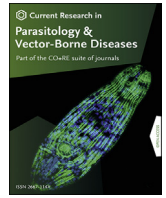


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## *Leishmania* infection in cats and feline leishmaniosis: An updated review with a proposal of a diagnosis algorithm and prevention guidelines



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### 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 sand flies. 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 in 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 herein proposed. 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.

### 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 “leishmaniasies” [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

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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 Moraes et al., 2013; Benassi et al., 2017; Metzendorf 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, 2010, 2014, 2015b; Tabar et al., 2008; Millán et al., 2011; Chatzis et al., 2014a; 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; Pralong 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; Mohebbi 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); and
- (vii) *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 (mainly 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

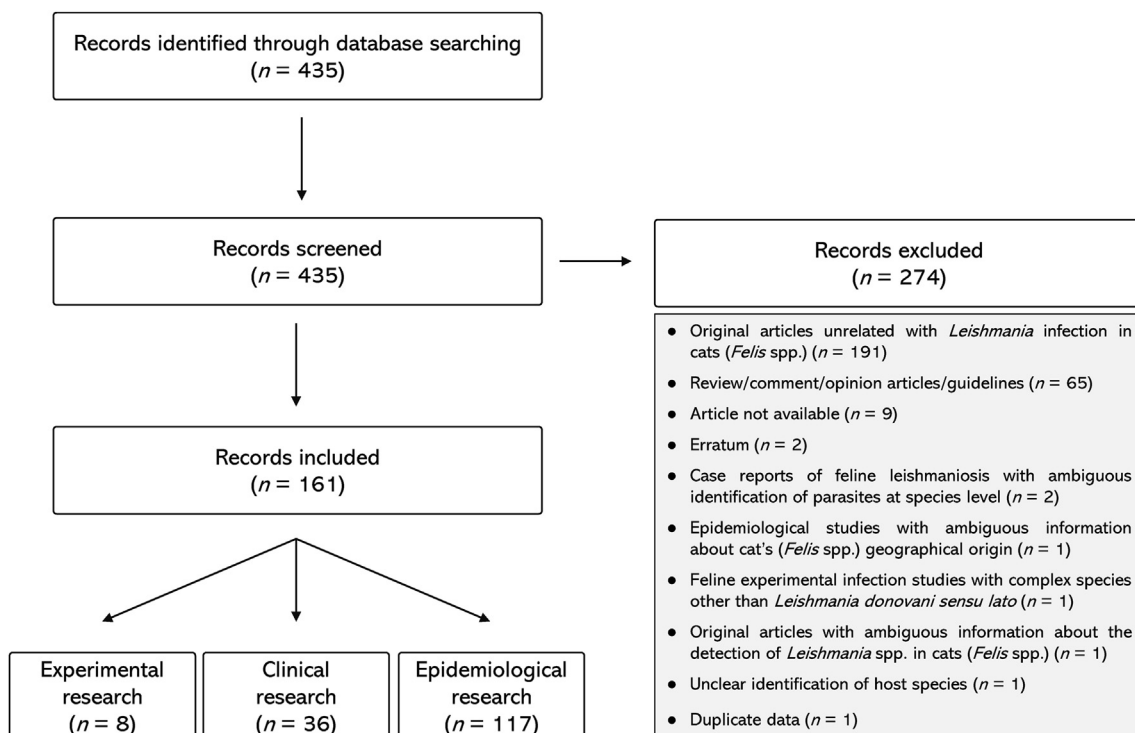


Fig. 1 Flow diagram of study searching and selection process

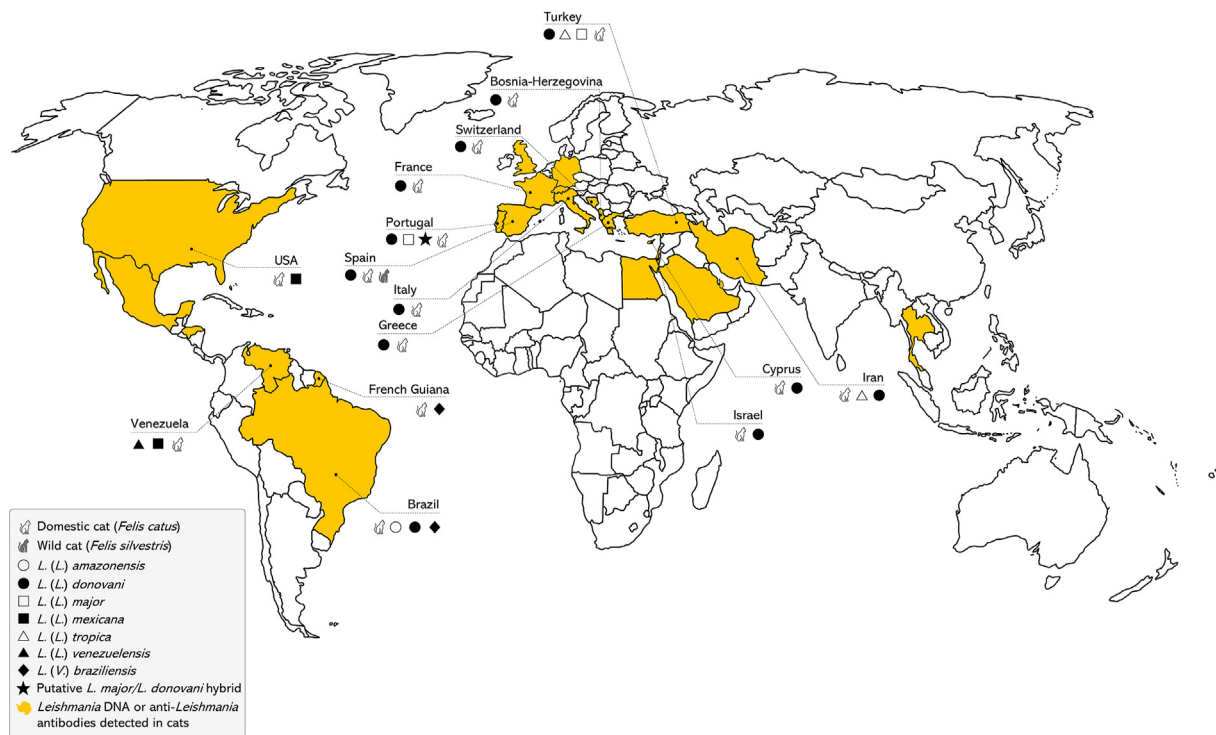


Fig. 2 Worldwide distribution of *Leishmania* infection in cats (*Felis* spp.)

