followed by restriction enzyme digestion (i.e. restriction fragment length polymorphism) and/or sequencing for parasite species identification (Metzdorf et al., 2017; Pereira et al., 2020).

The most common serological tests used to detect anti-*Leishmania* antibodies in cats are based on enzyme-linked immunosorbent assay (ELISA) and immunofluorescent antibody test (IFAT). The latter is considered as the reference test for the serodiagnosis of canine and human leishmaniosis (OIE, 2018; WHO, 2010). Persichetti et al. (2017) established 1:80 serum dilution as IFAT cut-off for FeL serodiagnosis, and demonstrated that this test helps to detect subclinical or early *Leishmania* infections in cats. More recently, Iatta et al. (2020) validated IFAT as an accurate test to assess the exposure of cats to *L. infantum*, reporting positive and negative predictive values of 80.7% and 89.9%, respectively. Compared to IFAT, ELISA (cut-off 40 ELISA units) presents a better performance for the serodiagnosis of clinical FeL (Persichetti et al., 2017). Western blot analysis is mainly intended for research but rarely available in routine practice. However, this test seems to offer the best diagnostic performance (considering an 18 kDa band as a marker for positivity) to detect antibodies against *L. infantum* in cats (Persichetti et al., 2017). Direct agglutination test has also occasionally been used in both clinical and epidemiological contexts for serological diagnosis of FeL (Pimenta et al., 2015; Asgari et al., 2020). Some authors have considered a cut-off value of 1:100 to distinguish infected from uninfected cats (Kongkaew et al., 2007; Cardoso et al., 2010; Maia et al., 2015a; Lopes et al., 2017; Asgari et al., 2020; Neves et al., 2020). Indirect hemagglutination was exclusively performed in epidemiological studies in domestic cats in Egypt (Michael et al., 1982; Morsy et al., 1988; Morsy & Abou el Seoud, 1994).

Cats with clinical leishmaniosis tend to present high antibody levels (Richter et al., 2014; Maia et al., 2015b; Pimenta et al., 2015; Basso et al., 2016), and specific treatment frequently leads to the reduction of anti-*Leishmania* antibodies (Pennisi et al., 2004; Richter et al., 2014; Basso et al., 2016; Pereira et al., 2019c). In some cases, an increase of antibody titres was associated with clinical relapse. Nevertheless, it is essential to emphasise that a positive

serological result formally only reflects exposure to pathogens and should be interpreted in a clinical context (Paltrinieri et al., 2016).

In conclusion, the diagnosis of FeL can be a real challenge for veterinarians and is seldom considered during the differential diagnosis. Therefore, the algorithm illustrated in Fig. 3 is proposed for clinically healthy cats used as blood donors or for breeding purposes, and for cats with suspected leishmaniosis.

7. Treatment and prognosis

Treatment should be considered only after confirmation of disease (see *Section 6*). Although several treatment regimens have been empirically used for FeL (Table 6), no controlled studies about their efficacy and safety have been performed. Long-term administration of allopurinol as monotherapy is the most common regimen prescribed for FeL (Pennisi et al., 2004; Leiva et al., 2005; Rüfenacht et al., 2005; Marcos et al., 2009; Pocholle et al., 2012; Richter et al., 2014; Maia et al., 2015b; Migliazzo et al., 2015; Pimenta et al., 2015; Basso et al., 2016; Attipa et al., 2017a; Leal et al., 2018; Brianti et al., 2019; Pereira et al., 2019c; Altuzarra et al., 2020; Fernandez-Gallego et al., 2020). This drug is generally well-tolerated, but possible cases of cutaneous adverse reactions (Leal et al., 2018; Brianti et al., 2019), coprostasis (Maia et al., 2015b), and elevated liver enzymes (Rüfenacht et al., 2005) have been sporadically reported. Favourable results (i.e. clinical cure or improvement of clinical status) with allopurinol as monotherapy have been commonly obtained (Pennisi et al., 2004; Leiva et al., 2005; Rüfenacht et al., 2005; Pocholle et al., 2012; Richter et al., 2014; Migliazzo et al., 2015; Pimenta et al., 2015; Attipa et al., 2017a; Fernandez-Gallego et al., 2020; Altuzarra et al., 2020). Nevertheless, relapse after discontinuation or low-dose administration (Pennisi et al., 2004; Leiva et al., 2005; Brianti et al., 2019; Pereira et al., 2019c) and no or poor response to allopurinol therapy have been occasionally reported, even in cats with no apparent history of concomitant infections or immunosuppressive therapies (Rüfenacht et al., 2005; Marcos et al., 2009; Basso et al., 2016; Fernandez-Gallego et al., 2020). Therefore, the combination of meglumine antimoniate and allopurinol has been proposed for FeL treatment, appearing to be more effective (Basso et al., 2016; Pereira et al., 2019c), but acute kidney injury has already been reported (Leal et al., 2018). Although controversial, this drug is suspected of inducing nephrotoxicity in dogs (reviewed by Roura et al., 2021). Thus, its use in cats with altered renal function should be carefully considered. Meglumine antimoniate plus ketoconazole was used in a cat with cutaneous and systemic signs of FeL, resulting in apparent clinical cure (Hervás et al., 1999). Miltefosine was recently adopted as an alternative to meglumine antimoniate in an azotemic cat, resulting in rapid

clinical improvement (Leal et al., 2018). In this case, transient vomiting episodes were reported in the first week of treatment with miltefosine but were managed using antiemetics (i.e. maropitant). Nevertheless, Fernandez-Gallego et al. (2020) recently reported a case of FeL with concomitant FIV infection not responsive to miltefosine plus allopurinol (combination therapy). Pennisi et al. (2004) reported treatment failure in a seropositive cat for FIV, *T. gondii* and *B. henselae* suffering from leishmaniosis. In this case, three distinct regimens were used (i.e. metronidazole plus spiramycin, fluconazole and itraconazole) (Pennisi et al., 2004). In another cat with leishmaniosis associated with an invasive squamous cell carcinoma, domperidone was used after unsuccessful allopurinol monotherapy, but clinical signs remained after one month of treatment (Maia et al., 2015b). The dietary supplement active hexose correlated compound (AHCC) was recently suggested as a possible alternative maintenance therapy to allopurinol (Leal et al., 2018). Surgical removal of lesions was also reported as an additional therapeutic approach (Hervás et al., 2001; Rüfenacht et al., 2005; Basso et al., 2016).

Like in dogs, *Leishmania* parasites may persist in treated cats (Pocholle et al., 2012; Pimenta et al., 2015; Attipa et al., 2017a), suggesting that treatment may lead to clinical cure but may not eliminate the infection.

Overall, FeL has a good prognosis even in cases with underlying viral infections (i.e. FIV or FeLV) (Hervás et al., 1999; Pennisi et al., 2004; Rüfenacht et al., 2005; Richter et al., 2014; Migliazzo et al., 2015; Pimenta et al., 2015; Basso et al., 2016; Attipa et al., 2017a; Leal et al., 2018; Pereira et al., 2019c; Altuzarra et al., 2020; Fernandez-Gallego et al., 2020). On the other hand, panleukopaenia, acute kidney injury and lack of treatment seem to be critical factors associated with poor prognosis (Ozon et al., 1998; Hervás et al., 1999; Poli et al., 2002; Pennisi et al., 2004; Pimenta et al., 2015; Fernandez-Gallego et al., 2020).

8. Prophylaxis and control

No vaccines or drugs preventing leishmaniosis are currently available for use in cats, and most repellents avoiding infection in dogs are toxic to these felids. In endemic areas, cats are frequently exposed to phlebotomine sand fly bites, and this is associated with an increased risk of *Leishmania* infection (Pereira et al., 2019b). Chemoprophylaxis may be achieved by using a matrix collar containing 10% imidacloprid and 4.5% flumethrin. This formulation showed to be safe and effective in reducing infection risk by *L. infantum* in cats (Brianti et al., 2017). Nevertheless, keeping cats indoors during the period of vector activity (April to November in Mediterranean areas, see Alten et al., 2016) from dusk to dawn, as well as using physical barriers such as nets (i.e. mesh size 1,240 holes/in^2) on windows and doors (Faiman et al., 2009) may

eschew exposure to phlebotomine sand fly bites, thereby minimising the risk of *Leishmania* infection. Spraying with residual insecticides on walls and roofs of human houses and animal shelters has been proposed as an additional measure for preventing CanL (Maroli et al., 2010). However, their use in environments with cats should be carefully considered since most of these products contain compounds (i.e. pyrethrins or pyrethroids) that can induce feline toxicosis. Isoxazolines, namely afoxolaner and fluralaner, have been regarded as a new promising class of drugs for controlling CanL and human leishmaniosis in endemic areas (Miglianico et al., 2018; Bongiorno et al., 2020; Queiroga et al., 2020). A spot-on formulation of fluralaner (112.5–500 mg) is licensed for ectoparasite (i.e. ticks, fleas and mites) control in cats. This systemic insecticide induced long-term mortality of *Lutzomyia longipalpis* and *Phlebotomus perniciosus* (vectors of *L. infantum* in the New and Old Worlds, respectively) after feeding on treated dogs (Bongiorno et al., 2020; Queiroga et al., 2020). Similar results are expected to be observed in cats. Although studies are undoubtedly needed, this drug may also hypothetically represent an affordable indirect method for reducing *Leishmania* infection in cats in endemic areas. The detection and treatment of cats with leishmaniosis is also likely a beneficial control measure, as they may serve as a source of infection to phlebotomine sand fly vectors (Maroli et al., 2007; da Silva et al., 2010; Mendonça et al., 2020). In the absence of evidence indicating otherwise, *Leishmania*-infected cats should not be used for breeding or as blood donors due to the potential risk of transmission through blood transfusion and venereal/congenital infection, as reported in dogs (Owens et al., 2001; Naucke & Lorentz, 2012).

In summary, and according to the current knowledge, the following prophylactic measures are proposed to prevent and control feline infection:

- In endemic areas, keeping cats indoors from dusk to dawn during the phlebotomine sand fly season should be encouraged.
- Use of physical barriers on houses and animal shelters located in endemic areas with high vector density.
- Use of a matrix collar containing 10% imidacloprid and 4.5% flumethrin as well topical solutions containing 112.5–500 mg of fluralaner in cats living in or travelling to (cover the time of travel) endemic areas during the known transmission season.
- After the return from endemic areas, cats should be clinically evaluated and tested.
- Cats eligible for breeding and blood transfusion should be periodically tested.
- Infected cats should not be used for breeding or as blood donors.
- Cats with leishmaniosis should be treated and periodically monitored.

9. Public health considerations

Zoonotic visceral leishmaniosis (ZVL) caused by *L. infantum* is a life-threatening human disease endemic in the Mediterranean Basin, the Middle East, western Asia, and Brazil (WHO, 2010). Domestic dogs are considered the primary source of human infection, which typically occurs *via* the bites of female phlebotomine sand flies (WHO, 2010). Nevertheless, during the last years, cats have been deserved attention due to their potential enrolment in ZVL epidemiology, appearing now as possible primary or secondary reservoir hosts (Asfaram et al., 2019). This hypothesis arises by the following reasons (Maroli et al., 2007; da Silva et al., 2010; GfK, 2016; Pereira et al., 2019b, 2019c, 2020; Carneiro et al., 2020; Fernandez-Gallego et al., 2020; Mendonça et al., 2020):

- Cats are frequently exposed to the bites of competent vectors.
- Cats are naturally susceptible to *L. infantum* infection.
- Feline infection often runs a subclinical course.
- Parasites are frequently found in the skin and blood of infected cats.
- Naturally infected cats are infectious to competent vectors.
- Naturally infected cats may be the source of infection to other mammals through competent vectors.
- Strains of feline origin seem to be indistinguishable from those isolated from dogs, humans, and competent vectors.
- Cats are among the most popular animals owned as a pet.
- Cats are often present in domestic/peridomestic areas where transmission cycles occur.

10. Conclusions

During the last years, several studies concerning *Leishmania* infection in cats were conducted. Feline leishmaniosis has also gained importance appearing nowadays as an emergent disease. Nevertheless, its immunopathogenesis is poorly known. This protozoonosis is manifested by a broad spectrum of clinical signs and clinicopathological abnormalities, which, associated with the lack of standardised protocols, make its diagnosis further challenging for veterinarians. In this review, a diagnostic algorithm for FeL is proposed for clinical decision support. Treatment options currently available are empirical and suboptimal. The main form to prevent disease is to avoid infection. However, in contrast to dogs, very limited options are currently available to keep infective sand flies away from cats. Thus, a set of prevention guidelines are herein suggested.

Funding

The Global Health and Tropical Medicine centre is funded by the Fundação para a Ciência e a Tecnologia, I.P. (FCT) (GHTM-UID/Multi/04413/2013). AP was supported by the Portuguese Ministry of Education and Science (*via* FCT) through a PhD grant (SFRH/BD/116516/2016).

CRediT author statement

André Pereira: Conceptualisation, Methodology, Validation, Formal analysis, Investigation, Writing – Original Draft, Writing - Review & Editing. Carla Maia: Conceptualisation, Methodology, Validation, Writing - Review & Editing, Supervision. The authors read and approved the final manuscript.

Declaration of competing interests

The authors declare that they have no competing interests.

References

Abbate, J.M., Arfuso, F., Napoli, E., Gaglio, G., Giannetto, S., Latrofa, M.S., et al., 2019. *Leishmania infantum* in wild animals in endemic areas of southern Italy. Comp. Immunol. Microbiol. Infect. Dis. 67, 101374. https://doi.org/10.1016/j.cimid.2019.101374.

Akhtardanesh, B., Kheirandish, R., Sharifi, I., Mohammadi, A., Mostafavi, A., Mahmoodi, T., Ebrahimi, M., 2018. Low susceptibility of domestic cats to experimental *Leishmania infantum* infection. J. Vector Borne Dis. 55, 230–234. https://doi.org/10.4103/0972-9062.249481.

Akhtardanesh, B., Moeini, E., Sharifi, I., Saberi, M., Sadeghi, B., Ebrahimi, M., Otranto, D., 2020. *Leishmania* infection in cats positive for immunodeficiency virus and feline leukemia virus in an endemic region of Iran. Vet. Parasitol. Reg. Stud. Reports 20, 100387. https://doi.org/10.1016/j.vprsr.2020.100387.

Akhtardanesh, B., Sharifi, I., Mohammadi, A., Mostafavi, M., Hakimmipour, M., Pourafshar, N.G., 2017. Feline visceral leishmaniasis in Kerman, southeast of Iran: Serological and

molecular study. J Vector Borne Dis. 54, 96–102.

Alcover, M.M., Ribas, A., Guillén, M.C., Berenguer, D., Tomás-Pérez, M., Riera, C., Fisa, R., 2020. Wild mammals as potential silent reservoirs of *Leishmania infantum* in a Mediterranean area. Prev. Vet. Med. 175, 104874. https://doi.org/10.1016/j.prevetmed.2019.104874.

Alten, B., Maia, C., Afonso, M.O., Campino, L., Jiménez, M., González, E., et al., 2016. Seasonal dynamics of phlebotomine sand fly species proven vectors of mediterranean leishmaniasis caused by *Leishmania infantum*. PLoS Negl. Trop. Dis. 10, e0004458. https://doi.org/10.1371/journal.pntd.0004458.

Altuzarra, R., Movilla, R., Roura, X., Espada, Y., Majo, N., Novellas, R., 2020. Computed tomographic features of destructive granulomatous rhinitis with intracranial extension secondary to leishmaniasis in a cat. Vet. Radiol. Ultrasound 61, E64–E68. https://doi.org/10.1111/vru.12666.

Asfaram, S., Fakhar, M., Teshnizi, S.H., 2019. Is the cat an important reservoir host for visceral leishmaniasis? A systematic review with meta-analysis. J. Venom. Anim. Toxins Incl. Trop. Dis. 25, e20190012. https://doi.org/10.1590/1678-9199-jvatitd-2019-0012.

Asgari, Q., Mohammadpour, I., Bozorg-Ghalati, F., Motazedian, M.H., Kalantari, M., Hosseini, S., 2020. Alarming: high prevalence of *Leishmania infantum* infection in cats from southern Iran based on molecular and serological methods. Ann. Parasitol. 66, 143–156. https://doi.org/10.17420/ap6602.249.

Attipa, C., Neofytou, K., Yiapanis, C., Martínez-Orellana, P., Baneth, G., Nachum-Biala, Y., et al., 2017a. Follow-up monitoring in a cat with leishmaniosis and coinfections with Hepatozoon felis and *Candidatus* Mycoplasma haemominutum. J. Feline Med. Surg. Open Reports 3, 205511691774045. https://doi.org/10.1177/2055116917740454.

Attipa, C., Papasouliotis, K., Solano-Gallego, L., Baneth, G., Nachum-Biala, Y., Sarvani, E., et al., 2017b. Prevalence study and risk factor analysis of selected bacterial, protozoal and viral, including vector-borne, pathogens in cats from Cyprus. Parasit. Vectors 10, 130. https://doi.org/10.1186/s13071-017-2063-2.

Ayllón, T., Diniz, P.P.V.P., Breitschwerdt, E.B., Villaescusa, A., Rodríguez-Franco, F., Sainz, A., 2012. Vector-borne diseases in client-owned and stray cats from Madrid, Spain. Vector Borne Zoonotic Dis. 12, 143–150. https://doi.org/10.1089/vbz.2011.0729.

Ayllón, T., Tesouro, M.A., Amusategui, I., Villaescusa, A., Rodriguez-Franco, F., Sainz, Á., 2008. Serologic and molecular evaluation of *Leishmania infantum* in cats from central Spain, Ann. N. Y. Acad. Sci. 1149, 361–364. https://doi.org/10.1196/annals.1428.019.

Baneth, G., Nachum-Biala, Y., Zuberi, A., Zipori-Barki, N., Orshan, L., Kleinerman, G., et al.,

2020. *Leishmania* infection in cats and dogs housed together in an animal shelter reveals a higher parasite load in infected dogs despite a greater seroprevalence among cats. Parasit. Vectors 13, 115. https://doi.org/10.1186/s13071-020-3989-3.

Basso, M.A., Marques, C., Santos, M., Duarte, A., Pissarra, H., Carreira, L.M., et al., 2016b. Successful treatment of feline leishmaniosis using a combination of allopurinol and N-methyl-glucamine antimoniate. J. Feline Med. Surg. Open Reports 2, 205511691663000. https://doi.org/10.1177/2055116916630002.

Benassi, J.C., Benvenga, G.U., Ferreira, H.L., Pereira, V.F., Keid, L.B., Soares, R., Oliveira, T.M.F. de S., 2017. Detection of *Leishmania infantum* DNA in conjunctival swabs of cats by quantitative real-time PCR. Exp. Parasitol. 177, 93–97. https://doi.org/10.1016/j.exppara.2017.04.004.

Berenguer, L.K.A.R., Gomes, C.F.C. de A., Nascimento, J. de O., Bernardi, J.C.M., Lima, V.F.S., de Oliveira, J.B., et al., 2020. *Leishmania infantum* infection in a domestic cat: a real threat or an occasional finding? Acta Parasitol. https://doi.org/10.1007/s11686-020-00294-z.

Bezerra, J.A.B., De Medeiros Oliveira, I.V.P., Yamakawa, A.C., Nilsson, M.G., Tomaz, K.L.R., De Oliveira, K.D.S., et al., 2019. Serological and molecular investigation of *Leishmania* spp. infection in cats from an area endemic for canine and human leishmaniasis in northeast Brazil. Rev. Bras. Parasitol. Vet. 28, 790–796. https://doi.org/10.1590/s1984-29612019082.

Bonfante-Garrido, R., Urdaneta, I., Urdaneta, R., Alvarado, J., 1991. Natural infection of cats with Leishmania in Barquisimeto, Venezuela. Trans. R. Soc. Trop. Med. Hyg. 85, 53. https://doi.org/10.1016/0035-9203(91)90153-p.

Bongiorno, G., Meyer, L., Evans, A., Lekouch, N., Bianchi, R., Khoury, C., Chiummo, R., Thomas, E., Gradoni, L., 2020. A single oral dose of fluralaner (Bravecto®) in dogs rapidly kills 100% of blood- fed *Phlebotomus perniciosus* , a main visceral leishmaniasis vector, for at least 1 month after treatment. Med. Vet. Entomol. 34, 240–243. https://doi.org/10.1111/mve.12420.

Braga, A.R.C., Corrêa, A.P.F.L., Camossi, L.G., Da Silva, R.C., Langoni, H., Lucheis, S.B., 2014a. Coinfection by *Toxoplasma gondii* and *Leishmania* spp. in domestic cats (*Felis catus*) in state of mato grosso do sul. Rev. Soc. Bras. Med. Trop. 47, 796–797. https://doi.org/10.1590/0037-8682-0041-2014.

Braga, A.R.C., Langoni, H., Lucheis, S.B., 2014b. Evaluation of canine and feline leishmaniasis by the association of blood culture, immunofluorescent antibody test and polymerase chain reaction. https://doi.org/10.1186/1678-9199-20-5.

Bresciani, K.D.S., Serrano, A.C.M., Matos, L.V.S. de, Savani, E.S.M.M., D'Auria, S.R.N., Perri, S.H. V., et al., 2010. Ocorrência de *Leishmania* spp. em felinos do município de Araçatuba, SP. Rev. Bras. Parasitol. Veterinária 19, 127–129. https://doi.org/10.4322/rbpv.01902012.

Brianti, E., Celi, N., Napoli, E., Abbate, J.M., Arfuso, F., Gaglio, G., et al., 2019. Treatment and long-term follow-up of a cat with leishmaniosis. Parasit. Vectors 12, 121. https://doi.org/10.1186/s13071-019-3388-9.

Brianti, E., Falsone, L., Napoli, E., Gaglio, G., Giannetto, S., Pennisi, M.G., et al., 2017. Prevention of feline leishmaniosis with an imidacloprid 10%/flumethrin 4.5% polymer matrix collar. Parasit. Vectors 10. https://doi.org/10.1186/s13071-017-2258-6.

BSAVA, 2020. Small animal formulary, Part A: canine and feline, 10th ed. British Small Animal Veterinary Association, Gloucester.

Can, H., Döşkaya, M., Özdemir, H.G., Şahar, E.A., Karakavuk, M., Pektaş, B., et al., 2016. Seroprevalence of *Leishmania* infection and molecular detection of *Leishmania tropica* and *Leishmania infantum* in stray cats of İzmir, Turkey. Exp. Parasitol. 167, 109–114. https://doi.org/10.1016/j.exppara.2016.05.011.

Cardia, D.F.F., Camossi, L.G., Neto, L. da S., Langoni, H., Bresciani, K.D.S., 2013. Prevalence of *Toxoplasma gondii* and *Leishmania* spp. infection in cats from Brazil. Vet. Parasitol. 197, 634–637. https://doi.org/10.1016/j.vetpar.2013.07.017.

Cardoso, L., Lopes, A.P., Sherry, K., Schallig, H., Solano-Gallego, L., 2010. Low seroprevalence of *Leishmania infantum* infection in cats from northern Portugal based on DAT and ELISA. Vet. Parasitol. 174, 37–42. https://doi.org/10.1016/j.vetpar.2010.08.022.

Carneiro, L.A., Vasconcelos dos Santos, T., do Rêgo Lima, L.V. do R., Ramos, P.K.S., Campos, M.B., Silveira, F.T., 2020. First report on feline leishmaniasis caused by *Leishmania* (*Leishmania*) *amazonensis* in Amazonian Brazil. Vet. Parasitol. Reg. Stud. Reports 19, 100360. https://doi.org/10.1016/j.vprsr.2019.100360.

Chatzis, M.K., Andreadou, M., Leontides, L., Kasabalis, D., Mylonakis, M., Koutinas, A.F., et al., 2014a. Cytological and molecular detection of *Leishmania infantum* in different tissues of clinically normal and sick cats. Vet. Parasitol. 202, 217–225. https://doi.org/10.1016/j.vetpar.2014.02.044.

Chatzis, M.K., Leontides, L., Athanasiou, L. V., Papadopoulos, E., Kasabalis, D., Mylonakis, M., et al., 2014b. Evaluation of indirect immunofluorescence antibody test and enzyme-linked immunosorbent assay for the diagnosis of infection by *Leishmania infantum* in clinically normal and sick cats. Exp. Parasitol. 147, 54–59.

https://doi.org/10.1016/j.exppara.2014.10.004.

Chatzis, M.K., Xenoulis, P.G., Leontides, L., Kasabalis, D., Mylonakis, M.E., Andreadou, M., et al., 2020. Evaluation of clinicopathological abnormalities in sick cats naturally infected by *Leishmania infantum*. Heliyon 6, e05177. https://doi.org/10.1016/j.heliyon.2020.e05177.

Coelho, W.M.D., de Lima, V.M.F., do Amarante, A.F.T., Langoni, H., Pereira, V.B.R., Abdelnour, A., Bresciani, K.D.S., 2010. Occurrence of *Leishmania* (*Leishmania*) *chagasi* in a domestic cat (*Felis catus*) in Andradina, São Paulo, Brazil: case report. Rev. Bras. Parasitol. Vet. 19, 256–258. https://doi.org/10.1590/s1984-29612010000400013.

Coelho, W.M.D., Do Amarante, A.F.T., De Carvalho Apolinário, J., Coelho, N.M.D., De Lima, V.M.F., Perri, S.H.V., Bresciani, K.D.S., 2011a. Seroepidemiology of *Toxoplasma gondii*, *Neospora caninum*, and *Leishmania* spp. infections and risk factors for cats from Brazil. Parasitol. Res. 109, 1009–1013. https://doi.org/10.1007/s00436-011-2461-x.

Coelho, W.M.D., Richini-Pereira, V.B., Langoni, H., Bresciani, K.D.S., 2011b. Molecular detection of *Leishmania* sp. in cats (*Felis catus*) from Andradina Municipality, São Paulo State, Brazil. Vet. Parasitol. 176, 281–282. https://doi.org/10.1016/j.vetpar.2010.10.052.

Colella, V., Hodžić, A., Iatta, R., Baneth, G., Alić, A., Otranto, D., 2019. Zoonotic leishmaniasis, Bosnia and Herzegovina. Emerg. Infect. Dis. https://doi.org/10.3201/eid2502.181481.

Coura, F.M., Passos, S.K.P., Pelegrino, M. de O.F., Leme, F. de O.P., Paz, G.F., Gontijo, C.M.F., da Costa-Val, A.P., 2018. Serological, molecular, and microscopic detection of leishmania in cats (*Felis catus*) in Belo Horizonte, Minas Gerais State, Brazil. Rev. Bras. Parasitol. Vet. 27, 570–574. https://doi.org/10.1590/s1984-296120180052.

Craig, M.T., Barton, C.L., Mercer, S.H., Droleskey, B.E., Jones, P., 1986. Dermal leishmaniasis in a Texas cat. Am. J. Trop. Med. Hyg. 35, 1100–1102. https://doi.org/10.4269/ajtmh.1986.35.1100.

da Costa-Val, A.P., Coura, F.M., Barbieri, J. de M., Diniz, L., Sampaio, A., Dos Reis, J.K.P., et al., 2020. Serological study of feline leishmaniasis and molecular detection of *Leishmania infantum* and *Leishmania braziliensis* in cats (*Felis catus*). Rev. Bras. Parasitol. Vet. 29, 1–12. https://doi.org/10.1590/s1984-29612020023.

da Silva, A.V.M., de Souza Cândido, C.D., de Pita Pereira, D., Brazil, R.P., Carreira, J.C.A., 2008. The first record of American visceral leishmaniasis in domestic cats from Rio de Janeiro, Brazil. Acta Trop. 105, 92–94. https://doi.org/10.1016/j.actatropica.2007.09.001.

da Silva, S.M., Rabelo, P.F.B., Gontijo, N. de F., Ribeiro, R.R., Melo, M.N., Ribeiro, V.M., Michalick, M.S.M., 2010. First report of infection of *Lutzomyia longipalpis* by *Leishmania* (*Leishmania*) *infantum* from a naturally infected cat of Brazil. Vet. Parasitol.

174, 150–154. https://doi.org/10.1016/j.vetpar.2010.08.005.

da Silveira Neto, L., Sobrinho, L.S.V., Martins, C.O., Machado, R.Z., Marcondes, M., De Lima, V.M.F., 2011. Use of crude, FML and rK39 antigens in ELISA to detect anti-*Leishmania* spp. antibodies in *Felis catus*. Vet. Parasitol. 177, 374–377. https://doi.org/10.1016/j.vetpar.2010.11.055.

De Matos, A.M.R.N., Caldart, E.T., Ferreira, F.P., Monteiro, K.C., De Souza, M., Brunieri, D.T.S.C., et al., 2018. Antibodies anti-trypanosomatides in domestic cats in Paraná: who is at highest risk of infection? Rev. Bras. Parasitol. Vet. 27, 232–236. https://doi.org/10.1590/S1984-296120180033.

de Morais, R.C.S., Gonçalves, S. da C., Costa, P.L., da Silva, K.G., da Silva, F.J., Silva, R.P.E., et al., 2013. Detection of *Leishmania infantum* in animals and their ectoparasites by conventional PCR and real time PCR. Exp. Appl. Acarol. 59, 473–481. https://doi.org/10.1007/s10493-012-9611-4.

de Sousa, K.C.M., Herrera, H.M., Domingos, I.H., Campos, J.B.V., dos Santos, I.M.C., Neves, H.H., et al., 2014. Detecção sorológica de *Toxoplasma gondii, Leishmania infantum* e *Neospora caninum* em gatos de uma área endêmica para leishmaniose no Brasil. Rev. Bras. Parasitol. Vet. 23, 449–455. https://doi.org/10.1590/S1984-29612014078.

De Sousa Oliveira, T.M.F., vanessa Pereira, F., Benvenga, G.U., Martin, M.F.A., Benassi, J.C., Da Silva, D.T., Starke-Buzetti, W.A., 2015. Conjunctival swab PCR to detect *Leishmania* spp. in cats. Rev. Bras. Parasitol. Vet. 24, 220–222. https://doi.org/10.1590/S1984-29612015016.

De Souza, A.I., Barros, E.M.S., Ishikawa, E., Novaes Ilha, I.M., Barbosa Marin, G.R., Brandão Nunes, V.L., 2005. Feline leishmaniasis due to *Leishmania* (*Leishmania*) *amazonensis* in Mato Grosso do Sul State, Brazil. Vet. Parasitol. 128, 41–45. https://doi.org/10.1016/j.vetpar.2004.11.020.

Dedola, C., Zobba, R., Varcasia, A., Visco, S., Alberti, A., Pipia, A.P., et al., 2018. Serological and molecular detection of *Leishmania infantum* in cats of Northern Sardinia, Italy. Vet. Parasitol. Reg. Stud. Reports 13, 120–123. https://doi.org/10.1016/j.vprsr.2018.05.003.

Del Río, L., Chitimia, L., Cubas, A., Victoriano, I., De la Rúa, P., Gerrikagoitia, X., et al., 2014. Evidence for widespread *Leishmania infantum* infection among wild carnivores in *L. infantum* periendemic northern Spain. Prev. Vet. Med. 113, 430–435. https://doi.org/10.1016/j.prevetmed.2013.12.001.

Di Mattia, D., Fondevila, D., Abramo, F., Fondati, A., 2018. A retrospective histopathological, immunohistochemical and molecular study of the presence of *Leishmania* spp. in the skin of cats with head and neck ulcerative dermatitis. Vet. Dermatol. 29, 212-e76.

https://doi.org/10.1111/vde.12535.

Diakou, A., Di Cesare, A., Accettura, P.M., Barros, L., Iorio, R., Paoletti, B., et al., 2017. Intestinal parasites and vector-borne pathogens in stray and free-roaming cats living in continental and insular Greece. PLoS Negl. Trop. Dis. 11, e0005335. https://doi.org/10.1371/journal.pntd.0005335.

Diakou, A., Papadopoulos, E., Lazarides, K., 2009. Specific anti-*Leishmania* spp. antibodies in stray cats in Greece. J. Feline Med. Surg. 11, 728–730. https://doi.org/10.1016/j.jfms.2008.01.009.

Dincer, E., Karapinar, Z., Oktem, M., Ozbaba, M., Ozkul, A., Ergunay, K., 2016. Canine infections and partial s segment sequence analysis of Toscana virus in Turkey. Vector Borne Zoonotic Dis. 16, 611–618. https://doi.org/10.1089/vbz.2016.1979.

Dincer, E., Ozkul, A., Gargari, S., Ergunay, K., 2015. Potential animal reservoirs of Toscana virus and coinfections with *Leishmania infantum* in Turkey. Am. J. Trop. Med. Hyg. 92, 690–697. https://doi.org/10.4269/ajtmh.14-0322.

Dinçer, D., Arca E., Koç E., Topal, Y., Özkan, A.T., Celebi, B. 2012. A case of cutaneous leishmaniasis caused by *Leishmania infantum* in a non-endemic province (Ankara) of Turkey. Mikrobiyol. Bul. 46, 499-506.

Duarte, A., Castro, I., Pereira da Fonseca, I.M., Almeida, V., Madeira de Carvalho, L.M., Meireles, J., et al., 2010. Survey of infectious and parasitic diseases in stray cats at the Lisbon Metropolitan Area, Portugal. J. Feline Med. Surg. 12, 441–446. https://doi.org/10.1016/j.jfms.2009.11.003.

Ebani, V.V., Guardone, L., Marra, F., Altomonte, I., Nardoni, S., Mancianti, F., 2020. Arthropod-borne pathogens in stray cats from northern italy: A serological and molecular survey. Animals 10, 2334. https://doi.org/10.3390/ani10122334.

Faiman, R., Cuño, R., Warburg, A., 2009. Control of phlebotomine sand flies with vertical fine-mesh nets. J. Med. Entomol. 46, 820–831. https://doi.org/10.1603/033.046.0412.