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, leukopenia, 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 leukemia 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- $\gamma$ ), tumour necrosis factor-alpha (TNF- $\alpha$ ), 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- $\gamma$  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

**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) <sup>a</sup>                                                                                                                                                            |
|------------------------|------------------------------|---------------|------------------------------------|----------------|---------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Albania                | Silaghi et al. (2014)        | 2008–2010     | <i>F. catus</i> (stray)            | 146            | Serological (IFAT, 1:64)<br>Molecular (qPCR, kDNA)                                                            | Serum<br>Whole blood                                                              | 0.7 ( <i>L. infantum</i> )<br>0                                                                                                                                                              |
| Angola                 | Lopes et al. (2017)          | 2014–2016     | <i>F. catus</i> (domestic)         | 102            | Serological (DAT, 1:100)                                                                                      | Serum                                                                             | 0                                                                                                                                                                                            |
| Bosnia and Herzegovina | Colella et al. (2019)        | 2017          | <i>F. catus</i> (domestic)         | 5              | Serological (IFAT)<br>Molecular (qPCR, kDNA)                                                                  | Serum<br>Whole blood                                                              | 0<br>20.0 ( <i>Leishmania</i> spp.)                                                                                                                                                          |
|                        |                              |               |                                    | 1 <sup>b</sup> | Molecular (PCR, kDNA)<br>Molecular (qPCR, ITS2)                                                               | Whole blood<br>Whole blood                                                        | 100 ( <i>L. infantum</i> )<br>100 ( <i>L. infantum</i> )                                                                                                                                     |
| Cyprus                 | Attipa et al. (2017a,b)      | 2014          | <i>F. catus</i> (domestic/shelter) | 164            | Serological (ELISA, 32 EU)<br>Molecular (qPCR, kDNA)                                                          | Serum<br>Whole blood                                                              | 4.4 ( <i>L. infantum</i> )<br>2.3 ( <i>L. infantum</i> )                                                                                                                                     |
| Egypt                  | Michael et al. (1982)        | na            | <i>F. catus</i> (stray)            | 80             | Serological (IHA)                                                                                             | Serum                                                                             | 3.8 ( <i>Leishmania</i> spp.)                                                                                                                                                                |
|                        | Morsy et al. (1988)          | na            | <i>F. catus</i> (stray)            | 28             | Serological (IHA)                                                                                             | Serum                                                                             | 3.6 ( <i>Leishmania</i> spp.)                                                                                                                                                                |
|                        | Morsy & Abou el Seoud (1994) | na            | <i>F. catus</i> (domestic/stray)   | 60             | Serological (IHA, 1:32)                                                                                       | Serum                                                                             | 10.0 ( <i>Leishmania</i> spp.)                                                                                                                                                               |
| Germany                | Schäfer et al. (2021)        | 2012–2020     | <i>F. catus</i> (domestic)         | 624            | Serological (IFAT, 1:64)                                                                                      | Serum                                                                             | 4.0 ( <i>Leishmania</i> spp.)                                                                                                                                                                |
| Greece                 | Chatzis et al. (2014a, b)    | 2009–2011     | <i>F. catus</i> (domestic)         | 100            | Parasitological (cytology)<br>Serological (ELISA, 0.145)<br>Serological (IFAT, 1:10)<br>Molecular (PCR, kDNA) | Bone marrow<br>Lymph node<br>Skin<br>Serum<br>Serum<br>Bone marrow<br>Whole blood | 0<br>0<br>0<br>1.0 ( <i>Leishmania</i> spp.)<br>10.0 ( <i>Leishmania</i> spp.)<br>16.0 ( <i>L. infantum</i> )<br>13.0 ( <i>L. infantum</i> )                                                 |
|                        |                              |               |                                    | 99             | Molecular (PCR, kDNA)                                                                                         | Skin                                                                              | 13.1 ( <i>L. infantum</i> )                                                                                                                                                                  |
|                        |                              |               |                                    | 96             |                                                                                                               | Conjunctival swab                                                                 | 3.1 ( <i>L. infantum</i> )                                                                                                                                                                   |
|                        | Diakou et al. (2017)         | 2015          | <i>F. catus</i> (stray)            | 148            | Serological (IFAT, 1:80)<br>Molecular (nPCR, SSU)                                                             | Serum<br>Whole blood                                                              | 6.1 ( <i>L. infantum</i> )<br>6.1 ( <i>L. infantum</i> )                                                                                                                                     |
|                        | Diakou et al. (2009)         | na            | <i>F. catus</i> (stray)            | 284            | Serological (ELISA)                                                                                           | Serum                                                                             | 3.9 ( <i>Leishmania</i> spp.)                                                                                                                                                                |
|                        | Morelli et al. (2020)        | na            | <i>F. catus</i>                    | 153            | Serological (IFAT, 1:80)                                                                                      | Serum                                                                             | 2.0 ( <i>L. infantum</i> )                                                                                                                                                                   |
| Iran                   | Mohebbali et al. (2017)      | 2013–2015     | <i>F. catus</i> (stray)            | 103            | Serological (DAT, 1:320)                                                                                      | Serum                                                                             | 3.9 ( <i>L. infantum</i> )                                                                                                                                                                   |
|                        |                              |               |                                    | 4 <sup>b</sup> | Parasitological (cytology)                                                                                    | Liver<br>Spleen                                                                   | 25.0 ( <i>L. infantum</i> )<br>25.0 ( <i>L. infantum</i> )                                                                                                                                   |
|                        |                              |               |                                    | 4 <sup>b</sup> | Parasitological (culture)                                                                                     | Liver<br>Spleen                                                                   | 0<br>0                                                                                                                                                                                       |
|                        |                              |               |                                    | 1 <sup>b</sup> | Molecular (nPCR, ITS2)                                                                                        | Liver<br>Spleen                                                                   | 100 ( <i>L. infantum</i> )<br>100 ( <i>L. infantum</i> )                                                                                                                                     |
|                        | Akhtardanesh et al. (2020)   | 2016          | <i>F. catus</i> (stray)            | 180            | Molecular (nPCR, kDNA)                                                                                        | Whole blood                                                                       | 13.9 ( <i>L. infantum</i> )                                                                                                                                                                  |
|                        | Asgari et al. (2020)         | 2016–2018     | <i>F. catus</i> (stray)            | 174            | Serological (DAT, 1:100)<br>Serological (ELISA)<br>Molecular (nPCR, kDNA)                                     | Serum<br>Serum<br>Buffy coat                                                      | 17.2 ( <i>L. infantum</i> )<br>27.6 ( <i>L. infantum</i> )<br>20.7 ( <i>L. infantum</i> )                                                                                                    |
|                        | Sarkari et al. (2009)        | na            | <i>F. catus</i> (stray)            | 40             | Serological (DAT, 1:20)<br>Serological (IFAT, 1:10)                                                           | Serum<br>Serum                                                                    | 20.0 ( <i>L. infantum</i> )<br>25.0 ( <i>L. infantum</i> )                                                                                                                                   |
|                        | Hatam et al. (2010)          | na            | <i>F. catus</i> (stray)            | 40             | Parasitological (cytology)<br>Parasitological (culture)<br>Molecular (PCR, kDNA)                              | Liver<br>Spleen<br>Liver<br>Spleen                                                | 2.5 ( <i>Leishmania</i> spp.)<br>2.5 ( <i>Leishmania</i> spp.)<br>7.5 ( <i>Leishmania</i> spp.)<br>2.5 ( <i>Leishmania</i> spp.)<br>7.5 ( <i>L. infantum</i> )<br>5.0 ( <i>L. infantum</i> ) |
|                        | Fatollahzadeh et al. (2016)  | na            | <i>F. catus</i> (stray)            | 65             | Parasitological (cytology)<br>Parasitological (culture)                                                       | Liver<br>Spleen<br>Liver<br>Spleen                                                | 0<br>0<br>0<br>0                                                                                                                                                                             |
|                        |                              |               |                                    |                | Serological (DAT, 1:320)<br>Molecular (PCR, kDNA)                                                             | Serum<br>Spleen                                                                   | 23.1 ( <i>L. infantum</i> )<br>0                                                                                                                                                             |
|                        | Akhtardanesh et al. (2017)   | na            | <i>F. catus</i> (stray)            | 60             | Serological (ELISA)<br>Molecular (nPCR, 7SL RNA)                                                              | Serum<br>Whole blood                                                              | 6.7 ( <i>L. infantum</i> )<br>16.7 ( <i>L. infantum</i> )<br>1.7 ( <i>L. tropica</i> )                                                                                                       |
| Iraq                   | Otranto et al. (2019)        | 2008          | <i>F. catus</i> (stray)            | 207            | Molecular (qPCR, kDNA)                                                                                        | Whole blood                                                                       | 0                                                                                                                                                                                            |
| Israel                 | Nasereddin et al. (2008)     | 1999–2000     | <i>F. catus</i> (domestic/stray)   | 104            | Serological (ELISA)                                                                                           | Serum                                                                             | 6.7 ( <i>L. infantum</i> )                                                                                                                                                                   |
|                        | Baneth et al. (2020)         | 2018          | <i>F. catus</i> (shelter)          | 67             | Serological (ELISA, 0.4)                                                                                      | Serum                                                                             | 75.0 ( <i>L. infantum</i> )                                                                                                                                                                  |

Table 1 (continued)

| Country                   | Study                     | Sampling year              | Species (origin)                 | No. tested                 | Method (test, cut-off/target gene) | Sample                        | % Positive (species) <sup>a</sup> |
|---------------------------|---------------------------|----------------------------|----------------------------------|----------------------------|------------------------------------|-------------------------------|-----------------------------------|
| Italy                     | Vita et al. (2005)        | 2002–2004                  | <i>F. catus</i> (domestic/stray) | 203                        | Molecular (qPCR, kDNA)             | Whole blood                   | 16.0 ( <i>L. infantum</i> )       |
|                           |                           |                            |                                  |                            | Molecular (HRM-PCR, ITS1)          | Whole blood                   | 0                                 |
|                           | Spada et al. (2013)       | 2008–2010                  | <i>F. catus</i> (stray)          | 233                        | Serological (IFAT, 1:40)           | Lymph node                    | 16.3 ( <i>L. infantum</i> )       |
|                           |                           |                            |                                  |                            | Molecular (PCR)                    | Whole blood                   | 100 ( <i>L. infantum</i> )        |
|                           | Morganti et al. (2019)    | 2010–2016                  | <i>F. catus</i> (shelter/stray)  | 286                        | Serological (IFAT, 1:40)           | Serum                         | 45.5 ( <i>L. infantum</i> )       |
|                           |                           |                            |                                  |                            | Molecular (qPCR, kDNA)             | Whole blood                   | 25.3 ( <i>L. infantum</i> )       |
|                           | Dedola et al. (2018)      | 2011–2013                  | <i>F. catus</i> (domestic)       | 90                         | Serological (IFAT, 1:40)           | Buffy coat                    | 9.1 ( <i>L. infantum</i> )        |
|                           |                           |                            |                                  |                            | Molecular (nPCR, ITS)              | Conjunctival swab             | 0                                 |
|                           | Veronesi et al. (2016)    | 2011–2014                  | <i>F. silvestris</i> (wild)      | 21                         | Serological (IFAT, 1:80)           | Serum                         | 15.7 ( <i>L. infantum</i> )       |
|                           |                           |                            |                                  |                            | Molecular (qPCR, COII)             | Whole blood                   | 10.0 ( <i>L. infantum</i> )       |
|                           | Persichetti et al. (2016) | 2012–2013                  | <i>F. catus</i> (domestic)       | 42                         | Serological (IFAT, 1:80)           | Spleen                        | 5.5 ( <i>L. infantum</i> )        |
|                           |                           |                            |                                  |                            | Molecular (qPCR, kDNA)             | Serum                         | 0                                 |
|                           | Persichetti et al. (2018) | 2012–2013                  | <i>F. catus</i> (domestic)       | 197                        | Parasitological (cytology)         | Whole blood                   | 2.4 ( <i>L. infantum</i> )        |
|                           |                           |                            |                                  |                            | Serological (IFAT, 1:80)           | Whole blood                   | 42.8 ( <i>L. infantum</i> )       |
|                           | Spada et al. (2016)       | 2014                       | <i>F. catus</i> (stray)          | 90                         | Molecular (qPCR, kDNA)             | Conjunctival swab             | 0                                 |
|                           |                           |                            |                                  |                            | Serological (IFAT, 1:40)           | Lymph node                    | 9.6 ( <i>L. infantum</i> )        |
|                           | Brianti et al. (2017)     | 2015                       | <i>F. catus</i> (domestic)       | 159                        | Molecular (qPCR, kDNA)             | Urine                         | 1.5 ( <i>L. infantum</i> )        |
|                           |                           |                            |                                  |                            | Serological (IFAT, 1:80)           | Oral swab                     | 1.7 ( <i>L. infantum</i> )        |
|                           | Otranto et al. (2017)     | 2015–2016                  | <i>F. catus</i> (domestic)       | 330                        | Molecular (qPCR, kDNA)             | Whole blood                   | 2.1 ( <i>L. infantum</i> )        |
|                           |                           |                            |                                  |                            | Serological (IFAT, 1:40)           | Whole blood                   | 1.5 ( <i>L. infantum</i> )        |
|                           | Abbate et al. (2019)      | 2015–2017                  | <i>F. silvestris</i> (wild)      | 11                         | Molecular (qPCR, kDNA)             | Whole blood                   | 2.0 ( <i>L. infantum</i> )        |
|                           |                           |                            |                                  |                            | Serological (IFAT, 1:80)           | Serum                         | 30.0 ( <i>L. infantum</i> )       |
|                           | Priolo et al. (2019)      | 2016–2017                  | <i>F. catus</i> (domestic/stray) | 66                         | Molecular (qPCR, kDNA)             | Conjunctival swab             | 0                                 |
|                           |                           |                            |                                  |                            | Serological (ELISA)                | Lymph node                    | 1.1 ( <i>L. infantum</i> )        |
|                           | Spada et al. (2020)       | 2016–2018                  | <i>F. catus</i> (stray)          | 102                        | Serology (IFAT, 1:80)              | Whole blood                   | 1.1 ( <i>L. infantum</i> )        |
|                           |                           |                            |                                  |                            | Molecular (qPCR, kDNA)             | Whole blood                   | 1.1 ( <i>L. infantum</i> )        |
|                           | Urbani et al. (2020)      | 2017                       | <i>F. catus</i> (domestic)       | 152                        | Molecular (qPCR, kDNA)             | Serum                         | 9.4 ( <i>L. infantum</i> )        |
|                           |                           |                            |                                  |                            | Serological (IFAT, 1:80)           | Conjunctival swab             | 3.8 ( <i>L. infantum</i> )        |