Fatollahzadeh, M., Khanmohammadi, M., Bazmani, A., Mirsamadi, N., Jafari, R., Mohebali, M., et al., 2016. Survey of feline visceral leishmaniasis in Azarshahr area, north west of Iran, 2013. J. Parasit. Dis. 40, 683–687. https://doi.org/10.1007/s12639-014-0559-7.

Fernandez-Gallego, A., Feo Bernabe, L., Dalmau, A., Esteban-Saltiveri, D., Font, A., Leiva, M., et al., 2020. Feline leishmaniosis: diagnosis, treatment and outcome in 16 cats. J. Feline Med. Surg. 22, 993–1007. https://doi.org/10.1177/1098612X20902865.

Figueiredo, F.B., Bonna, I.C.F., Nascimento, L.D., Da Costa, T., Baptista, C., Pacheco, T.M.V., et al., 2009. Avaliação sorológica para detecção de anticorpos anti-*Leishmania* em cães e gatos no bairro de Santa Rita de Cássia, Município de Barra Mansa, Estado do Rio de

Janeiro. Rev. Soc. Bras. Med. Trop. 42, 141–145. https://doi.org/10.1590/S0037-86822009000200009.

Galluzzi, L., Ceccarelli, M., Diotallevi, A., Menotta, M., Magnani, M., 2018. Real-time PCR applications for diagnosis of leishmaniasis. Parasit. Vectors, 11, 273. https://doi.org/10.1186/s13071-018-2859-8.

GfK, 2016. Pet ownership. URL https://cdn2.hubspot.net/hubfs/2405078/cms-pdfs/fileadmin/user_upload/country_one_pager/nl/documents/global-gfk-survey_pet-ownership_2016.pdf.

Grevot, A., Jaussaud Hugues, P., Marty, P., Pratlong, F., Ozon, C., Haas, P., et al., 2005. Leishmaniosis due to *Leishmania infantum* in a FIV and FelV positive cat with a squamous cell carcinoma diagnosed with histological, serological and isoenzymatic methods. Parasite 12, 271–275. https://doi.org/10.1051/parasite/2005123271.

Hatam, G.R., Adnani, S.J., Asgari, Q., Fallah, E., Motazedian, M.H., Sadjjadi, S.M., Sarkari, B., 2010. First report of natural infection in cats with *Leishmania infantum* in Iran. Vector Borne Zoonotic Dis. 10, 313–316. https://doi.org/10.1089/vbz.2009.0023.

Headley, S.A., Pimentel, L.A., de Amorim, I.F.G., Amude, A.M., Viana, N.E., Muraro, L.S., et al., 2019. Immunohistochemical characterization of cutaneous leishmaniasis in cats from Central-west Brazil. Vet. Parasitol. Reg. Stud. Reports 17, 100290. https://doi.org/10.1016/j.vprsr.2019.100290.

Hervás, J., Chacón-M De Lara, F., Sdnchez-lsarria, M.A., Pellicer, S., Carrasco, L., Castillo, J.A., et al., 1999. Two cases of feline visceral and cutaneous leishmaniosis in Spain. J Feline Med. Surg. 1, 101–105. doi: 10.1016/S1098-612X(99)90066-9.

Hervás, J., Chacón-Manrique De Lara, F., López, J., Gómez-Villamandos, J.C., Guerrero, M.J., Moreno, A., 2001. Granulomatous (pseudotumoral) iridociclitis associated with leishmaniasis in a cat. Vet. Rec. 149, 624–625. https://doi.org/10.1136/vr.149.20.624.

Iatta, R., Furlanello, T., Colella, V., Tarallo, V.D., Latrofa, M.S., Brianti, E., et al., 2019. A nationwide survey of *Leishmania infantum* infection in cats and associated risk factors in Italy. PLoS Negl. Trop. Dis. 13. https://doi.org/10.1371/journal.pntd.0007594.

Iatta, R., Trerotoli, P., Lucchese, L., Natale, A., Buonavoglia, C., Nachum-Biala, Y., et al., 2020. Validation of a new immunofluorescence antibody test for the detection of *Leishmania infantum* infection in cats. Parasitol. Res. 119, 1381–1386. https://doi.org/10.1007/s00436-020-06627-1.

Junsiri, W., Wongnarkpet, S., Chimnoi, W., Kengradomkij, C., Kajeerum, W., Pangjai, D., Nimsuphan, B., 2017. Seroprevalence of *Leishmania* infection in domestic animals in Songkhla and Satun provinces, southern Thailand. Trop. Biomed. 34, 352–362.

Karakuş, M., Arserim, S.K., Erişöz Kasap, Ö., Pekağırbaş, M., Aküzüm, D., Alten, B., et al., 2019. Vector and reservoir surveillance study in a canine and human leishmaniasis endemic area in most western part of Turkey, Karaburun. Acta Trop. 190, 177–182. https://doi.org/10.1016/j.actatropica.2018.11.020.

Kirkpatrick, C.E., Farrell, J.P., Goldschmidt, M.H., 1984. *Leishmania chagasi* and *L. donovani*: experimental infections in domestic cats. Exp. Parasitol. 58, 125–131. https://doi.org/10.1016/0014-4894(84)90027-4.

Kongkaew, W., Siriarayaporn, P., Leelayoova, S., Supparatpinyo, K., Areechokchai, D., Duang-ngern, P., et al., 2007. Autochthonous visceral leishmaniasis: a report of a second case in Thailand. Southeast Asian J. Trop. Med. Public Health 38, 8–12.

Kovalenko, D.A., Nasyrova, R.M., Ponomareva, V.I., Fatullaeva, A.A., Razakov, S.A., Ponirovskiĭ, E.N., et al., 2011. Human and canine visceral leishmaniasis in the Papsky District, Namangan Region, Uzbekistan: seroepidemiological and seroepizootological surveys. Med. Parazitol. (Mosk.) 3, 32-37.

Latrofa, M.S., Iatta, R., Toniolo, F., Furlanello, T., Ravagnan, S., Capelli, G., et al., 2020. A molecular survey of vector-borne pathogens and haemoplasmas in owned cats across Italy. Parasit. Vectors 13, 116. https://doi.org/10.1186/s13071-020-3990-x.

Leal, R.O., Pereira, H., Cartaxeiro, C., Delgado, E., Peleteiro, M. da C., Pereira da Fonseca, I., 2018. Granulomatous rhinitis secondary to feline leishmaniosis: report of an unusual presentation and therapeutic complications. JFMS Open Reports 4, 2055116918811374. https://doi.org/10.1177/2055116918811374.

Leiva, M., Lloret, A., Peña, T., Roura, X., 2005. Therapy of ocular and visceral leishmaniasis in a cat. Vet. Ophthalmol. 8, 71–75. https://doi.org/10.1111/j.1463-5224.2005.00342.x.

Leonel, J.A.F., Vioti, G., Alves, M.L., Benassi, J.C., Silva, D.T. da, Spada, J.C.P., et al., 2020. Leishmaniasis in cat shelters: A serological, molecular and entomological study. Transbound. Emerg. Dis. 67, 2013–2019. https://doi.org/10.1111/tbed.13544.

Lima, C., Colella, V., Latrofa, M.S., Cardoso, L., Otranto, D., Alho, A.M., 2019. Molecular detection of *Leishmania* spp. in dogs and a cat from Doha, Qatar. Parasit. Vectors 12, 125. https://doi.org/10.1186/s13071-019-3394-y.

Longoni, S.S., López-Cespedes, A., Sánchez-Moreno, M., Bolio-Gonzalez, M.E., Sauri-Arceo, C.H., Rodríguez-Vivas, R.I., Marín, C., 2012. Detection of different *Leishmania* spp. and *Trypanosoma cruzi* antibodies in cats from the Yucatan Peninsula (Mexico) using an iron superoxide dismutase excreted as antigen. Comp. Immunol. Microbiol. Infect. Dis. 35, 469–476. https://doi.org/10.1016/j.cimid.2012.04.003.

Lopes, A.P., Oliveira, A.C., Granada, S., Rodrigues, F.T., Papadopoulos, E., Schallig, H., et al.,

2017. Antibodies to *Toxoplasma gondii* and *Leishmania* spp. in domestic cats from Luanda, Angola. Vet. Parasitol. 239, 15–18. https://doi.org/10.1016/j.vetpar.2017.04.009.

Maia, C., Campino, L., 2018. Biomarkers associated with *Leishmania infantum* exposure, infection, and disease in dogs. Front. Cell. Infect. Microbiol. 8, 302. https://doi.org/10.3389/fcimb.2018.00302.

Maia, C., Cardoso, L., 2015. Spread of *Leishmania infantum* in Europe with dog travelling. Vet. Parasitol. 213, 2–11. https://doi.org/10.1016/j.vetpar.2015.05.003.

Maia, C., Gomes, J., Cristóvão, J., Nunes, M., Martins, A., Rebêlo, E., Campino, L., 2010. Feline *Leishmania* infection in a canine leishmaniasis endemic region, Portugal. Vet. Parasitol. 174, 336–40. https://doi.org/10.1016/j.vetpar.2010.08.030.

Maia, C., Nunes, M., Campino, L., 2008. Importance of cats in zoonotic leishmaniasis in Portugal. Vector Borne Zoonotic Dis. 8, 555–560. https://doi.org/10.1089/vbz.2007.0247.

Maia, C., Ramos, C., Coimbra, M., Bastos, F., Martins, Â., Pinto, P., et al., 2014. Bacterial and protozoal agents of feline vector-borne diseases in domestic and stray cats from southern Portugal. Parasit. Vectors 7, 115. https://doi.org/10.1186/1756-3305-7-115.

Maia, C., Ramos, C., Coimbra, M., Cardoso, L., Campino, L., 2015a. Prevalence of *Dirofilaria immitis* antigen and antibodies to *Leishmania infantum* in cats from southern Portugal. Parasitol. Int. 64, 154–6. https://doi.org/10.1016/j.parint.2014.11.006.

Maia, C., Sousa, C., Ramos, C., Cristóvão, J.M., Faísca, P., Campino, L., 2015b. First case of feline leishmaniosis caused by *Leishmania infantum* genotype E in a cat with a concurrent nasal squamous cell carcinoma. JFMS Open Reports 1, 2055116915593969. https://doi.org/10.1177/2055116915593969.

Marcondes, M., Hirata, K.Y., Vides, J.P., Sobrinho, L.S.V., Azevedo, J.S., Vieira, T.S.W.J., Vieira, R.F.C., 2018. Infection by *Mycoplasma* spp., feline immunodeficiency virus and feline leukemia virus in cats from an area endemic for visceral leishmaniasis. Parasit. Vectors 11. https://doi.org/10.1186/s13071-018-2716-9.

Marcos, R., Santos, M., Malhaõ, F., Pereira, R., Fernandes, A.C., Montenegro, L., Roccabianca, P., 2009. Pancytopenia in a cat with visceral leishmaniasis. Vet. Clin. Pathol. 38, 201–205. https://doi.org/10.1111/j.1939-165X.2009.00111.x.

Marenzoni, M.L., Lauzi, S., Miglio, A., Coletti, M., Arbia, A., Paltrinieri, S., Antognoni, M.T., 2018. Comparison of three blood transfusion guidelines applied to 31 feline donors to minimise the risk of transfusion-transmissible infections. J. Feline Med. Surg. 20, 663–673. https://doi.org/10.1177/1098612X17727233.

Maroli, M., Gradoni, L., Oliva, G., Castagnaro, M., Crotti, A., Lubas, G., et al., 2010. Guidelines for prevention of leishmaniasis in dogs. J. Am. Vet. Med. Assoc. 236, 1200–1206.

https://doi.org/10.2460/javma.236.11.1200.

Maroli, M., Pennisi, M.G., Di Muccio, T., Khoury, C., Gradoni, L., Gramiccia, M., 2007. Infection of sandflies by a cat naturally infected with *Leishmania infantum*. Vet. Parasitol. 145, 357–360. https://doi.org/10.1016/j.vetpar.2006.11.009.

Martín-Sánchez, J., Acedo, C., Muñoz-Pérez, M., Pesson, B., Marchal, O., Morillas-Márquez, F., 2007. Infection by *Leishmania infantum* in cats: Epidemiological study in Spain. Vet. Parasitol. 145, 267–273. https://doi.org/10.1016/j.vetpar.2006.11.005.

Mccown, M., Grzeszak, B., 2010. Zoonotic and infectious disease surveillance in central america: honduran feral cats positive for *Toxoplasma*, *Trypanosoma*, *Leishmania*, *Rickettsia*, and Lyme disease. J. Spec. Oper. Med. 10, 41–43.

Mendonça, I.L. de, Batista, J.F., Lopes, K.S.P. do P., Magalhães Neto, F. das C.R., Alcântara, D.S., Merigueti, Y.F.F.B., Costa, C.H.N., 2020. Infection of *Lutzomyia longipalpis* in cats infected with *Leishmania infantum*. Vet. Parasitol. 280, 109058. https://doi.org/10.1016/j.vetpar.2020.109058.

Mesa-Sanchez, I., Ferreira, R.R.F., Cardoso, I., Morais, M., Flamínio, M., Vieira, S., et al., 2020. Transfusion transmissible pathogens are prevalent in healthy cats eligible to become blood donors. J. Small Anim. Pract. https://doi.org/10.1111/jsap.13257.

Metzdorf, I.P., da Costa Lima, M.S., de Fatima Cepa Matos, M., de Souza Filho, A.F., de Souza Tsujisaki, R.A., Franco, K.G., et al., 2017. Molecular characterization of *Leishmania infantum* in domestic cats in a region of Brazil endemic for human and canine visceral leishmaniasis. Acta Trop. 166, 121–125. https://doi.org/10.1016/j.actatropica.2016.11.013.

Michael, S.A., Morsy, T.A., El-Seoud, A.F., Saleh, M.S., 1982. Leishmaniasis antibodies in stray cats in Ismailiya Governorate, Egypt. J. Egypt. Soc. Parasitol. 12, 283–286.

Miglianico, M., Eldering, M., Slater, H., Ferguson, N., Ambrose, P., Lees, R.S., et al., 2018. Repurposing isoxazoline veterinary drugs for control of vector-borne human diseases. Proc. Natl. Acad. Sci. USA 115, E6920–E6926. https://doi.org/10.1073/pnas.1801338115.

Migliazzo, A., Vitale, F., Calderone, S., Puleio, R., Binanti, D., Abramo, F., 2015. Feline leishmaniosis: a case with a high parasitic burden. Vet. Dermatol. 26, 69–70. https://doi.org/10.1111/vde.12180.

Millán, J., Zanet, S., Gomis, M., Trisciuoglio, A., Negre, N., Ferroglio, E., 2011. An Investigation into alternative reservoirs of canine leishmaniasis on the endemic Island of Mallorca (Spain). Transbound. Emerg. Dis. 58, 352–357. https://doi.org/10.1111/j.1865-1682.2011.01212.x.

Minard, H.M., Daniel, A.K., Pool, R.R., Snowden, K.F., Levine, G.J., 2017. Pathology in practice. J. Am. Vet. Med. Assoc. 251, 57–59. https://doi.org/10.2460/javma.251.1.57.

Miró, G., Hernández, L., Montoya, A., Arranz-Solís, D., Dado, D., Rojo-Montejo, S., Mendoza-Ibarra, J.A., et al., 2011. First description of naturally acquired *Tritrichomonas foetus* infection in a Persian cattery in Spain. Parasitol. Res. 109, 1151–1154. https://doi.org/10.1007/s00436-011-2359-7.

Miró, G., Rupérez, C., Checa, R., Gálvez, R., Hernández, L., García, M., et al., 2014. Current status of *L. infantum* infection in stray cats in the Madrid region (Spain): Implications for the recent outbreak of human leishmaniosis? Parasit. Vectors 7, 112. https://doi.org/10.1186/1756-3305-7-112.

Mohebali, M., Malmasi, A., Khodabakhsh, M., Zarei, Z., Akhoundi, B., Hajjaran, H., Azarm, A., 2017. Feline leishmaniosis due to *Leishmania infantum* in Northwest Iran: the role of cats in endemic areas of visceral leishmaniosis. Vet. Parasitol. Reg. Stud. Reports 9, 13–16. https://doi.org/10.1016/j.vprsr.2017.03.010.

Montoya, A., García, M., Gálvez, R., Checa, R., Marino, V., Sarquis, J., et al., 2018a. Implications of zoonotic and vector-borne parasites to free-roaming cats in central Spain. Vet. Parasitol. 251, 125–130. https://doi.org/10.1016/j.vetpar.2018.01.009.

Montoya, A., Miró, G., Saugar, J.M., Fernández, B., Checa, R., Gálvez, R., et al., 2018b. Detection and molecular characterization of *Acanthamoeba* spp. in stray cats from Madrid, Spain. Exp. Parasitol. 188, 8–12. https://doi.org/10.1016/j.exppara.2018.02.011.

Morelli, S., Colombo, M., Dimzas, D., Barlaam, A., Traversa, D., Di Cesare, A., et al., 2020. *Leishmania infantum* seroprevalence in cats from touristic areas of Italy and Greece. Front. Vet. Sci. 7, 616566. https://doi.org/10.3389/fvets.2020.616566.