|                           | Iatta et al. (2019)       | 2017–2018                  | <i>F. catus</i> (domestic)       | 2,659                      | Molecular (qPCR, kDNA)             | Whole blood                   | 7.5 ( <i>L. infantum</i> )        |
|                           |                           |                            |                                  |                            | Serological (IFAT, 1:80)           | Serum                         | 25.7 ( <i>L. infantum</i> )       |
|                           | Ebani et al. (2020)       | 2018–2019                  | <i>F. catus</i> (stray)          | 85                         | Molecular (qPCR, kDNA)             | Conjunctival swab             | 1.8 ( <i>L. infantum</i> )        |
|                           |                           |                            |                                  |                            | Serological (IFAT)                 | Whole blood                   | 2.1 ( <i>L. infantum</i> )        |
| Persichetti et al. (2017) | 2013                      | na                         | 76                               | Serological (ELISA, 40 EU) | Lymph node/skin/spleen             | 0                             |                                   |
|                           |                           |                            |                                  | Serological (IFAT, 1:80)   | Serum                              | 17.0 ( <i>L. infantum</i> )   |                                   |
| Poli et al. (2002)        | na                        | <i>F. catus</i> (domestic) | 110                              | Serological (IFAT, 1:80)   | Serum                              | 14.0 ( <i>L. infantum</i> )   |                                   |
|                           |                           |                            |                                  | Serological (WB)           | Whole blood                        | 4.0 ( <i>L. infantum</i> )    |                                   |
| Morelli et al. (2019)     | na                        | <i>F. catus</i> (domestic) | 167                              | Molecular (qPCR, kDNA)     | Serum                              | 4.9 ( <i>L. infantum</i> )    |                                   |
|                           |                           |                            |                                  | Serology (IFAT, 1:80)      | Conjunctival swab                  | 0                             |                                   |
| Morelli et al. (2020)     | na                        | <i>F. catus</i>            | 116                              | Molecular (qPCR, kDNA)     | Lymph node                         | 4.3 ( <i>L. infantum</i> )    |                                   |
|                           |                           |                            |                                  | Serological (IFAT, 1:80)   | Whole blood                        | 0                             |                                   |
| Persichetti et al. (2017) | 2013                      | na                         | 21 <sup>b</sup>                  | Serological (IFAT, 1:80)   | Serum                              | 11.8 ( <i>L. infantum</i> )   |                                   |
|                           |                           |                            |                                  | Serological (WB)           | Conjunctival swab                  | 0                             |                                   |
| Persichetti et al. (2017) | 2013                      | na                         | 21 <sup>b</sup>                  | Serological (ELISA, 40 EU) | Hair                               | 0.7 ( <i>L. infantum</i> )    |                                   |
|                           |                           |                            |                                  | Serological (IFAT, 1:80)   | Whole blood                        | 0                             |                                   |
| Persichetti et al. (2017) | 2013                      | na                         | 21 <sup>b</sup>                  | Serological (IFAT, 1:80)   | Serum                              | 3.3 ( <i>L. infantum</i> )    |                                   |
|                           |                           |                            |                                  | Serological (WB)           | Whole blood                        | 0.8 ( <i>L. infantum</i> )    |                                   |
| Persichetti et al. (2017) | 2013                      | na                         | 21 <sup>b</sup>                  | Serological (ELISA, 40 EU) | Serum                              | 2.4 ( <i>Leishmania</i> spp.) |                                   |
|                           |                           |                            |                                  | Serological (IFAT, 1:80)   | Blood <sup>c</sup>                 | 5.9 ( <i>Leishmania</i> spp.) |                                   |
| Poli et al. (2002)        | na                        | <i>F. catus</i> (domestic) | 110                              | Serological (IFAT, 1:80)   | Serum                              | 2.6 ( <i>L. infantum</i> )    |                                   |
|                           |                           |                            |                                  | Serological (WB)           | Serum                              | 17.1 ( <i>L. infantum</i> )   |                                   |
| Morelli et al. (2019)     | na                        | <i>F. catus</i> (domestic) | 167                              | Serological (IFAT, 1:80)   | Serum                              | 18.4 ( <i>L. infantum</i> )   |                                   |
|                           |                           |                            |                                  | Serological (WB)           | Serum                              | 100 ( <i>L. infantum</i> )    |                                   |
| Morelli et al. (2020)     | na                        | <i>F. catus</i>            | 116                              | Serological (IFAT, 1:80)   | Serum                              | 95.2 ( <i>L. infantum</i> )   |                                   |
|                           |                           |                            |                                  | Serological (IFAT, 1:80)   | Serum                              | 100 ( <i>L. infantum</i> )    |                                   |
| Morelli et al. (2020)     | na                        | <i>F. catus</i>            | 116                              | Serological (IFAT, 1:80)   | Serum                              | 0.9 ( <i>Leishmania</i> spp.) |                                   |
|                           |                           |                            |                                  | Serological (IFAT, 1:80)   | Serum                              | 3.0 ( <i>L. infantum</i> )    |                                   |
| Morelli et al. (2020)     | na                        | <i>F. catus</i>            | 116                              | Serological (IFAT, 1:80)   | Serum                              | 4.3 ( <i>L. infantum</i> )    |                                   |
|                           |                           |                            |                                  | Serological (IFAT, 1:80)   | Serum                              | 4.3 ( <i>L. infantum</i> )    |                                   |

(continued on next page)

Table 1 (continued)