Morelli, S., Crisi, P.E., Di Cesare, A., De Santis, F., Barlaam, A., Santoprete, G., et al., 2019. Exposure of client-owned cats to zoonotic vector-borne pathogens: clinic-pathological alterations and infection risk analysis. Comp. Immunol. Microbiol. Infect. Dis. 66, 101344. https://doi.org/10.1016/j.cimid.2019.101344.

Moreno, I., Álvarez, J., García, N., de la Fuente, S., Martínez, I., Marino, E., et al., 2014. Detection of anti-*Leishmania* infantum antibodies in sylvatic lagomorphs from an epidemic area of Madrid using the indirect immunofluorescence antibody test. Vet. Parasitol. 199, 264–267. https://doi.org/10.1016/j.vetpar.2013.10.010.

Morganti, G., Veronesi, F., Stefanetti, V., Di Muccio, T., Fiorentino, E., Diaferia, M., et al., 2019. Emerging feline vector-borne pathogens in Italy. Parasit. Vectors 12, 193. https://doi.org/10.1186/s13071-019-3409-8.

Morsy, T., Aldakhil, M.A., el-Bahrawy, A., 1999. Natural *Leishmania* infection in sand cats

captured in Riyadh district, Saudi Arabia. J. Egypt. Soc. Parasitol. 29, 69–74.

Morsy, T.A., Abou el Seoud, S.M., 1994. Natural infection in two pet cats in a house of a zoonotic cutaneous leishmaniasis patient in Imbaba area, Giza Governorate, Egypt. J. Egypt. Soc. Parasitol. 24, 199–204.

Morsy, T.A., Michael, S.A., Makhlouf, M., Sibai, M., 1988. *Leishmania* infection sought in non human hosts in Suez Governorate, Egypt. J. Egypt. Soc. Parasitol. 18, 539–545.

Nasereddin, A., Salant, H., Abdeen, Z., 2008. Feline leishmaniasis in Jerusalem: serological investigation. Vet. Parasitol. 158, 364–369. https://doi.org/10.1016/j.vetpar.2008.09.022.

Naucke, T.J., Lorentz, S., 2012. First report of venereal and vertical transmission of canine leishmaniosis from naturally infected dogs in Germany. Parasit. Vectors 5, 67. https://doi.org/10.1186/1756-3305-5-67.

Navarro, J.A., Sánchez, J., Peñafiel-Verdú, C., Buendía, A.J., Altimira, J., Vilafranca, M., 2010. Histopathological lesions in 15 cats with leishmaniosis. J. Comp. Pathol. 143, 297–302. https://doi.org/10.1016/j.jcpa.2010.03.003.

Neves, M., Lopes, A.P., Martins, C., Fino, R., Paixão, C., Damil, L., et al., 2020. Survey of *Dirofilaria immitis* antigen and antibodies to *Leishmania infantum* and *Toxoplasma gondii* in cats from Madeira Island, Portugal. Parasit. Vectors 13, 117. https://doi.org/10.1186/s13071-020-3988-4.

OIE, 2018. Leishmaniosis. In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. pp. 491–502. https://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.01.11_LEISHMANIOSIS.pdf.

Oliveira, G.C., Paiz, L.M., Menozzi, B.D., Lima, M. de S., de Moraes, C.C.G., Langoni, H., 2015. Antibodies to *Leishmania* spp. in domestic felines. Rev. Bras. Parasitol. Vet. 24, 464–470. https://doi.org/10.1590/S1984-29612015071.

Otranto, D., Iatta, R., Baneth, G., Cavalera, M.A., Bianco, A., Parisi, A., et al., 2019. High prevalence of vector-borne pathogens in domestic and wild carnivores in Iraq. Acta Trop. 197, 105058. https://doi.org/10.1016/j.actatropica.2019.105058.

Otranto, D., Napoli, E., Latrofa, M.S., Annoscia, G., Tarallo, V.D., Greco, G., et al., 2017. Feline and canine leishmaniosis and other vector-borne diseases in the Aeolian Islands: pathogen and vector circulation in a confined environment. Vet. Parasitol. 236, 144–151. https://doi.org/10.1016/j.vetpar.2017.01.019.

Owens, S.D., Oakley, D.A., Marryott, K., Hatchett, W., Walton, R., Nolan, T.J., et al., 2001. Transmission of visceral leishmaniasis through blood transfusions from infected English Foxhounds to anemic dogs. J. Am. Vet. Med. Assoc. 219, 1076–1083.

https://doi.org/10.2460/javma.2001.219.1076.

Ozon, C., Marty, P., Pratlong, F., Breton, C., Blein, M., Lelièvre, A., Haas, P., 1998. Disseminated feline leishmaniosis due to *Leishmania infantum* in southern France. Vet. Parasitol. 75, 273–277. https://doi.org/10.1016/S0304-4017(97)00174-X.

Paltrinieri, S., Gradoni, L., Roura, X., Zatelli, A., Zini, E., 2016. Laboratory tests for diagnosing and monitoring canine leishmaniasis. Vet. Clin. Pathol. 45, 552–578. https://doi.org/10.1111/vcp.12413.

Paniz Mondolfi, A.E., Colmenares Garmendia, A., Mendoza Pérez, Y., Hernández-Pereira, C.E., Medina, C., Vargas, F., et al., 2019. Autochthonous cutaneous leishmaniasis in urban domestic animals (*Felis catus* / *Canis lupus familiaris*) from central-western Venezuela. Acta Trop. 191, 252–260. https://doi.org/10.1016/j.actatropica.2019.01.006.

Paşa, S., Tetik Vardarlı, A., Erol, N., Karakuş, M., Töz, S., Atasoy, A., et al., 2015. Detection of *Leishmania major* and *Leishmania tropica* in domestic cats in the Ege Region of Turkey. Vet. Parasitol. 212, 389–392. https://doi.org/10.1016/j.vetpar.2015.07.042.

Pedrassani, D., Biolchi, J., Gonçalves, L.R., Mendes, N.S., Zanatto, D.C. de S., Calchi, A.C., et al., 2019. Molecular detection of vector-borne agents in cats in southern Brazil. Rev. Bras. Parasitol. Vet. 28, 632–643. https://doi.org/10.1590/s1984-29612019077.

Pennisi, M.G., Persichetti, M.F., 2018. Feline leishmaniosis: Is the cat a small dog? Vet. Parasitol. 251, 131–137. https://doi.org/10.1016/J.VETPAR.2018.01.012.

Pennisi, M.G., Venza, M., Reale, S., Vitale, F., Lo Giudice, S., 2004. Case report of leishmaniasis in four cats, Vet. Res. Commun. 28, 363–366.

Pereira, A., Ayhan, N., Cristóvão, J.M.J.M., Vilhena, H., Martins, Â., Cachola, P., et al., 2019a. Antibody response to Toscana virus and sandfly fever Sicilian virus in cats naturally exposed to phlebotomine sand fly bites in Portugal. Microorganisms 7. https://doi.org/10.3390/microorganisms7090339.

Pereira, A., Cristóvão, J.M.J.M.J.M., Vilhena, H., Martins, Â., Cachola, P., Henriques, J., et al., 2019b. Antibody response to *Phlebotomus perniciosus* saliva in cats naturally exposed to phlebotomine sand flies is positively associated with *Leishmania* infection. Parasit. Vectors 12, 128. https://doi.org/10.1186/s13071-019-3376-0.

Pereira, A., Parreira, R., Cristóvão, J.M., Castelli, G., Bruno, F., Vitale, F., Campino, L., Maia, C., 2020. Phylogenetic insights on *Leishmania* detected in cats as revealed by nucleotide sequence analysis of multiple genetic markers. Infect. Genet. Evol. 77, 104069. https://doi.org/10.1016/j.meegid.2019.104069.

Pereira, A., Valente, J., Parreira, R., Cristovão, J.M.J.M., Azinheira, S., Campino, L., Maia, C., 2019c. An unusual case of feline leishmaniosis with involvement of the mammary

glands. Top. Companion Anim. Med. 37, 2017–2020. https://doi.org/10.1016/j.tcam.2019.100356.

Persichetti, M.-F., Solano-Gallego, L., Serrano, L., Altet, L., Reale, S., Masucci, M., Pennisi, M.-G., 2016. Detection of vector-borne pathogens in cats and their ectoparasites in southern Italy. Parasit. Vectors 9, 247. https://doi.org/10.1186/s13071-016-1534-1.

Persichetti, M.F., Pennisi, M.G., Vullo, A., Masucci, M., Migliazzo, A., Solano-Gallego, L., 2018. Clinical evaluation of outdoor cats exposed to ectoparasites and associated risk for vector-borne infections in southern Italy. Parasit. Vectors 11, 136. https://doi.org/10.1186/s13071-018-2725-8.

Persichetti, M.F., Solano-Gallego, L., Vullo, A., Masucci, M., Marty, P., Delaunay, P., et al., 2017. Diagnostic performance of ELISA, IFAT and Western blot for the detection of anti-*Leishmania infantum* antibodies in cats using a Bayesian analysis without a gold standard. Parasit. Vectors 10. https://doi.org/10.1186/s13071-017-2046-3.

Pimenta, P., Alves-Pimenta, S., Barros, J., Barbosa, P., Rodrigues, A., Pereira, M.J., et al., 2015. Feline leishmaniosis in Portugal: 3 cases (year 2014). Vet. Parasitol. Reg. Stud. Reports 1–2, 65–69. https://doi.org/10.1016/j.vprsr.2016.02.003.

Pocholle, E., Reyes-Gomez, E., Giacomo, A., Delaunay, P., Hasseine, L., Marty, P., 2012. A case of feline leishmaniasis in the south of France. Parasite 19, 77–80. https://doi.org/10.1051/parasite/2012191077.

Poli, A., Abramo, F., Barsotti, P., Leva, S., Gramiccia, M., Ludovisi, A., Mancianti, F., 2002. Feline leishmaniosis due to *Leishmania infantum* in Italy. Vet. Parasitol. 106, 181–191. https://doi.org/10.1016/S0304-4017(02)00081-X.

Pratlong, F., Rioux, J.A., Marry, P., Faraut-Gambarelli, F., Dereure, J., Lanotte, G., Dedet, J.P., 2004. Isoenzymatic analysis of 712 strains of *Leishmania infantum* in the south of France and relationship of enzymatic polymorphism to clinical and epidemiological features. J. Clin. Microbiol. 42, 4077–4082. https://doi.org/10.1128/JCM.42.9.4077-4082.2004.

Priolo, V., Martínez-Orellana, P., Pennisi, M.G., Masucci, M., Prandi, D., Ippolito, D., et al., 2019. *Leishmania infantum*-specific IFN-γ production in stimulated blood from cats living in areas where canine leishmaniosis is endemic. Parasit. Vectors 12, 133. https://doi.org/10.1186/s13071-019-3386-y.

Queiroga, T.B.D., Ferreira, H.R.P., dos Santos, W.V., de Assis, A.B.L., de Araújo Neto, V.T., da Câmara, A.C.J., et al., 2020. Fluralaner (Bravecto®) induces long-term mortality of *Lutzomyia longipalpis* after a blood meal in treated dogs. Parasit. Vectors 13, 609. https://doi.org/10.1186/s13071-020-04489-1.

Richter, M., Schaarschmidt-Kiener, D., Krudewig, C., 2014. Ocular signs, diagnosis and long-

term treatment with allopurinol in a cat with leishmaniasis. Schweiz. Arch. Tierheilkd. 156, 289–294. https://doi.org/10.1024/0036-7281/a000593.

Risueño, J., Ortuño, M., Pérez-Cutillas, P., Goyena, E., Maia, C., Cortes, S., et al., 2018. Epidemiological and genetic studies suggest a common *Leishmania infantum* transmission cycle in wildlife, dogs and humans associated to vector abundance in Southeast Spain. Vet. Parasitol. 259, 61–67. https://doi.org/10.1016/j.vetpar.2018.05.012.

Rivas, A.K., Alcover, M., Martínez-Orellana, P., Montserrat-Sangrà, S., Nachum-Biala, Y., Bardagí, M., et al., 2018. Clinical and diagnostic aspects of feline cutaneous leishmaniosis in Venezuela. Parasit. Vectors 11. https://doi.org/10.1186/s13071-018-2747-2.

Rocha, A.V.V.O., Moreno, B.F.S., Cabral, A.D., Louzeiro, N.M., Miranda, L.M., Santos, V.M.B. dos, et al., 2019. Diagnosis and epidemiology of *Leishmania infantum* in domestic cats in an endemic area of the Amazon region, Brazil. Vet. Parasitol. 273, 80–85. https://doi.org/10.1016/j.vetpar.2019.08.007.

Rougeron, V., Catzeflis, F., Hide, M., De Meeus, T., Bañuls, A.L., 2011. First clinical case of cutaneous leishmaniasis due to *Leishmania* (*Viannia*) *braziliensis* in a domestic cat from French Guiana. Vet. Parasitol. 181, 325–328. https://doi.org/10.1016/j.vetpar.2011.04.028.

Roura, X., Cortadellas, O., Day, M.J., Benali, S.L., D'Anna, N., Fondati, A., et al., 2021. Canine leishmaniosis and kidney disease: Q&A for an overall management in clinical practice. J. Small Anim. Pract. https://doi.org/10.1111/jsap.13237.

Rüfenacht, S., Sager, H., Müller, N., Schaerer, V., Heier, A., Welle, M.M., Roosje, P.J., 2005. Two cases of feline leishmaniosis in Switzerland. Vet. Rec. 156, 542–545. https://doi.org/10.1136/vr.156.17.542.

Sarkari, B., Hatam, G.R., Adnani, S.J., Asgari, Q., 2009. Seroprevalence of feline leishmaniasis in areas of Iran where *Leishmania infantum* is endemic. Ann. Trop. Med. Parasitol. 103, 275–277. https://doi.org/10.1179/136485909X398276.

Savani, E.S.M.M., De Oliveira Camargo, M.C.G., De Carvalho, M.R., Zampieri, R.A., Dos Santos, M.G., D'Áuria, S.R.N., et al., 2004. The first record in the Americas of an autochthonous case of *Leishmania* (*Leishmania*) *infantum chagasi* in a domestic cat (*Felix catus*) from Cotia County, São Paulo State, Brazil. Vet. Parasitol. 120, 229–233. https://doi.org/10.1016/j.vetpar.2004.01.008.

Schäfer, I., Kohn, B., Volkmann, M., Müller, E., 2021. Retrospective evaluation of vector-borne pathogens in cats living in Germany (2012–2020). Parasit. Vectors 14, 123. https://doi.org/10.1186/s13071-021-04628-2.

Schubach, T.M.P., Figueiredo, F.B., Pereira, S.A., Madeira, M.F., Santos, I.B., Andrade, M. V., et al., 2004. American cutaneous leishmaniasis in two cats from Rio de Janeiro, Brazil: First report of natural infection with *Leishmania* (*Viannia*) *braziliensis*. Trans. R. Soc. Trop. Med. Hyg. 98, 165–167. https://doi.org/10.1016/S0035-9203(03)00040-3.

Sherry, K., Miró, G., Trotta, M., Miranda, C., Montoya, A., Espinosa, C., et al., 2011. A serological and molecular study of leishmania infantum infection in cats from the island of Ibiza (Spain). Vector Borne Zoonotic Dis. 11, 239–245. https://doi.org/10.1089/vbz.2009.0251.

Silaghi, C., Knaus, M., Rapti, D., Kusi, I., Shukullari, E., Hamel, D., et al., 2014. Survey of *Toxoplasma gondii* and *Neospora caninum*, haemotropic mycoplasmas and other arthropod-borne pathogens in cats from Albania. Parasit. Vectors 7, 62. https://doi.org/10.1186/1756-3305-7-62.

Silva, R. de C.N., Ramos, R.A.N., Pimentel, D. de S., Oliveira, G.M. de A., de Carvalho, G.A., Santana, M. de A., et al., 2014. Detection of antibodies against *Leishmania infantum* in cats (*Felis catus*) from the state of Pernambuco, Brazil. Rev. Soc. Bras. Med. Trop. 47, 108–109. https://doi.org/10.1590/0037-8682-0005-2012.

Silva, R.B.S., Portela, R. de A., Arruda, L.F.B., Ferreira, J. da S., Souto, E.P.F., de Araújo, A.L., et al., 2020. Natural infection by *Leishmania infantum* in domestic cats (*Felis catus*) in a municipality of moderate transmission in the Brazilian semi-arid region. Rev. Bras. Parasitol. Vet. 29, e016620. https://doi.org/10.1590/S1984-29612020102.

Sobrinho, L.S.V., Rossi, C.N., Vides, J.P., Braga, E.T., Gomes, A.A.D., de Lima, V.M.F., et al., 2012. Coinfection of *Leishmania chagasi* with *Toxoplasma gondii*, Feline Immunodeficiency Virus (FIV) and Feline Leukemia Virus (FeLV) in cats from an endemic area of zoonotic visceral leishmaniasis. Vet. Parasitol. 187, 302–306. https://doi.org/10.1016/j.vetpar.2012.01.010.