| Country                 | Study                                        | Sampling year               | Species (origin)                                         | No. tested                      | Method (test, cut-off/target gene)                 | Sample                                                                                                                                 | % Positive (species) <sup>a</sup>                                                                                                                                |                                |
|-------------------------|----------------------------------------------|-----------------------------|----------------------------------------------------------|---------------------------------|----------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------|
| Portugal                | Duarte et al. (2010)<br>Maia et al. (2008)   | 2003–2005                   | <i>F. catus</i> (stray)                                  | 180                             | Serology (IFAT, 1:40)                              | Serum                                                                                                                                  | 0.6 ( <i>L. infantum</i> )                                                                                                                                       |                                |
|                         |                                              | 2004                        | <i>F. catus</i> (stray)                                  | 20                              | Serological (IFAT, 1:64)                           | Serum                                                                                                                                  | 0                                                                                                                                                                |                                |
|                         |                                              |                             |                                                          |                                 | 23                                                 | Molecular (PCR, ITS1)                                                                                                                  | Blood on filter paper                                                                                                                                            | 30.4 ( <i>Leishmania</i> spp.) |
|                         |                                              |                             |                                                          |                                 |                                                    | Molecular (PCR, kDNA)                                                                                                                  | Blood on filter paper                                                                                                                                            | 30.4 ( <i>Leishmania</i> spp.) |
|                         |                                              |                             |                                                          |                                 | 4 <sup>b</sup>                                     | Molecular (PCR–RFLP, ITS1)                                                                                                             | Blood on filter paper                                                                                                                                            | 100 ( <i>L. infantum</i> )     |
|                         | Cardoso et al. (2010)                        | 2004–2008                   | <i>F. catus</i> (domestic)                               | 316                             | Serological (DAT, 1:100)                           | Serum                                                                                                                                  | 1.9 ( <i>L. infantum</i> )                                                                                                                                       |                                |
|                         |                                              |                             |                                                          |                                 |                                                    | Serological (ELISA)                                                                                                                    | Serum                                                                                                                                                            | 2.8 ( <i>L. infantum</i> )     |
|                         | Maia et al. (2010)                           | 2007–2008                   | <i>F. catus</i> (domestic/stray)                         | 76                              | Serological (IFAT, 1:64)                           | Serum                                                                                                                                  | 1.3 ( <i>Leishmania</i> spp.)                                                                                                                                    |                                |
|                         |                                              |                             |                                                          | 138                             | Molecular (PCR, kDNA)                              | Whole blood                                                                                                                            | 20.3 ( <i>L. infantum</i> )                                                                                                                                      |                                |
|                         | Maia et al. (2015a,b)                        | 2011–2014                   | <i>F. catus</i> (domestic/stray)                         | 271                             | Serological (DAT, 1:100)                           | Serum                                                                                                                                  | 3.7 ( <i>L. infantum</i> )                                                                                                                                       |                                |
|                         | Maia et al. (2014)                           | 2012–2013                   | <i>F. catus</i> (domestic/stray)                         | 649                             | Molecular (nPCR, SSU)                              | Whole blood                                                                                                                            | 9.9 ( <i>Leishmania</i> spp.)                                                                                                                                    |                                |
|                         | Pereira et al. (2019a, b, 2020)              | 2017–2018                   | <i>F. catus</i> (domestic/shelter/stray)                 | 373                             | Serological (IFAT, 1:64)                           | Serum                                                                                                                                  | 1.6 ( <i>Leishmania</i> spp.)                                                                                                                                    |                                |
|                         |                                              |                             |                                                          | 465                             | Molecular (nPCR, SSU)                              | Buffy coat                                                                                                                             | 5.4 ( <i>Leishmania</i> spp.)                                                                                                                                    |                                |
|                         |                                              |                             |                                                          | 25 <sup>b</sup>                 | Molecular (nPCR, <i>cytB</i> )                     | Buffy coat                                                                                                                             | 12.0 ( <i>L. donovani</i> s.l.)<br>4.0 ( <i>L. major</i> )<br>4.0 ( <i>L. major</i> /<br><i>L. donovani</i> s.l.) <sup>f</sup><br>4.0 ( <i>L. donovani</i> s.l.) |                                |
|                         |                                              |                             |                                                          |                                 | Molecular (PCR, <i>g6pdh</i> )                     | Buffy coat                                                                                                                             | 4.0 ( <i>L. donovani</i> s.l.)                                                                                                                                   |                                |
|                         |                                              |                             |                                                          | Molecular (nPCR, <i>hsp70</i> ) | Buffy coat                                         | 12.0 ( <i>L. donovani</i> s.l.)<br>4.0 ( <i>L. major</i> /<br><i>L. donovani</i> s.l.) <sup>f</sup><br>12.0 ( <i>L. donovani</i> s.l.) |                                                                                                                                                                  |                                |
|                         |                                              |                             |                                                          | Molecular (nPCR, ITS)           | Buffy coat                                         | 4.0 ( <i>L. major</i> /<br><i>L. donovani</i> s.l.) <sup>f</sup><br>12.0 ( <i>L. donovani</i> s.l.)<br>4.0 ( <i>L. major</i> )         |                                                                                                                                                                  |                                |
|                         | Neves et al. (2020)<br>Vilhena et al. (2013) | 2018–2019<br>na             | <i>F. catus</i> (domestic)<br><i>F. catus</i> (domestic) | 141<br>320                      | Serological (DAT, 1:100)<br>Molecular (qPCR, kDNA) | Serum<br>Whole blood                                                                                                                   | 0<br>0.3 ( <i>L. infantum</i> )                                                                                                                                  |                                |
| Portugal/Spain          | Mesa-Sanchez et al. (2020)                   | 2015–2020                   | <i>F. catus</i> (domestic) <sup>g</sup>                  | 173                             | Molecular (nPCR, SSU)                              | Whole blood                                                                                                                            | 0                                                                                                                                                                |                                |
| Qatar                   | Lima et al. (2019)                           | 2016–2018                   | <i>F. catus</i> (domestic/stray)                         | 79                              | Molecular (qPCR, kDNA)                             | Whole blood/on dried spot                                                                                                              | 1.3 ( <i>Leishmania</i> spp.)                                                                                                                                    |                                |
| Saudi Arabia            | Morsy et al. (1999)                          | na                          | <i>F. margarita</i> (wild)                               | 10                              | Parasitological (cytology)                         | Liver                                                                                                                                  | 20.0 ( <i>Leishmania</i> spp.)                                                                                                                                   |                                |
|                         |                                              |                             |                                                          |                                 |                                                    | Spleen                                                                                                                                 | 40.0 ( <i>Leishmania</i> spp.)                                                                                                                                   |                                |
|                         |                                              |                             |                                                          |                                 | Serological (IHA, 1:64)                            | Serum                                                                                                                                  | 40.0 ( <i>Leishmania</i> spp.)                                                                                                                                   |                                |
| Spain                   | Del Río et al. (2014)                        | 2001–2006                   | <i>Felis silvestris</i> (wild)                           | 4                               | Molecular (qPCR, kDNA)                             | Liver and/or spleen                                                                                                                    | 25.0 ( <i>L. infantum</i> )                                                                                                                                      |                                |
|                         |                                              |                             |                                                          | 1 <sup>b</sup>                  | Molecular (PCR, ITS2)                              | Liver and/or spleen                                                                                                                    | 100 ( <i>L. infantum</i> )                                                                                                                                       |                                |
|                         | Martín-Sánchez et al. (2007)                 | 2003–2004                   | <i>F. catus</i> (domestic)                               | 183                             | Serological (IFAT, 1:40)                           | Serum                                                                                                                                  | 28.3 ( <i>Leishmania</i> spp.)                                                                                                                                   |                                |
|                         |                                              |                             |                                                          |                                 | Molecular (PCR-ELISA, kDNA)                        | Whole blood                                                                                                                            | 25.7 ( <i>L. infantum</i> )                                                                                                                                      |                                |
|                         |                                              |                             |                                                          |                                 | 7 <sup>b</sup>                                     | Parasitological (culture)                                                                                                              | Leucoconcentrate                                                                                                                                                 | 0                              |
|                         |                                              |                             |                                                          |                                 |                                                    | Parasitological (cytology)                                                                                                             | Leucoconcentrate                                                                                                                                                 | 42.9 ( <i>Leishmania</i> spp.) |
|                         | Ayllón et al. (2008)                         | 2005–2006                   | <i>F. catus</i> (domestic)                               | 233                             | Serological (IFAT, 1:100)                          | Serum                                                                                                                                  | 1.3 ( <i>L. infantum</i> )                                                                                                                                       |                                |
|                         |                                              |                             |                                                          |                                 |                                                    | Molecular (PCR, kDNA)                                                                                                                  | Whole blood                                                                                                                                                      | 0.4 ( <i>L. infantum</i> )     |
|                         | Ayllón et al. (2012)                         | 2005–2008                   | <i>F. catus</i> (domestic/stray)                         | 680                             | Serological (IFAT, 1:50)                           | Serum                                                                                                                                  | 3.7 ( <i>L. infantum</i> )                                                                                                                                       |                                |
|                         |                                              |                             |                                                          |                                 |                                                    | Molecular (PCR, kDNA)                                                                                                                  | Whole blood                                                                                                                                                      | 0.6 ( <i>L. infantum</i> )     |
|                         | Tabar et al. (2008)                          | 2006                        | <i>F. catus</i> (domestic)                               | 100                             | Molecular (qPCR, kDNA)                             | Whole blood                                                                                                                            | 3.0 ( <i>L. infantum</i> )                                                                                                                                       |                                |
|                         | Sherry et al. (2011)                         | 2008                        | <i>F. catus</i> (shelter)                                | 105                             | Serological (ELISA)                                | Serum                                                                                                                                  | 13.2 ( <i>L. infantum</i> )                                                                                                                                      |                                |
|                         |                                              |                             |                                                          | 104                             | Molecular (qPCR, kDNA)                             | Whole blood                                                                                                                            | 8.7 ( <i>L. infantum</i> )                                                                                                                                       |                                |
|                         | Millán et al. (2011)                         | 2008–2009                   | <i>F. catus</i> (stray)                                  | 83                              | Serological (WB)                                   | Serum                                                                                                                                  | 15.7 ( <i>L. infantum</i> )                                                                                                                                      |                                |
|                         |                                              |                             |                                                          | 73                              | Molecular (PCR, kDNA)                              | Blood and/or spleen                                                                                                                    | 25.6 ( <i>L. infantum</i> )                                                                                                                                      |                                |
|                         |                                              |                             | 14 <sup>b</sup>                                          | Molecular (PCR–RFLP, kDNA)      | Blood and/or spleen                                | 100 ( <i>L. infantum</i> )                                                                                                             |                                                                                                                                                                  |                                |
| Miró et al. (2014)      | 2012–2013                                    | <i>F. catus</i> (stray)     | 346                                                      | Serological (IFAT, 1:100)       | Serum                                              | 3.2 ( <i>L. infantum</i> )                                                                                                             |                                                                                                                                                                  |                                |
|                         |                                              |                             | 57 <sup>d</sup>                                          | Molecular (nested PCR, ITS1)    | Whole blood                                        | 0                                                                                                                                      |                                                                                                                                                                  |                                |
|                         |                                              |                             |                                                          | Molecular (nested PCR, SSU)     | Whole blood                                        | 0                                                                                                                                      |                                                                                                                                                                  |                                |
| Risueño et al. (2018)   | 2013–2015                                    | <i>F. silvestris</i> (wild) | 2                                                        | Molecular (qPCR, kDNA)          | Skin<br>Other organs <sup>e</sup>                  | 50.0 ( <i>L. infantum</i> )<br>0                                                                                                       |                                                                                                                                                                  |                                |
| Marenzoni et al. (2018) | 2014–2015                                    | <i>F. catus</i> (domestic)  | 31 <sup>g</sup>                                          | Molecular (PCR, kDNA)           | Whole blood                                        | 0                                                                                                                                      |                                                                                                                                                                  |                                |