Solano-Gallego, L., Montserrrat-Sangrà, S., Ordeix, L., Martínez-Orellana, P., 2016. *Leishmania infantum*-specific production of IFN-γ and IL-10 in stimulated blood from dogs with clinical leishmaniosis. Parasit. Vectors 9. https://doi.org/10.1186/s13071-016-1598-y.

Solano-Gallego, L., Rodríguez-Cortés, A., Iniesta, L., Quintana, J., Pastor, J., Espada, Y., et al., 2007. Cross-sectional serosurvey of feline leishmaniasis in ecoregions around the Northwestern Mediterranean. Am. J. Trop. Med. Hyg. 76, 676–680.

Spada, E., Canzi, I., Baggiani, L., Perego, R., Vitale, F., Migliazzo, A., Proverbio, D., 2016. Prevalence of *Leishmania infantum* and co-infections in stray cats in northern Italy. Comp. Immunol. Microbiol. Infect. Dis. 45, 53–58. https://doi.org/10.1016/j.cimid.2016.03.001.

Spada, E., Perego, R., Vitale, F., Bruno, F., Castelli, G., Tarantola, G., et al., 2020. Feline *Leishmania* spp. infection in a non-endemic area of Northern Italy. Animals 10, 817. https://doi.org/10.3390/ani10050817.

Spada, E., Proverbio, D., Migliazzo, A., Della Pepa, A., Perego, R., Bagnagatti De Giorgi, G., 2013. Serological and molecular evaluation of *Leishmania infantum* infection in stray cats in a nonendemic area in northern Italy . ISRN Parasitol. 2013, 916376. https://doi.org/10.5402/2013/916376.

Sukmee, T., Siripattanapipong, S., Mungthin, M., Worapong, J., Rangsin, R., Samung, Y., et al., 2008. A suspected new species of *Leishmania*, the causative agent of visceral leishmaniasis in a Thai patient. Int. J. Parasitol. 38, 617–622. https://doi.org/10.1016/j.ijpara.2007.12.003.

Tabar, M.D., Altet, L., Francino, O., Sánchez, A., Ferrer, L., Roura, X., 2008. Vector-borne infections in cats: Molecular study in Barcelona area (Spain). Vet. Parasitol. 151, 332–336. https://doi.org/10.1016/j.vetpar.2007.10.019.

Tirado, T.C., Bavia, L., Ambrosio, A.R., Campos, M.P., de Almeida Santiago, M., Messias-Reason, I.J., Figueiredo, F.B., 2021. A comparative approach on the activation of the three complement system pathways in different hosts of visceral leishmaniasis after stimulation with *Leishmania infantum*. Dev. Comp. Immunol. 120, 104061. https://doi.org/10.1016/j.dci.2021.104061.

Trainor, K.E., Porter, B.F., Logan, K.S., Hoffman, R.J., Snowden, K.F., 2010. Eight cases of feline cutaneous leishmaniasis in Texas. Vet. Pathol. 47, 1076–1081. https://doi.org/10.1177/0300985810382094.

Urbani, L., Tirolo, A., Salvatore, D., Tumbarello, M., Segatore, S., Battilani, M., et al., 2020. Serological, molecular and clinicopathological findings associated with *Leishmania infantum* infection in cats in northern Italy. J. Feline Med. Surg. 22, 935–943. https://doi.org/10.1177/1098612X19895067.

Verneuil, M., 2013. Leishmaniose oculaire féline: À propos d'un cas. J. Fr. Ophtalmol. 36, e67–e72. https://doi.org/10.1016/j.jfo.2012.09.006.

Veronesi, F., Ravagnan, S., Cerquetella, M., Carli, E., Olivieri, E., Santoro, A., et al., 2016. First detection of *Cytauxzoon* spp. infection in European wildcats (*Felis silvestris silvestris*) of Italy. Ticks Tick Borne Dis. 7, 853–858. https://doi.org/10.1016/j.ttbdis.2016.04.003.

Vides, J.P., Schwardt, T.F., Sobrinho, L.S.V., Marinho, M., Laurenti, M.D., Biondo, A.W., et al., 2011. *Leishmania chagasi* infection in cats with dermatologic lesions from an endemic area of visceral leishmaniosis in Brazil. Vet. Parasitol. 178, 22–28. https://doi.org/10.1016/j.vetpar.2010.12.042.

Viettri, M., Herrera, L., Aguilar, C.M., Morocoima, A., Reyes, J., Lares, M., et al., 2018. Molecular diagnosis of *Trypanosoma cruzi*/*Leishmania* spp. coinfection in domestic, peridomestic and wild mammals of Venezuelan co-endemic areas. Vet. Parasitol. Reg. Stud. Reports 14, 123–130. https://doi.org/10.1016/j.vprsr.2018.10.002.

Vilhena, H., Martinez-Díaz, V.L., Cardoso, L., Vieira, L., Altet, L., Francino, O., et al., 2013. Feline vector-borne pathogens in the north and centre of Portugal. Parasit. Vectors 6, 99. https://doi.org/10.1186/1756-3305-6-99.

Villanueva- Saz, S., Giner, J., Tobajas, A.P., Pérez, M.D., González- Ramírez, A.M., Macías-León, J., et al., 2021. Serological evidence of SARS- CoV- 2 and co- infections in stray cats in Spain. Transbound. Emerg. Dis. tbed.14062. https://doi.org/10.1111/tbed.14062.

Vita, S., Santori, D., Aguzzi, I., Petrotta, E., Luciani, A., 2005. Feline leishmaniasis and ehrlichiosis: serological investigation in Abruzzo region. Vet. Res. Commun. 29, 319–321. https://doi.org/10.1007/s11259-005-0071-8.

WHO, 2010. Control of the Leishmaniasis: Report of the WHO Expert Committee on the control of leishmaniases. WHO Technical Report Series. https://apps.who.int/iris/bitstream/handle/10665/44412/WHO_TRS_949_eng.pdf?sequence=1&isAllowed=y.

Legends to figures

Fig. 1 Flow diagram of study searching and selection process

Fig. 2 Worldwide distribution of *Leishmania* infection in cats (*Felis* spp.)

Fig. 3 Proposed diagnostic algorithm for clinically healthy cats used as blood donors or for breeding, and cats with suspected leishmaniosis

Table 1

Epidemiological studies on the frequency of *Leishmania* infection in cats (*Felis* spp.) in the Old World

Country	Study	Sampling year	Species (origin)	No. tested	Method (test, cut-off/target gene)	Sample	% Positive (species)[a]
Albania	Silaghi et al. (2014)	2008–2010	*F. catus* (stray)	146	Serological (IFAT, 1:64)	Serum	0.7 (*L. infantum*)
					Molecular (qPCR, kDNA)	Whole blood	0
Angola	Lopes et al. (2017)	2014–2016	*F. catus* (domestic)	102	Serological (DAT, 1:100)	Serum	0
Bosnia and Herzegovina	Colella et al. (2019)	2017	*F. catus* (domestic)	5	Serological (IFAT)	Serum	0
					Molecular (qPCR, kDNA)	Whole blood	20.0 (*Leishmania* spp.)
				1[b]	Molecular (PCR, kDNA)	Whole blood	100 (*L. infantum*)
					Molecular (qPCR, ITS2)	Whole blood	100 (*L. infantum*)
Cyprus	Attipa et al. (2017)	2014	*F. catus* (domestic/shelter)	164	Serological (ELISA, 32 EU)	Serum	4.4 (*L. infantum*)
				174	Molecular (qPCR, kDNA)	Whole blood	2.3 (*L. infantum*)
Egypt	Michael et al. (1982)	na	*F. catus* (stray)	80	Serological (IHA)	Serum	3.8 (*Leishmania* spp.)
	Morsy et al. (1988)	na	*F. catus* (stray)	28	Serological (IHA)	Serum	3.6 (*Leishmania* spp.)
	Morsy & Abou el Seoud (1994)	na	*F. catus* (domestic/stray)	60	Serological (IHA, 1:32)	Serum	10.0 (*Leishmania* spp.)
Germany	Schäfer et al. (2021)	2012–2020	*F. catus* (domestic)	624	Serological (IFAT, 1:64)	Serum	4.0 (*Leishmania* spp.)
Greece	Chatzis et al. (2014b, 2014a)	2009–2011	*F. catus* (domestic)	100	Parasitological (cytology)	Bone marrow	0
						Lymph node	0
						Skin	0
					Serological (ELISA, 0.145)	Serum	1.0 (*Leishmania* spp.)
					Serological (IFAT, 1:10)	Serum	10.0 (*Leishmania* spp.)
					Molecular (PCR, kDNA)	Bone marrow	16.0 (*L. infantum*)
						Whole blood	13.0 (*L. infantum*)
				99	Molecular (PCR, kDNA)	Skin	13.1 (*L. infantum*)
				96		Conjunctival swab	3.1 (*L. infantum*)
	Diakou et al. (2017)	2015	*F. catus* (stray)	148	Serological (IFAT, 1:80)	Serum	6.1 (*L. infantum*)
					Molecular (nPCR, SSU)	Whole blood	6.1 (*L. infantum*)
	Diakou et al. (2009)	na	*F. catus* (stray)	284	Serological (ELISA)	Serum	3.9 (*Leishmania* spp.)
	Morelli et al. (2020)	na	*F. catus*	153	Serological (IFAT, 1:80)	Serum	2.0 (*L. infantum*)
Iran	Mohebali et al. (2017)	2013–2015	*F. catus* (stray)	103	Serological (DAT, 1:320)	Serum	3.9 (*L. infantum*)
				4[b]	Parasitological (cytology)	Liver	25.0 (*L. infantum*)
						Spleen	25.0 (*L. infantum*)
				4[b]	Parasitological (culture)	Liver	0

						Spleen	0
				1[b]	Molecular (nPCR, ITS2)	Liver	100 (*L. infantum*)
						Spleen	100 (*L. infantum*)
	Akhtardanesh et al. (2020)	2016	*F. catus* (stray)	180	Molecular (nPCR, kDNA)	Whole blood	13.9 (*L. infantum*)
	Asgari et al. (2020)	2016–2018	*F. catus* (stray)	174	Serological (DAT, 1:100)	Serum	17.2 (*L. infantum*)
					Serological (ELISA)	Serum	27.6 (*L. infantum*)
					Molecular (nPCR, kDNA)	Buffy coat	20.7 (*L. infantum*)
	Sarkari et al. (2009)	na	*F. catus* (stray)	40	Serological (DAT, 1:20)	Serum	20.0 (*L. infantum*)
					Serological (IFAT, 1:10)	Serum	25.0 (*L. infantum*)
	Hatam et al. (2010)	na	*F. catus* (stray)	40	Parasitological (cytology)	Liver	2.5 (*Leishmania* spp.)
						Spleen	2.5 (*Leishmania* spp.)
					Parasitological (culture)	Liver	7.5 (*Leishmania* spp.)
						Spleen	2.5 (*Leishmania* spp.)
					Molecular (PCR, kDNA	Liver	7.5 (*L. infantum*)
						Spleen	5.0 (*L. infantum*)
	Fatollahzadeh et al. (2016)	na	*F. catus* (stray)	65	Parasitological (cytology)	Liver	0
						Spleen	0
					Parasitological (culture)	Liver	0
						Spleen	0
					Serological (DAT, 1:320)	Serum	23.1 (*L. infantum*)
					Molecular (PCR, kDNA)	Spleen	0
	Akhtardanesh et al. (2017)	na	*F. catus* (stray)	60	Serological (ELISA)	Serum	6.7 (*L. infantum*)
					Molecular (nPCR, 7SL RNA)	Whole blood	16.7 (*L. infantum*)
							1.7 (*L. tropica*)
Iraq	Otranto et al. (2019)	2008	*F. catus* (stray)	207	Molecular (qPCR, kDNA)	Whole blood	0
Israel	Nasereddin et al. (2008)	1999–2000	*F. catus* (domestic/stray)	104	Serological (ELISA)	Serum	6.7 (*L. infantum*)
	Baneth et al. (2020)	2018	*F. catus* (shelter)	67	Serological (ELISA, 0.4)	Serum	75.0 (*L. infantum*)
					Molecular (qPCR, kDNA)	Whole blood	16.0 (*L. infantum*)
					Molecular ($_{HRM}$PCR, ITS1)	Whole blood	0
Italy	Vita et al. (2005)	2002–2004	*F. catus* (domestic/stray)	203	Serological (IFAT, 1:40)	Serum	16.3 (*L. infantum*)
				11[b]	Molecular (PCR)	Lymph node	100 (*L. infantum*)
						Whole blood	45.5 (*L. infantum*)
	Spada et al. (2013)	2008–2010	*F. catus* (stray)	233	Serological (IFAT, 1:40)	Serum	25.3 (*L. infantum)*
					Molecular (qPCR, kDNA)	Whole blood	0
	Morganti et al. (2019)	2010–2016	*F. catus* (shelter/stray)	286	Serological (IFAT, 1:40)	Serum	9.1 (*L. infantum*)
					Molecular (nPCR, SSU)	Buffy coat	0
						Conjunctival swab	15.7 (*L. infantum*)
	Dedola et al. (2018)	2011–2013	*F. catus* (domestic)	90	Serological (IFAT, 1:40)	Serum	10.0 (*L. infantum*)
					Molecular (nPCR, ITS)	Whole blood	5.5 (*L. infantum*)
	Veronesi et al. (2016)	2011–2014	*F. silvestris* (wild)	21	Molecular (qPCR, COII)	Spleen	0
	Persichetti et al. (2016)	2012–2013	*F. catus* (domestic)	42	Serological (IFAT, 1:80)	Serum	2.4 (*L. infantum*)

				Molecular (qPCR, kDNA)	Whole blood	42.8 (*L. infantum*)
Persichetti et al. (2018)	2012–2013	*F. catus* (domestic)	197	Parasitological (cytology)	Whole blood	0
				Serological (IFAT, 1:80)	Serum	9.6 (*L. infantum*)
				Molecular (qPCR, kDNA)	Conjunctival swab	1.5 (*L. infantum*)
			181	Molecular (qPCR, kDNA)	Lymph node	1.7 (*L. infantum*)
			143	Molecular (qPCR, kDNA)	Urine	2.1 (*L. infantum*)
			197	Molecular (qPCR, kDNA)	Oral swab	1.5 (*L. infantum*)
					Whole blood	2.0 (*L. infantum*)
Spada et al. (2016)	2014	*F. catus* (stray)	90	Serological (IFAT, 1:40)	Serum	30.0 (*L. infantum*)
				Molecular (qPCR, kDNA)	Conjunctival swab	0
					Lymph node	1.1 (*L. infantum*)
					Whole blood	1.1 (*L. infantum*)
Brianti et al. (2017)	2015	*F. catus* (domestic)	159	Serological (IFAT, 1:80)	Serum	9.4 (*L. infantum*)
				Molecular (qPCR, kDNA)	Conjunctival swab	3.8 (*L. infantum*)
					Whole blood	7.5 (*L. infantum*)
Otranto et al. (2017)	2015–2016	*F. catus* (domestic)	330	Serological (IFAT, 1:40)	Serum	25.7 (*L. infantum*)
				Molecular (qPCR, kDNA)	Conjunctival swab	1.8 (*L. infantum*)
					Whole blood	2.1 (*L. infantum*)
Abbate et al. (2019)	2015–2017	*F. silvestris* (wild)	11	Molecular (qPCR, kDNA)	Lymph node/skin/spleen	0
Priolo et al. (2019)	2016–2017	*F. catus* (domestic/stray)	66	Serological (ELISA)	Serum	17.0 (*L. infantum*)
				Serological (IFAT, 1:80)	Serum	14.0 (*L. infantum*)
				Molecular (qPCR, kDNA)	Whole blood	4.0 (*L. infantum*)
Spada et al. (2020)	2016–2018	*F. catus* (stray)	102	Serology (IFAT, 1:80)	Serum	4.9 (*L. infantum*)
			117	Molecular (qPCR, kDNA)	Conjunctival swab	0
			115	Molecular (qPCR, kDNA)	Lymph node	4.3 (*L. infantum*)
			109	Molecular (qPCR, kDNA)	Whole blood	0
Urbani et al. (2020)	2017	*F. catus* (domestic)	152	Serological (IFAT, 1:80)	Serum	11.8 (*L. infantum*)
			150	Molecular (qPCR, kDNA)	Conjunctival swab	0
					Hair	0.7 (*L. infantum*)
			146	Molecular (qPCR, kDNA)	Whole blood	0
Iatta et al. (2019)	2017–2018	*F. catus* (domestic)	2659	Serological (IFAT, 1:80)	Serum	3.3 (*L. infantum*)
				Molecular (qPCR, kDNA)	Whole blood	0.8 (*L. infantum*)
Ebani et al. (2020)	2018–2019	*F. catus* (stray)	85	Serological (IFAT)	Serum	2.4 (*Leishmania* spp.)
				Molecular (PCR, SSU)	Blood[c]	5.9 (*Leishmania* spp.)
Persichetti et al. (2017)	2013	na	76	Serological (ELISA, 40 EU)	Serum	2.6 (*L. infantum*)
				Serological (IFAT, 1:80)	Serum	17.1 (*L. infantum*)
				Serological (WB)	Serum	18.4 (*L. infantum*)
			21[b]	Serological (ELISA, 40 EU)	Serum	100 (*L. infantum*)
				Serological (IFAT, 1:80)	Serum	95.2 (*L. infantum*)
				Serological (WB)	Serum	100 (*L. infantum*)
Poli et al. (2002)	na	*F. catus* (domestic)	110	Serological (IFAT, 1:80)	Serum	0.9 (*Leishmania* spp.)
Morelli et al. (2019)	na	*F. catus* (domestic)	167	Serological (IFAT, 1:80)	Serum	3.0 (*L. infantum*)
Morelli et al. (2020)	na	*F. catus*	116	Serological (IFAT, 1:80)	Serum	4.3 (*L. infantum*)