Table 1 (continued)

| Country    | Study                        | Sampling year | Species (origin)                   | No. tested     | Method (test, cut-off/target gene) | Sample            | % Positive (species) <sup>a</sup> |
|------------|------------------------------|---------------|------------------------------------|----------------|------------------------------------|-------------------|-----------------------------------|
|            | Montoya et al. (2018a)       | 2014–2017     | <i>F. catus</i> (stray)            | 249            | Serological (IFAT, 1:100)          | Serum             | 4.8 ( <i>L. infantum</i> )        |
|            | Priolo et al. (2019)         | 2016–2017     | <i>F. catus</i> (domestic/stray)   | 113            | Molecular (PCR, ITS)               | Skin/whole blood  | 0                                 |
|            |                              |               |                                    |                | Serological (ELISA)                | Serum             | 7.0 ( <i>L. infantum</i> )        |
|            |                              |               |                                    |                | Serological (IFAT, 1:80)           | Serum             | 19.0 ( <i>L. infantum</i> )       |
|            |                              |               |                                    |                | Molecular (qPCR, kDNA)             | Whole blood       | 5.0 ( <i>L. infantum</i> )        |
|            | Villanueva-Saz et al. (2021) | 2020          | <i>F. catus</i> (stray)            | 114            | Serological (ELISA, 13 EU)         | Serum             | 16.7 ( <i>L. infantum</i> )       |
|            | Solano-Gallego et al. (2007) | na            | <i>F. catus</i> (domestic/stray)   | 445            | Serological (ELISA-IgG, 53 EU)     | Serum             | 5.3 ( <i>L. infantum</i> )        |
|            |                              | na            |                                    |                | Serological (ELISA-Prot A, 44 EU)  | Serum             | 6.3 ( <i>L. infantum</i> )        |
|            | Alcover et al. (2020)        | na            | <i>F. catus</i> (wild)             | 1              | Molecular (qPCR, kDNA)             | Liver             | 100 ( <i>Leishmania</i> spp.)     |
|            |                              | na            |                                    |                |                                    | Skin              | 100 ( <i>Leishmania</i> spp.)     |
|            |                              | na            |                                    |                |                                    | Spleen            | 100 ( <i>Leishmania</i> spp.)     |
|            | Miró et al. (2011)           | na            | <i>F. catus</i> (breeding)         | 20             | Serological (IFAT, 1:100)          | Serum             | 15.0 ( <i>L. infantum</i> )       |
|            | Moreno et al. (2014)         | na            | <i>F. catus</i> (stray)            | 43             | Serological (IFAT, 1:50)           | Serum             | 4.3 ( <i>L. infantum</i> )        |
|            | Montoya et al. (2018b)       | na            | <i>F. catus</i> (stray)            |                | Serological (IFAT, 1:100)          | Serum             | 0                                 |
| Thailand   | Sukmee et al. (2008)         | 2006          | <i>F. catus</i>                    | 15             | Serological (DAT; 1:100)           | Serum             | 60.0 ( <i>Leishmania</i> spp.)    |
|            |                              |               |                                    | 9 <sup>b</sup> | Molecular (PCR, ITS1)              | Whole blood       | 0                                 |
|            |                              |               |                                    |                | Molecular (PCR, kDNA)              | Whole blood       | 0                                 |
|            | Junsiri et al. (2017)        | 2013          | <i>F. catus</i> (domestic)         | 250            | Serological (ELISA, 0.2)           | Serum             | 5.6 ( <i>L. infantum</i> )        |
|            |                              |               |                                    |                | Molecular (PCR, kDNA)              | Whole blood       | 0                                 |
|            | Kongkaew et al. (2007)       | na            | <i>F. catus</i>                    | 5              | Serological (DAT, 1:100)           | Serum             | 20.0 ( <i>Leishmania</i> spp.)    |
|            |                              |               |                                    | 1 <sup>b</sup> | Molecular (PCR)                    | Whole blood       | 0                                 |
| Turkey     | Dincer et al. (2015)         | 2013          | <i>F. catus</i> (domestic/shelter) | 22             | Molecular (nPCR, kDNA)             | Whole blood       | 4.5 ( <i>L. infantum</i> )        |
|            | Karakuş et al. (2019)        | 2014          | <i>F. catus</i> (stray)            | 5              | Molecular (nPCR, SSU)              | Conjunctival swab | 0                                 |
|            |                              | 2015          |                                    | 8              | Molecular (qPCR, ITS1)             | Conjunctival swab | 12.5 ( <i>L. infantum</i> )       |
|            |                              | 2016          |                                    | 6              | Molecular (qPCR, ITS1)             | Conjunctival swab | 0                                 |
|            | Dincer et al. (2016)         | 2015          | <i>F. catus</i> (domestic/shelter) | 50             | Molecular (nPCR, kDNA)             | na                | 0                                 |
|            | Dinçer et al. (2012)         | na            | <i>F. catus</i> (domestic)         | 1              | Serological (IFAT)                 | Serum             | 0                                 |
|            |                              |               |                                    |                | Molecular (PCR)                    | na                | 0                                 |
|            | Paşa et al. (2015)           | na            | <i>F. catus</i> (domestic)         | 147            | Molecular (qPCR, ITS1)             | Whole blood       | 2.7 ( <i>L. major</i> )           |
|            |                              |               |                                    |                | Molecular (qPCR, <i>hsp70</i> )    | Whole blood       | 8.8 ( <i>L. tropica</i> )         |
|            |                              |               |                                    |                |                                    |                   | 2.0 ( <i>L. major</i> )           |
|            |                              |               |                                    |                |                                    |                   | 2.7 ( <i>L. tropica</i> )         |
|            |                              |               |                                    |                |                                    |                   | 2.7 ( <i>Leishmania</i> spp.)     |
|            | Can et al. (2016)            | na            | <i>F. catus</i> (shelter)          | 1,101          | Serological (ELISA)                | Serum             | 10.8                              |
|            |                              |               |                                    |                | Serological (IFAT, 1:40)           | Serum             | 15.2                              |
|            |                              |               |                                    |                | Molecular (qPCR, ITS1)             | Whole blood       | 0.1 ( <i>L. tropica</i> )         |
|            |                              |               |                                    |                | Molecular (nPCR, kDNA)             | Whole blood       | 0.1 ( <i>L. infantum</i> )        |
|            |                              |               |                                    |                |                                    |                   | 0.5 ( <i>L. tropica</i> )         |
| UK         | Persichetti et al. (2017)    | 2013          | <i>F. catus</i>                    | 64             | Serological (ELISA, 40 EU)         | Serum             | 1.6 ( <i>L. infantum</i> )        |
|            |                              |               |                                    |                | Serological (IFAT, 1:80)           | Serum             | 0                                 |
|            |                              |               |                                    |                | Serological (WB)                   | Serum             | 3.1 ( <i>L. infantum</i> )        |
| Uzbekistan | Kovalenko et al. (2011)      | na            | <i>F. catus</i>                    | 1              | Serological (ELISA)                | Serum             | 0                                 |

<sup>a</sup> Species defined according to the original study.

<sup>b</sup> Previously identified as positive by another test.

<sup>c</sup> DNA extracted from the sediment obtained after centrifugation of the blood samples.

<sup>d</sup> Seropositive for *L. infantum* and/or for feline retrovirus (feline leukemia virus and/or feline immunodeficiency virus).

<sup>e</sup> Not specified.

<sup>f</sup> Putative hybrid.