Portugal	Duarte et al. (2010)	2003–2005	*F. catus* (stray)	180	Serology (IFAT, 1:40)	Serum	0.6 (*L. infantum*)
	Maia et al. (2008)	2004	*F. catus* (stray)	20	Serological (IFAT, 1:64)	Serum	0
				23	Molecular (PCR, ITS1)	Blood on filter paper	30.4 (*Leishmania* spp.)
					Molecular (PCR, kDNA)	Blood on filter paper	30.4 (*Leishmania* spp.)
				4[b]	Molecular (PCR–RFLP, ITS1)	Blood on filter paper	100 (*L. infantum*)
	Cardoso et al. (2010)	2004–2008	*F. catus* (domestic)	316	Serological (DAT, 1:100)	Serum	1.9 (*L. infantum*)
					Serological (ELISA)	Serum	2.8 (*L. infantum*)
	Maia et al. (2010)	2007–2008	*F. catus* (domestic/stray)	76	Serological (IFAT, 1:64)	Serum	1.3 (*Leishmania* spp.)
				138	Molecular (PCR, kDNA)	Whole blood	20.3 (*L. infantum*)
	Maia et al. (2015)	2011–2014	*F. catus* (domestic/stray)	271	Serological (DAT, 1:100)	Serum	3.7 (*L. infantum*)
	Maia et al. (2014)	2012–2013	*F. catus* (domestic/stray)	649	Molecular (nPCR, SSU)	Whole blood	9.9 (*Leishmania* spp.)
	Pereira et al. (2019a, b, 2020)	2017–2018	*F. catus* (domestic/ shelter/stray)	373	Serological (IFAT, 1:64)	Serum	1.6 (*Leishmania* spp.)
				465	Molecular (nPCR, SSU)	Buffy coat	5.4 (*Leishmania* spp.)
				25[b]	Molecular (nPCR, *cytB*)	Buffy coat	12.0 (*L. donovani s.l.*) 4.0 (*L. major*) 4.0 (*L. major*/*L. donovani s.l.*)[f]
					Molecular (PCR, *g6pdh*)	Buffy coat	4.0 (*L. donovani s.l.*)
					Molecular (nPCR, *hsp70*)	Buffy coat	12.0 (*L. donovani s.l.*) 4.0 (*L. major*/*L. donovani s.l.*)[f]
					Molecular (nPCR, ITS)	Buffy coat	12.0 (*L. donovani s.l.*) 4.0 (*L. major*)
	Neves et al. (2020)	2018–2019	*F. catus* (domestic)	141	Serological (DAT, 1:100)	Serum	0
	Vilhena et al. (2013)	na	*F. catus* (domestic)	320	Molecular (qPCR, kDNA)	Whole blood	0.3 (*L. infantum*)
Portugal/Spain	Mesa-Sanchez et al. (2020)	2015–2020	*F. catus* (domestic)[g]	173	Molecular (nPCR, SSU)	Whole blood	0
Qatar	Lima et al. (2019)	2016–2018	*F. catus* (domestic/stray)	79	Molecular (qPCR, kDNA)	Whole blood/on dried spot	1.3 (*Leishmania* spp.)
Saudi Arabia	Morsy et al. (1999)	na	*F. margarita* (wild)	10	Parasitological (cytology)	Liver	20.0 (*Leishmania* spp.)
						Spleen	40.0 (*Leishmania* spp.)
					Serological (IHA, 1:64)	Serum	40.0 (*Leishmania* spp.)
Spain	Del Río et al. (2014)	2001–2006	*Felis silvestris* (wild)	4	Molecular (qPCR, kDNA)	Liver and/or spleen	25.0 (*L. infantum*)
				1[b]	Molecular (PCR, ITS2)	Liver and/or spleen	100 (*L. infantum*)
	Martín-Sánchez et al. (2007)	2003–2004	*F. catus* (domestic)	183	Serological (IFAT, 1:40)	Serum	28.3 (*Leishmania* spp.)
					Molecular (PCR-ELISA, kDNA)	Whole blood	25.7 (*L. infantum*)
				7[b]	Parasitological (culture)	Leucoconcentrate	0
					Parasitological (cytology)	Leucoconcentrate	42.9 (*Leishmania* spp.)

	Ayllón et al. (2008)	2005–2006	*F. catus* (domestic)	233	Serological (IFAT, 1:100)	Serum	1.3 (*L. infantum*)
					Molecular (PCR, kDNA)	Whole blood	0.4 (*L. infantum*)
	Ayllón et al. (2012)	2005–2008	*F. catus* (domestic/stray)	680	Serological (IFAT, 1:50)	Serum	3.7 (*L. infantum*)
					Molecular (PCR, kDNA)	Whole blood	0.6 (*L. infantum*)
	Tabar et al. (2008)	2006	*F. catus* (domestic)	100	Molecular (qPCR, kDNA)	Whole blood	3.0 (*L. infantum*)
	Sherry et al. (2011)	2008	*F. catus* (shelter)	105	Serological (ELISA)	Serum	13.2 (*L. infantum*)
				104	Molecular (qPCR, kDNA)	Whole blood	8.7 (*L. infantum*)
	Millán et al. (2011)	2008–2009	*F. catus* (stray)	83	Serological (WB)	Serum	15.7 (*L. infantum*)
				73	Molecular (PCR, kDNA)	Blood and/or spleen	25.6 (*L. infantum*)
				14[b]	Molecular (PCR–RFLP, kDNA)	Blood and/or spleen	100 (*L. infantum*)
	Miró et al. (2014)	2012–2013	*F. catus* (stray)	346	Serological (IFAT, 1:100)	Serum	3.2 (*L. infantum*)
				57[d]	Molecular (nested PCR, ITS1)	Whole blood	0
					Molecular (nested PCR, SSU)	Whole blood	0
	Risueño et al. (2018)	2013–2015	*F. silvestris* (wild)	2	Molecular (qPCR, kDNA)	Skin	50.0 (*L. infantum*)
						Other organs[e]	0
	Marenzoni et al. (2018)	2014–2015	*F. catus* (domestic)	31[g]	Molecular (PCR, kDNA)	Whole blood	0
	Montoya et al. (2018a)	2014–2017	*F. catus* (stray)	249	Serological (IFAT, 1:100)	Serum	4.8 (*L. infantum*)
					Molecular (PCR, ITS)	Skin/whole blood	0
	Priolo et al. (2019)	2016–2017	*F. catus* (domestic/stray)	113	Serological (ELISA)	Serum	7.0 (*L. infantum*)
					Serological (IFAT, 1:80)	Serum	19.0 (*L. infantum*)
					Molecular (qPCR, kDNA)	Whole blood	5.0 (*L. infantum*)
	Villanueva- Saz et al. (2021)	2020	*F. catus* (stray)	114	Serological (ELISA, 13 EU)	Serum	16.7 (*L. infantum*)
	Solano-Gallego et al. (2007)	na	*F. catus* (domestic/stray)	445	Serological (ELISA-IgG, 53 EU)	Serum	5.3 (*L. infantum*)
		na			Serological (ELISA-Prot A, 44 EU)	Serum	6.3 (*L. infantum*)
	Alcover et al. (2020)	na	*F. catus* (wild)	1	Molecular (qPCR, kDNA)	Liver	100 (*Leishmania* spp.)
		na				Skin	100 (*Leishmania* spp.)
		na				Spleen	100 (*Leishmania* spp.)
	Miró et al. (2011)	na	*F. catus* (breeding)	20	Serological (IFAT, 1:100)	Serum	15.0 (*L. infantum*)
	Moreno et al. (2014)	na	*F. catus* (stray)	43	Serological (IFAT, 1:50)	Serum	4.3 (*L. infantum*)
	Montoya et al. (2018b)	na	*F. catus* (stray)		Serological (IFAT, 1:100)	Serum	0
Thailand	Sukmee et al. (2008)	2006	*F. catus*	15	Serological (DAT; 1:100)	Serum	60.0 (*Leishmania* spp.)
				9[b]	Molecular (PCR, ITS1)	Whole blood	0
					Molecular (PCR, kDNA)	Whole blood	0
	Junsiri et al. (2017)	2013	*F. catus* (domestic)	250	Serological (ELISA, 0.2)	Serum	5.6 (*L. infantum*)
					Molecular (PCR, kDNA)	Whole blood	0
	Kongkaew et al. (2007)	na	*F. catus*	5	Serological (DAT, 1:100)	Serum	20.0 (*Leishmania* spp.)
				1[b]	Molecular (PCR)	Whole blood	0

Turkey	Dincer et al. (2015)	2013	*F. catus* (domestic/shelter)	22	Molecular (nPCR, kDNA)	Whole blood	4.5 (*L. infantum*)
	Karakuş et al. (2019)	2014	*F. catus* (stray)	5	Molecular (nPCR, SSU)	Conjunctival swab	0
		2015		8	Molecular (qPCR, ITS1)	Conjunctival swab	12.5 (*L. infantum*)
		2016		6	Molecular (qPCR, ITS1)	Conjunctival swab	0
	Dincer et al. (2016)	2015	*F. catus* (domestic/shelter)	50	Molecular (nPCR, kDNA)	na	0
	Dinçer et al. (2012)	na	*F. catus* (domestic)	1	Serological (IFAT)	Serum	0
					Molecular (PCR)	na	0
	Paşa et al. (2015)	na	*F. catus* (domestic)	147	Molecular (qPCR, ITS1)	Whole blood	2.7 (*L. major*)
							8.8 (*L. tropica*)
					Molecular (qPCR, *hsp70*)	Whole blood	2.0 (*L. major*)
							2.7 (*L. tropica*)
							2.7 (*Leishmania* spp.)
	Can et al. (2016)	na	*F. catus* (shelter)	1101	Serological (ELISA)	Serum	10.8
					Serological (IFAT, 1:40)	Serum	15.2
					Molecular (qPCR, ITS1)	Whole blood	0.1 (*L. tropica*)
					Molecular (nPCR, kDNA)	Whole blood	0.1 (*L. infantum*)
							0.5 (*L. tropica*)
UK	Persichetti et al. (2017)	2013	*F. catus*	64	Serological (ELISA, 40 EU)	Serum	1.6 (*L. infantum*)
					Serological (IFAT, 1:80)	Serum	0
					Serological (WB)	Serum	3.1 (*L. infantum*)
Uzbekistan	Kovalenko et al. (2011)	na	*F. catus*	1	Serological (ELISA)	Serum	0

[a] Species defined according to the original study.
[b] Previously identified as positive by another test.
[c] DNA extracted from the sediment obtained after centrifugation of the blood samples.
[d] Seropositive for *L. infantum* and/or for feline retrovirus (feline leukemia virus and/or feline immunodeficiency virus).
[e] Not specified.
[f] Putative hybrid.
[g] Cats eligible for blood donation.

Abbreviations: COII, cytochrome oxidase II; *cytB*, cytochrome b; DAT, direct agglutination test; ELISA, enzyme-linked immunosorbent assay; EU, ELISA units; *F.*, *Felis*; *g6pdh*, glucose-6-phosphate dehydrogenase; $_{HRM}$PCR, high resolution melt PCR; *hsp70*, heat-shock protein 70; IFAT, immunofluorescence antibody test; IgG, Immunoglobulin G; IHA, indirect hemagglutination; ITS, internal transcriber spacers; ITS1, internal transcriber spacer 1; ITS2, internal transcriber spacer 2; kDNA, kinetoplast minicircle DNA; *L.*, *Leishmania*; na, not available; nPCR, nested PCR; PCR, one-step PCR (polymerase chain reaction); Prot A, Protein A; qPCR, real-time PCR; RFLP, restriction fragment length polymorphism; *s.l.*, *sensu lato*; SSU, small subunit ribosomal DNA; WB, western blot.

Table 2

Epidemiological studies on the frequency of *Leishmania* infection in cats (*Felis* spp.) in the New World

Country	Study	Sampling year	Species (origin)	No. tested	Method (test, cut-off/target gene)	Sample	% Positive (species)[a]
Brazil	De Matos et al. (2018)	2004–2014	*F. catus*	679	Serological (ELISA)	Serum	43.4 (*Leishmania* spp.)
					Serological (IFAT, 1:40)	Serum	15.8 (*Leishmania* spp.)
	Figueiredo et al. (2009)	2005	*F. catus* (domestic)	43	Serological (ELISA)	Serum	2.4 (*Leishmania* spp.)
					Serological (IFAT, 1:40)	Serum	0
	Coelho et al. (2011a)	2007–2009	*F. catus*	70	Serological (ELISA)	Serum	4.2 (*Leishmania* spp.)
					Serological (IFAT, 1:40)	Serum	0.0 (*Leishmania* spp.)
	Vides et al. (2011)	2008–2009	*F. catus*	55	Parasitological (cytology)	Bone marrow	12.7 (*Leishmania* spp.)
						Liver	3.6 (*Leishmania* spp.)
						Lymph node	5.5 (*Leishmania* spp.)
						Spleen	7.3 (*Leishmania* spp.)
					Parasitological (IHC)	Skin	16.4 (*Leishmania* spp.)
					Serological (ELISA, 0.277)	Serum	25.4 (*Leishmania* spp.)
					Serological (IFAT, 1:40)	Serum	10.9 (*Leishmania* spp.
				3	Molecular (qPCR, *gp63*)	Whole blood	100 (*L. chagasi*)
	Cardia et al. (2013)	2010	*F. catus* (shelter/stray)	386	Serological (IFAT, 1:40)	Serum	0.5 (*Leishmania* spp.)
	Silva et al. (2014)	2010	*F. catus* (domestic/shelter)	153	Serological (ELISA)	Serum	3.9 (*L. infantum*)
	De Sousa Oliveira et al. (2015)	2012	*F. catus*	52	Molecular (PCR, kDNA)	Conjunctival swab	13.5 (*Leishmania* spp.)
	de Sousa et al. (2014)	2013	*F. catus* (domestic/stray)	151	Serological (IFAT, 1:40)	Serum	6.6 (*L. infantum*)
	Metzdorf et al. (2017)	2013–2014	*F. catus* (domestic/shelter)	100	Parasitological (cytology)	Bone marrow	4.0 (*Leishmania* spp.)
						Lymph node	4.0 (*Leishmania* spp.)
						Whole blood	4.0 (*Leishmania* spp.)
					Molecular (PCR-RFLP, kDNA)	Bone marrow	6.0 (*L. infantum*)
						Lymph node	3.0 (*L. infantum*)
						Whole blood	4.0 (*L. infantum*)
	Leonel et al. (2020)	2014	*F. catus* (shelter)	94	Serological (ELISA)	Serum	31.9 (*Leishmania* spp.)
					Serological (IFAT, 1:40)	Serum	29.8 (*Leishmania* spp.)
					Molecular (PCR, kDNA)	Conjunctival swab	0
						Whole blood	0
	Marcondes et al. (2018)	2014–2015	*F. catus* (domestic/shelter)	50[b]	Parasitological (cytology)	Bone marrow	14.0 (*Leishmania* spp.)
					Molecular (qPCR, kDNA)	Bone marrow	86.0 (*L. infantum*)
						Whole blood	72.0 (*L. infantum*)
	Rocha et al. (2019)	2016–2017	*F. catus* (domestic)	105	Serological (IFAT, 1:40)	Serum	30.5 (*L. infantum*)
					Molecular (PCR, CH1)	Whole blood	2.9 (*L. infantum*)
					Molecular (PCR, ITS1)	Whole blood	5.7 (*L. infantum*)

Pedrassani et al. (2019)	2017	*F. catus* (domestic)	30	Serological (IFAT, 1:80)	Serum	6.6 (*L. infantum*)
				Molecular (PCR, kDNA)	Whole blood	0
Berenguer et al. (2020)	2017	*F. catus* (domestic)	128	Molecular (PCR, kDNA)	Conjunctival swab	0
					Whole blood	0.8 (*L. infantum*)
			3[c]	Parasitological (cytology)	Lymph node	33.3 (*Leishmania* ssp.)
				Molecular (PCR, kDNA)	Lymph node	33.3 (*L. infantum*)
Bezerra et al. (2019)	2017–2018	*F. catus* (domestic)	91	Serological (IFAT, 1:40)	Serum	15.4 (*Leishmania* spp.)
				Molecular (PCR, kDNA)	Whole blood	0
da Silva et al. (2008)	na	*F. catus* (domestic)	8	Serological (IFAT, 1:40)	Serum	25.0 (*Leishmania* spp.)
			3	Molecular (multiplex PCR, kDNA)	Whole blood	66.7 (*Leishmania* spp.)
			2[b]	Molecular (DB)	Whole blood	100 (*L. chagasi*)
Bresciani et al. (2010)	na	*F. catus* (domestic)	283	Parasitological (cytology)	Lymph node	0.7 (*Leishmania* spp.)
				Serological (IFAT, 1:40)	Serum	0
Neto et al. (2011)	na	*F. catus* (shelter)	130	Serological (CAG-ELISA, 0.449)	Serum	23.0 (*Leishmania* spp.)
				Serological (FML-ELISA, 0.215)	Serum	13.3 (*Leishmania* spp.)
				Serological (rK39-ELISA, 0.347)	Serum	15.9 (*Leishmania* spp.)
Coelho et al. (2011b)	na	*F. catus* (domestic)	52	Parasitological (cytology)	Bone marrow	0
					Lymph node	3.8 (*Leishmania* spp.)
					Spleen	0
				Molecular (PCR, kDNA)	Bone marrow	0
					Lymph node	3.8 (*L. chagasi*)
					Spleen	1.9 (*L. chagasi*)
Sobrinho et al. (2012)	na	*F. catus* (shelter/stray)	302	Parasitological (Cytology)	Bone marrow	7.0 (*Leishmania* spp.)
					Lymph node	7.9 (*Leishmania* spp.)
				Serological (ELISA, 0.301)	Serum	13.0 (*Leishmania* spp.)
				Serological (IFAT, 1:40)	Serum	4.6 (*Leishmania* spp.)
			5[b]	Molecular (qPCR, *gp63*)	Whole blood	100 (*L. infantum*)
de Morais et al. (2013)	na	*F. catus* (domestic)	5	Molecular (qPCR, kDNA)	Whole blood	80.0 (*L. infantum*)
				Molecular (PCR, kDNA)	Whole blood	80.0 (*L. infantum*)
Braga et al. (2014a)	na	*F. catus* (domestic)	50	Serological (IFAT, 1:40)	Serum	4.0 (*Leishmania* spp.)
Braga et al. (2014b)	na	*F. catus*	100	Parasitological (culture)	Whole blood	2.0 (*Leishmania* spp.)
				Serological (IFAT, 1:40)	Serum	15.0 (*Leishmania* spp.)
				Molecular, PCR, kDNA)	Whole blood	0
Oliveira et al. (2015)	na	*F. catus* (domestic)	443	Serological (DAT, 1:40)	Serum	5.6 (*Leishmania* spp.)
				Serological (IFAT, 1:40)	Serum	4.1 (*Leishmania* spp.)
Benassi et al. (2017)	na	*F. catus* (domestic/stray)	108	Molecular (PCR, kDNA)	Conjunctival swab	1.9 (*Leishmania* spp.)
					Whole blood	0
				Molecular (qPCR, kDNA)	Conjunctival swab	1.9 (*Leishmania* spp.)
					Whole blood	0
			2[b]	Molecular (PCR, ITS1)	Conjunctival swab	50.0 (*L. infantum*)
Coura et al. (2018)	na	*F. catus* (shelter)	100	Parasitological (cytology)	Bone marrow	0

					Parasitological (culture)	Bone marrow	0
					Serological (IFAT, 1:40)	Serum	54.0 (*Leishmania* spp.)
				54[b]	Molecular (PCR, kDNA)	Bone marrow/skin	0
	da Costa-Val et al. (2020)	na	*F. catus* (domestic)	64	Serological (ELISA, 0.955)	Serum	29.8 (*Leishmania* spp.)
				64	Molecular (PCR, kDNA)	Conjunctival swab	6.3 (*Leishmania* spp.)
				64	Molecular (PCR, kDNA)	Oral swab	4.7 (*Leishmania* spp.)
				8[b]	Molecular (PCR-RFLP, ITS1)	Conjunctival swab	12.5 (*L. infantum*)
						Oral swab	37.5 (*L. infantum*) 12.5 (*L. braziliensis*)
Honduras	Mccown & Grzeszak, 2010)	na	*F. catus* (stray)	12	Serological (IFAT, 1:32)	Serum	25.0 (*L. donovani*)
Mexico	Longoni et al. (2012)	2008–2009	*F. catus* (stray)	95	Serological (ELISA-H)	Serum	5.3 (*L. braziliensis*) 13.7 (*L. infantum*) 1.1 (*L. mexicana*)
					Serological (ELISA-SODe)	Serum	11.6 (*L. baziliensis*) 22.1 (*L. infantum*) 10.5 (*L. mexicana*)
					Serological (WB)	Serum	10.5 (*L. baziliensis*) 20.0 (*L. infantum*) 10.5 (*L. mexicana*)
Venezuela	Viettri et al. (2018)	na	na	5	Molecular (nested-PCR, ITS1)	Blood on filter paper	20.0 (*Leishmania* spp.)
					Molecular (nPCR, *SSU* rDNA)	Blood on filter paper	20.0 (*Leishmania* spp.)
	Rivas et al. (2018)		*F. catus* (domestic/stray)	6	Parasitological (cytology)	Skin lesions	66.7 (*Leishmania* spp.)
				5	Parastiological (histology)	Skin lesions	80.0 (*Leishmania* spp.)
				5	Parasitological (IHC)	Skin lesions	100 (*Leishmania* spp.)
				30	Serological (ELISA, 15.3 EU)	Serum	6.7 (*L. braziliensis*)
					Serological (ELISA, 15.3 EU)	Serum	6.7 (*L. infantum*)
					Serological (WB)	Serum	33.3 (*L. braziliensis*)
					Serological (WB)	Serum	33.3 (*L. infantum*)
				31	Molecular (qPCR, kDNA)	Whole blood	9.7 (*Leishmania* spp.)
				5	Molecular (qPCR, kDNA)	Skin lesions	100 (*Leishmania* spp.)
					Molecular (qPCR, ITS1)	Skin lesions	40.0 (*L. mexicana*)
				2[b]	Molecular (PCR-RFLP, ITS1)	Skin lesions	50.0 (*L. mexicana*)
	Paniz Mondolfi et al. (2019)	na	na	12	Molecular (nPCR, *cytB*)	Skin lesions	83.3 (*L. mexicana*) 16.7 (*Leishmania* spp.)

[a] Species defined according to the original study.
[b] Previously identified as positive by another test.

[c] Cats with lymphadenomegaly.

Abbreviations: CAG, crude antigen; CH1, chitinase; *cytB*, cytochrome b; DAT, direct agglutination test; DB, dot blot; ELISA, enzyme-linked immunosorbent assay; EU, ELISA units; *F.*, *Felis*; FML, fucose-mannose ligand; *gp63*, metalloprotease gp63; H, total parasite extract; IFAT, immunofluorescence antibody test; IHC, immunohistochemistry; ITS1, internal transcriber spacer 1; kDNA, kinetoplast minicircle DNA; *L.*, *Leishmania*; na, not available; nPCR, nested-PCR; PCR, one-step PCR (polymerase chain reaction); qPCR, real-time PCR; RFLP, restriction fragment length polymorphism; rK39, recombinant K39; SODe - superoxide dismutase excreted; *SSU*, small subunit ribosomal DNA; WB, western blot.

Table 3

Frequency of clinical signs in domestic cats (*Felis catus*) with clinical leishmaniosis caused by *Leishmania infantum*

Historical or physical signs	Frequency (%)[a]	Reference
Dermatological		
Nodules	38	Poli et al. (2002); Savani et al. (2004); Rüfenacht et al. (2005); Richter et al. (2014); Pimenta et al. (2015); Basso et al. (2016); Attipa et al. (2017); Leal et al. (2018); Brianti et al. (2019); Headley et al. (2019); Pereira et al. (2019); Fernandez-Gallego et al. (2020); Silva et al. (2020)
Erosive/ulcerative skin disease	37	Ozon et al. (1998); Hervás et al. (1999, 2001); Pennisi et al. (2004); Grevot et al. (2005); Rüfenacht et al. (2005); Coelho et al. (2010); Pocholle et al. (2012); Maia et al. (2015); Basso et al. (2016); Brianti et al. (2019); Headley et al. (2019); Fernandez-Gallego et al. (2020); Silva et al. (2020)
Scaling/crusting	21	Ozon et al. (1998; Hervás et al. (1999); Pennisi et al. (2004); Rüfenacht et al. (2005); Coelho et al. (2010); da Silva et al. (2010); Headley et al. (2019); Fernandez-Gallego et al. (2020)
Alopecia	12	Hervás et al. (1999); Pennisi et al. (2004); Rüfenacht et al. (2005); Fernandez-Gallego et al. (2020)
Onychogryphosis	6	da Silva et al. (2010); Headley et al. (2019)
Bloody cyst	4	Pennisi et al. (2004)
Depigmentation	4	Rüfenacht et al. (2005); Pocholle et al. (2012)
Pruritus	4	Rüfenacht et al. (2005); Pocholle et al. (2012)
Pustule/papule	4	Rüfenacht et al. (2005); Pocholle et al. (2012)
Footpad hyperkeratosis	2	Fernandez-Gallego et al. (2020)
General/miscellaneous		
Lymphadenomegaly	27	Hervás et al. (1999, 2001); Poli et al. (2002); Pennisi et al. (2004); Savani et al. (2004); Maroli et al. (2007); da Silva et al. (2010); Brianti et al. (2019); Fernandez-Gallego et al. (2020); Silva et al. (2020)
Lethargy/depression	25	Poli et al. (2002); Pennisi et al. (2004); Leiva et al. (2005); Rüfenacht et al. (2005); Marcos et al. (2009); Pocholle et al. (2012); Richter et al. (2014); Fernandez-Gallego et al. (2020)
Anorexia/inappetence	21	Pennisi et al. (2004); Rüfenacht et al. (2005); Marcos et al. (2009); da Silva et al. (2010); Fernandez-Gallego et al. (2020)
Weight loss	21	Ozon et al. (1998); Hervás et al. (1999); Pennisi et al. (2004); Savani et al. (2004); da Silva et al. (2010); Fernandez-Gallego et al. (2020); Silva et al. (2020)
Hyperthermia	12	Leiva et al. (2005); Basso et al. (2016); Headley et al. (2019); Fernandez-Gallego et al. (2020)
Hepatomegaly	4	Pennisi et al. (2004); Leiva et al. (2005)
Splenomegaly	4	Poli et al. (2002); Leal et al. (2018)
Bruising	2	Maia et al. (2015)
Mastitis	2	Pereira et al. (2019)
Ocular		
Uveitis	27	Hervás et al. (2001); Pennisi et al. (2004); Verneuil (2013); Richter et al. (2014); Pimenta et al. (2015); Leal et al. (2018); Pereira et al. (2019); Fernandez-Gallego et al. (2020)
Corneal oedema	10	Hervás et al. (2001); Pimenta et al. (2015); Fernandez-Gallego et al. (2020)
Conjunctivitis	8	Migliazzo et al. (2015); Brianti et al. (2019); Fernandez-Gallego et al. (2020)
Chorioretinitis	4	Pennisi et al. (2004); Fernandez-Gallego et al. (2020)

Corneal opacification	4	Hervás et al. (2001); Pimenta et al. (2015)
Glaucoma	4	Leiva et al. (2005); Richter et al. (2014)
Keratitis	4	Richter et al. (2014); Fernandez-Gallego et al. (2020)
Blepharitis	2	Brianti et al. (2019)
Chemosis	2	Fernandez-Gallego et al. (2020)
Masse	2	Hervás et al. (2001)
Gastrointestinal/abdominal		
Stomatitis	21	Hervás et al. (2001); Leiva et al. (2005); Maroli et al. (2007); Verneuil (2013); Maia et al. (2015); Migliazzo et al. (2015); Fernandez-Gallego et al. (2020)
Glossitis	4	Fernandez-Gallego et al. (2020)
Jaundice	4	Hervás et al. (1999); Fernandez-Gallego et al. (2020)
Vomiting	4	Hervás et al. (1999); Fernandez-Gallego et al. (2020)
Abdominal distension	2	Leiva et al. (2005)
Diarrhoea	2	Fernandez-Gallego et al. (2020)
Cardiorespiratory		
Dispnoea/tachypnoea	12	da Silva et al. (2010); Basso et al. (2016); Leal et al. (2018); Headley et al. (2019); Silva et al. (2020)
Pallor	10	Hervás et al. (2001); Pennisi et al. (2004); Marcos et al. (2009); Maia et al. (2015); Richter et al. (2014)
Abnormal respiratory sounds	4	Leal et al. (2018); Altuzarra et al. (2020)
Nasal discharge	4	Migliazzo et al. (2015); Altuzarra et al. (2020)
Sneezing	2	Leal et al. (2018)
Musculoskeletal		
Muscle atrophy	2	da Silva et al. (2010)
Neurological		
Ataxia	2	Fernandez-Gallego et al. (2020)
Urogenital		
Vaginal bleeding	2	Maia et al. (2015)

[a] $n = 52$

Table 4

Frequency of clinicopathological abnormalities in domestic cats (*Felis catus*) with leishmaniosis caused by *Leishmania infantum*

Parameter	Frequency (%)[a]	Reference
Hemogram		
Anaemia	31	Hervás et al. (1999); Pennisi et al. (2004); Marcos et al. (2009); Richter et al. (2014); Pereira et al. (2019); Fernandez-Gallego et al. (2020); Pimenta et al. (2015)
Neutrophilia	19	Poli et al. (2002); Leiva et al. (2005); da Silva et al. (2010); Verneuil (2013); Fernandez-Gallego et al. (2020); Silva et al. (2020)
Thrombocytopaenia	17	Pennisi et al. (2004); Marcos et al. (2009); Richter et al. (2014); Pimenta et al. (2015); Basso et al. (2016); Pereira et al. (2019)
Leukocytosis	10	Ozon et al. (1998); da Silva et al. (2010); Fernandez-Gallego et al. (2020)
Leukopaenia	10	Pennisi et al. (2004); Rüfenacht et al. (2005); Richter et al. (2014)
Eosinophilia	7	Ozon et al. (1998); Hervás et al. (1999); Marcos et al. (2009); Altuzarra et al. (2020)
Neutropaenia	5	Fernandez-Gallego et al. (2020)
Lymphopaenia	2	Rüfenacht et al. (2005)
Monocytosis	2	Leiva et al. (2005)
Blood chemistry		
Hyperproteinaemia	36	Ozon et al. (1998); Hervás et al. (1999); Poli et al. (2002); Pennisi et al. (2004); Pimenta et al. (2015); Attipa et al. (2017); Leal et al. (2018); Brianti et al. (2019); Pereira et al. (2019); Fernandez-Gallego et al. (2020)
Hyperglobulinaemia	31	Pennisi et al. (2004); Leiva et al. (2005); Richter et al. (2014); Pimenta et al. (2015); Brianti et al. (2019); Altuzarra et al. (2020)
Azotemia	21	Pennisi et al. (2004); Leiva et al. (2005); Marcos et al. (2009); da Silva et al. (2010); Leal et al. (2018); Fernandez-Gallego et al. (2020)
Hypoalbuminaemia	10	Hervás et al. (1999; Rüfenacht et al. (2005); Richter et al. (2014); Fernandez-Gallego et al. (2020)
Hyperglycaemia	8	Leiva et al. (2005); Richter et al. (2014); Fernandez-Gallego et al. (2020)
Bilirrubinaemia	5	Fernandez-Gallego et al. (2020)
Hyperphosphataemia	3	Fernandez-Gallego et al. (2020)
Hypophosphataemia	3	Fernandez-Gallego et al. (2020)
Increased alanine aminotransferase	3	Fernandez-Gallego et al. (2020)
Increased aspartate transaminase	3	da Silva et al. (2010)
Increased creatinine kinase	3	Fernandez-Gallego et al. (2020)
Protein electrophoresis		
Hypergammaglobulinaemia	84	Ozon et al. (1998); Hervás et al. (1999); Poli et al. (2002); Pennisi et al. (2004); Leiva et al. (2005); Marcos et al. (2009); Richter et al. (2014); Basso et al. (2016); Leal et al. (2018); Brianti et al. (2019); Pereira et al. (2019); Altuzarra et al. (2020); Fernandez-Gallego et al. (2020)
Increased α_2 globulins	13	Basso et al. (2016); Fernandez-Gallego et al. (2020)
Hyperbetaglobulinaemia	3	Hervás et al. (1999)
Urinalysis		
Proteinuria	25	Marcos et al. (2009); Leal et al. (2018); Fernandez-Gallego et al. (2020)
Bilirrubinuria	4	Marcos et al. (2009)
Glycosuria	4	Leiva et al. (2005)

[a] Hemogram, $n = 42$; Blood chemistry, $n = 39$; Serum protein electrophoresis, $n = 32$; Urianalysis, $n = 24$.

Table 5

Common laboratory tests performed for diagnostic of *Leishmania* infection in domestic cats (*Felis catus*)

Type/test	Aim	Confirmation of clinical disease	Confirmation of subclinical disease	Preferential sample	Advantages	Disadvantages	Observations
Parasitological							
Cytology	Detection of parasites	+++	+	‣ Bone-marrow (FNB); ‣ Lymph node (FNB); ‣ Nodular lesions (FNB); ‣ Erosive/ ulcerative skin lesions (scraping)	‣ Does not require specific laboratory equipment; ‣ Low cost; ‣ Rapid; ‣ High specificity	‣ Requires experienced observers; ‣ Strictly qualitative; ‣ Not suitable for identification at the species level	‣ Amastigotes can be found in both intracellular and extracellular areas
Histopathology	Detection of parasites	+++	+	‣ Skin/ocular lesions; ‣ Bone marrow; ‣ Lymph-nodes; ‣ Spleen	‣ Preserves structure and maintains tissue pathology; ‣ High specificity; ‣ Good sensitivity using IHC	‣ Invasive; ‣ Requires experienced observer; ‣ Requires specific laboratory equipment; ‣ More laborious and time-consuming; ‣ IHC is not widely available; ‣ Only qualitative; ‣ Not suitable for identification at the species level	
Parasite culture	Isolation of viable parasites	++	+	‣ Biopsy lesions; ‣ Bone marrow; ‣ Lymph nodes	‣ Provides parasites for further analysis; ‣ Confirms active infection; ‣ High specificity	‣ Labour-intensive; ‣ Restricted to specialised reference laboratories; ‣ Up to more than 30 days to provide a result; ‣ Only qualitative; ‣ Not suitable for identification at the species level	‣ Aseptic sampling should be ensured; ‣ Biopsy sample must be homogenised in saline or culture medium under sterile conditions
Molecular							
PCR	Detection of parasite DNA	+++	+++	‣ Biopsy lesions; ‣ Bone marrow; ‣ Lymph nodes	‣ Allows identification at the species level; ‣ High sensitivity and specificity	‣ Transient infection cannot be excluded; ‣ Requires specific laboratory equipment; ‣ Requires vigilance against false-positive results; ‣ Only qualitative; ‣ Expensive	‣ Protocols targeting multicopy genes are preferable for diagnosis; ‣ Nested PCR has more sensitivity than conventional PCR

qPCR	Detection of parasite DNA	+++	+++	‣ Biopsy lesions ‣ Bone marrow ‣ Lymph nodes	‣ Allows identification at the species level; ‣ High sensitivity and specificity; ‣ Quantification of parasite load; ‣ Reduced cross-contamination probability; ‣ Valuable for treatment follow-up; ‣ Qualitative/quantitative	‣ Transient infection cannot be excluded; ‣ Standardised methods to parasite load quantification may not be offered by some laboratories; ‣ Expensive	‣ Protocols targeting multicopy genes are preferable for diagnosis
Serological							
ELISA	Detection of specific antibodies	+++	++	‣ Serum; ‣ Plasma	‣ Valuable for treatment follow-up; ‣ Relatively low cost; ‣ Qualitative/quantitative	‣ Possible cross-reactivity; ‣ Difficult to assess results at threshold of positivity; ‣ Not suitable for unambiguous identification at the species level	‣ Established cut-off (40 EU)
IFAT	Detection of specific antibodies	++	+++	‣ Serum; ‣ Plasma	‣ Valuable for treatment follow-up; ‣ Relatively low cost; ‣ Qualitative/quantitative	‣ Requires experienced observers; ‣ Subjective interpretation; ‣ Possible cross-reactivity; ‣ Not suitable for unambiguous identification at the species level	‣ Reference method for the serodiagnosis of human and canine leishmanioses; ‣ Established cut-off (1:80)
Western blot	Detection of specific antibodies	+++	+++	‣ Serum; ‣ Plasma	‣ High sensitivity and specificity	‣ Labour-intensive; ‣ Expensive; ‣ Not available in routine practice;	‣ Marker for positivity: 18 kDA band

Abbreviations: ELISA, enzyme-linked immunosorbent assay; EU, ELISA units; FNB, Fine needle biopsy; IFAT, immunofluorescence antibody test; IHC, immunohistochemistry, KDa, kilodaltons; PCR, conventional/nested polymerase chain reaction; qPCR, real time polymerase chain reaction; WB, western blot. +++, recommended test; ++ suitable test; +, limited test.

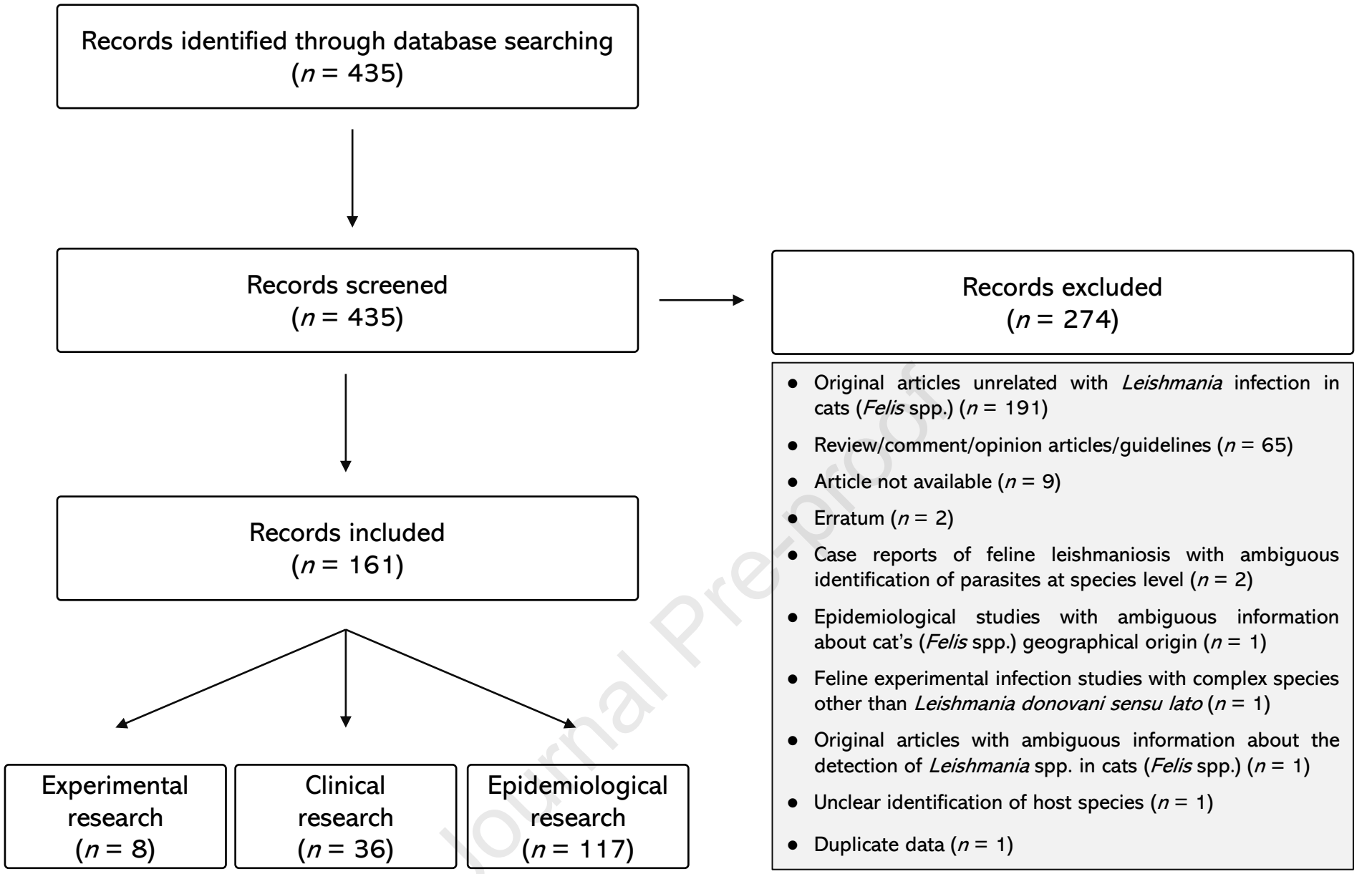
Table 6

Treatment regimens used for feline leishmaniosis

Type	Drug (regimen and dose)	Outcome	Adverse reactions[a]	Issues to consider	Reference
Monotherapy					
	Allopurinol (10–30 mg/kg or 100 mg/cat PO q12–24h; for long-term)	Variable (no response to clinical cure)	Increased liver enzymes; coprostasis[b]; toxidermia	Secondary xanthine urolithiasis has been reported in dogs	Pennisi et al. (2004); Rüfenacht et al. (2005); Marcos et al. (2009); Pocholle et al. (2012); Richter et al. (2014); Maia et al. (2015); Migliazzo et al. (2015); Pimenta et al. (2015); Basso et al. (2016); Leal et al. (2018); Attipa et al. (2017); Brianti et al. (2019); Pereira et al. (2019); Altuzarra et al. (2020); Fernandez-Gallego et al. (2020)
	Domperidone (0.5 mg/kg PO q24h for 1 month)	No improvement	Not reported	Immunomodulatory drug used on prevention and treatment of CanL	Maia et al. (2015)
	Fluconazole (5 mg/kg PO q24h for 2 months)	No response	Not reported	May be hepatotoxic	Pennisi et al. (2004)
	Itraconazole (50 mg/cat PO q24h for 2 months)	No response	Not reported	Hepatotoxic drug; may lead to suppression of adrenal function	Pennisi et al. (2004)
	Meglumine antimoniate (50 mg/kg SC q24h for 25 days)	Not applicable	AKI - suspected	Treatment stopped due to AKI development; painful to administer; may be nephrotoxic (controversial)	Leal et al. (2018)
	Meglumine antimoniate (300 mg/cat SC q24h for 4 months)	Resolution of clinical signs	See previous line	See previous line	Fernandez-Gallego et al. (2020)
Combination therapy					
	Meglumine antimoniate (50 mg/kg SC q24h for 30 days) plus allopurinol (10 mg/kg PO q12–24h for long-term)	Variable (partial resolution of clinical signs to clinical cure)	See meglumine antimoniate and allopurinol monotherapy	Proposed for FeL refractory cases	Pimenta et al. (2015); Basso et al. (2016); Pereira et al. (2019); Fernandez-Gallego et al. (2020)
	Meglumine antimoniate (5 mg/kg SC q24h)	Resolution of lesions	Not reported; see meglumine antimoniate	According to BSAVA (2020) ketoconazole is not recommended for cats	Hervás et al. (1999)

plus ketoconazole (10 mg/kg q24h); 3 cycles of 4 weeks, 10 days apart		monotherapy		
Metronidazole (25 mg/kg PO q24h for 35 days) plus spiramycin (150,000 IU/kg PO q24h for 35 days)	No response	Not reported		Pennisi et al. (2004)
Miltefosine (2 mg/kg PO q24h for 28 days) plus N-AHCC (½ tablet once daily for long-term)	Resolution of clinical signs	Transient vomiting associated with miltefosine administration	Miltefosine licenced formulations for CanL contain propylene glycol which can hypothetically induce Heinz body haemolytic anaemia in cats (Pennisi & Persichetti, 2018)	Leal et al. (2018)
Miltefosine (2 mg/kg PO q24h for 28 days) plus allopurinol (10 mg/kg PO q12 for long-term)	No response	See previous line	See previous line	Fernandez-Gallego et al. (2020)

[a] Reported during treatment of cats with clinical leishmaniosis.
[b] Associated with high doses (50 mg/kg q24h).
Abbreviations: AHCC, active hexose correlated compounds; AKI, acute kidney injury; CanL, canine leishmanosis; FeL, feline leishmaniosis; IU, internacional unit; PO, *per os*; SC, subcutaneous.


Records identified through database searching
(n = 435)
Records screened
(n = 435)
Records excluded
(n = 274)
Original articles unrelated with Leishmania infection in cats (Felis spp.) (n = 191)
Review/comment/opinion articles/guidelines (n = 65)
Article not available (n = 9)
Erratum (n = 2)
Case reports of feline leishmaniosis with ambiguous identification of parasites at species level (n = 2)
Epidemiological studies with ambiguous information about cat's (Felis spp.) geographical origin (n = 1)
Feline experimental infection studies with complex species other than Leishmania donovani sensu lato (n = 1)
Original articles with ambiguous information about the detection of Leishmania spp. in cats (Felis spp.) (n = 1)
Unclear identification of host species (n = 1)
Duplicate data (n = 1)
Records included
(n = 161)
Experimental research
(n = 8)
Clinical research
(n = 36)
Epidemiological research
(n = 117)

Turkey

Bosnia-Herzegovina

Switzerland

France

Portugal

USA

Spain

Italy

Greece

French Guiana

Venezuela

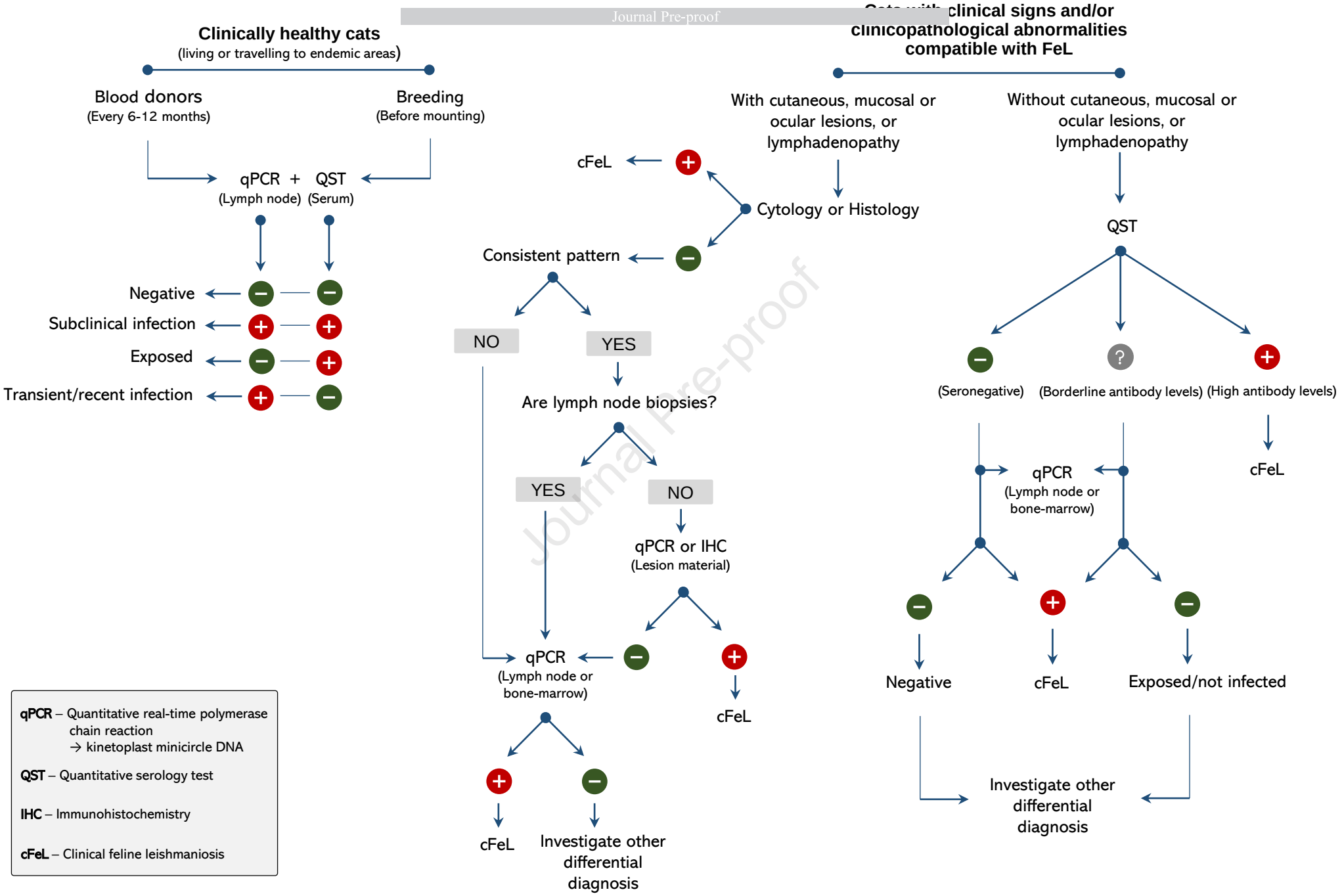
Brazil

Cyprus

Iran

Israel

- Domestic cat (*Felis catus*)
- Wild cat (*Felis silvestris*)
- *L.* (*L.*) *amazonensis*
- *L.* (*L.*) *donovani*
- *L.* (*L.*) *major*
- *L.* (*L.*) *mexicana*
- *L.* (*L.*) *tropica*
- *L.* (*L.*) *venezuelensis*
- *L.* (*V.*) *braziliensis*
- Putative *L. major*/*L. donovani* hybrid
- *Leishmania* DNA or anti-*Leishmania* antibodies detected in cats



Highlights

- A comprehensive review on epidemiology, immunopathogenesis, diagnosis, treatment, and prevention of feline leishmaniosis.
- An algorithm for assisting medical diagnosis of leishmaniosis in cats is suggested.
- Guidelines for the prevention of *Leishmania* infection in cats are provided.
- Dermatological lesions are the most common clinical manifestations.
- Most cats with clinical leishmaniosis present hypergammaglobulinemia.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.