<sup>g</sup> 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; <sub>HRM</sub>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) <sup>a</sup> |
|--------------------------------|---------------------------------|------------------------------------|------------------------------------|----------------------------|------------------------------------|--------------------------------|-----------------------------------|
| Brazil                         | De Matos et al. (2018)          | 2004–2014                          | <i>F. catus</i>                    | 679                        | Serological (ELISA)                | Serum                          | 43.4 ( <i>Leishmania</i> spp.)    |
|                                |                                 |                                    |                                    |                            | Serological (IFAT, 1:40)           | Serum                          | 15.8 ( <i>Leishmania</i> spp.)    |
|                                | Figueiredo et al. (2009)        | 2005                               | <i>F. catus</i> (domestic)         | 43                         | Serological (ELISA)                | Serum                          | 2.4 ( <i>Leishmania</i> spp.)     |
|                                | Coelho et al. (2011a)           | 2007–2009                          | <i>F. catus</i>                    | 70                         | Serological (IFAT, 1:40)           | Serum                          | 0                                 |
|                                |                                 |                                    |                                    |                            | Serological (ELISA)                | Serum                          | 4.2 ( <i>Leishmania</i> spp.)     |
|                                | Vides et al. (2011)             | 2008–2009                          | <i>F. catus</i>                    | 55                         | Parasitological (cytology)         | Bone marrow                    | 0.0 ( <i>Leishmania</i> spp.)     |
|                                |                                 |                                    |                                    |                            |                                    | Liver                          | 12.7 ( <i>Leishmania</i> spp.)    |
|                                |                                 |                                    |                                    |                            |                                    | Lymph node                     | 3.6 ( <i>Leishmania</i> spp.)     |
|                                |                                 |                                    |                                    |                            |                                    | Spleen                         | 5.5 ( <i>Leishmania</i> spp.)     |
|                                |                                 |                                    |                                    |                            | Parasitological (IHC)              | Skin                           | 7.3 ( <i>Leishmania</i> spp.)     |
|                                |                                 |                                    |                                    |                            |                                    |                                | 16.4 ( <i>Leishmania</i> spp.)    |
|                                |                                 |                                    |                                    |                            |                                    | Serological (ELISA, 0.277)     | 25.4 ( <i>Leishmania</i> spp.)    |
|                                |                                 |                                    |                                    |                            |                                    | Serological (IFAT, 1:40)       | Serum                             |
|                                | Cardia et al. (2013)            | 2010                               | <i>F. catus</i> (shelter/stray)    | 3                          | Molecular (qPCR, <i>gp63</i> )     | Whole blood                    | 100 ( <i>L. chagasi</i> )         |
|                                |                                 |                                    |                                    |                            |                                    | Serological (IFAT, 1:40)       | Serum                             |
|                                | Silva et al. (2014)             | 2010                               | <i>F. catus</i> (domestic/shelter) | 153                        | Serological (ELISA)                | Serum                          | 3.9 ( <i>L. infantum</i> )        |
|                                | De Sousa Oliveira et al. (2015) | 2012                               | <i>F. catus</i>                    | 52                         | Molecular (PCR, kDNA)              | Conjunctival swab              | 13.5 ( <i>Leishmania</i> spp.)    |
|                                | de Sousa et al. (2014)          | 2013                               | <i>F. catus</i> (domestic/stray)   | 151                        | Serological (IFAT, 1:40)           | Serum                          | 6.6 ( <i>L. infantum</i> )        |
|                                | Metzdorf et al. (2017)          | 2013–2014                          | <i>F. catus</i> (domestic/shelter) | 100                        | Parasitological (cytology)         | Bone marrow                    | 4.0 ( <i>Leishmania</i> spp.)     |
|                                |                                 |                                    |                                    |                            |                                    | Lymph node                     | 4.0 ( <i>Leishmania</i> spp.)     |
|                                |                                 |                                    |                                    |                            |                                    | Whole blood                    | 4.0 ( <i>Leishmania</i> spp.)     |
|                                |                                 |                                    |                                    |                            |                                    | Bone marrow                    | 6.0 ( <i>L. infantum</i> )        |
|                                |                                 |                                    |                                    |                            | Molecular (PCR-RFLP, kDNA)         | Lymph node                     | 3.0 ( <i>L. infantum</i> )        |
|                                |                                 |                                    |                                    |                            |                                    | Whole blood                    | 4.0 ( <i>L. infantum</i> )        |
|                                | Leonel et al. (2020)            | 2014                               | <i>F. catus</i> (shelter)          | 94                         | Serological (ELISA)                | Serum                          | 31.9 ( <i>Leishmania</i> spp.)    |
|                                |                                 |                                    |                                    |                            |                                    | Serological (IFAT, 1:40)       | Serum                             |
|                                |                                 |                                    |                                    |                            |                                    | Conjunctival swab              | 0                                 |
| Whole blood                    |                                 |                                    |                                    |                            |                                    | 0                              |                                   |
| Marcondes et al. (2018)        | 2014–2015                       | <i>F. catus</i> (domestic/shelter) | 50 <sup>b</sup>                    | Parasitological (cytology) | Bone marrow                        | 14.0 ( <i>Leishmania</i> spp.) |                                   |
|                                |                                 |                                    |                                    |                            | Molecular (qPCR, kDNA)             | Bone marrow                    | 86.0 ( <i>L. infantum</i> )       |
|                                |                                 |                                    |                                    |                            | Whole blood                        | 72.0 ( <i>L. infantum</i> )    |                                   |
|                                |                                 |                                    |                                    |                            | Serum                              | 30.5 ( <i>L. infantum</i> )    |                                   |
| Rocha et al. (2019)            | 2016–2017                       | <i>F. catus</i> (domestic)         | 105                                | Molecular (PCR, CH1)       | Whole blood                        | 2.9 ( <i>L. infantum</i> )     |                                   |
| Pedrassani et al. (2019)       | 2017                            | <i>F. catus</i> (domestic)         | 30                                 | Serological (IFAT, 1:80)   | Whole blood                        | 5.7 ( <i>L. infantum</i> )     |                                   |
|                                |                                 |                                    |                                    |                            | Serum                              | 6.6 ( <i>L. infantum</i> )     |                                   |
| Berenguer et al. (2020)        | 2017                            | <i>F. catus</i> (domestic)         | 128                                | Molecular (PCR, kDNA)      | Whole blood                        | 0                              |                                   |
|                                |                                 |                                    |                                    |                            | Conjunctival swab                  | 0                              |                                   |
|                                |                                 |                                    |                                    |                            | Whole blood                        | 0.8 ( <i>L. infantum</i> )     |                                   |
|                                |                                 |                                    |                                    |                            | Lymph node                         | 33.3 ( <i>Leishmania</i> spp.) |                                   |
| Bezerra et al. (2019)          | 2017–2018                       | <i>F. catus</i> (domestic)         | 91                                 | Molecular (PCR, kDNA)      | Lymph node                         | 33.3 ( <i>L. infantum</i> )    |                                   |
|                                |                                 |                                    |                                    |                            | Serological (IFAT, 1:40)           | Serum                          | 15.4 ( <i>Leishmania</i> spp.)    |
| da Silva et al. (2008)         | na                              | <i>F. catus</i> (domestic)         | 8                                  | Molecular (PCR, kDNA)      | Whole blood                        | 0                              |                                   |
|                                |                                 |                                    |                                    |                            | Serological (IFAT, 1:40)           | Serum                          | 25.0 ( <i>Leishmania</i> spp.)    |
|                                |                                 |                                    |                                    |                            | Whole blood                        | 66.7 ( <i>Leishmania</i> spp.) |                                   |
|                                |                                 |                                    |                                    |                            | Molecular (multiplex PCR, kDNA)    | Whole blood                    | 100 ( <i>L. chagasi</i> )         |
| Bresciani et al. (2010)        | na                              | <i>F. catus</i> (domestic)         | 283                                | Parasitological (cytology) | Whole blood                        | 0.7 ( <i>Leishmania</i> spp.)  |                                   |
| da Silveira Neto et al. (2011) | na                              | <i>F. catus</i> (shelter)          | 130                                | Serological (IFAT, 1:40)   | Lymph node                         | 0                              |                                   |
|                                |                                 |                                    |                                    |                            | Serological (CAG-ELISA, 0.449)     | Serum                          | 23.0 ( <i>Leishmania</i> spp.)    |
|                                |                                 |                                    |                                    |                            | Serum                              | 13.3 ( <i>Leishmania</i> spp.) |                                   |
|                                |                                 |                                    |                                    |                            | Serological (FML-ELISA, 0.215)     | Serum                          | 15.9 ( <i>Leishmania</i> spp.)    |
|                                |                                 |                                    |                                    |                            | Serum                              | 15.9 ( <i>Leishmania</i> spp.) |                                   |



Table 2 (continued)

| Country   | Study                        | Sampling year | Species (origin)                 | No. tested      | Method (test, cut-off/target gene) | Sample                | % Positive (species) <sup>a</sup> |
|-----------|------------------------------|---------------|----------------------------------|-----------------|------------------------------------|-----------------------|-----------------------------------|
|           | Coelho et al. (2011b)        | na            | <i>F. catus</i> (domestic)       | 52              | Parasitological (cytology)         | Bone marrow           | 0                                 |
|           |                              |               |                                  |                 |                                    | Lymph node            | 3.8 ( <i>Leishmania</i> spp.)     |
|           |                              |               |                                  |                 |                                    | Spleen                | 0                                 |
|           |                              |               |                                  |                 | Molecular (PCR, kDNA)              | Bone marrow           | 0                                 |
|           |                              |               |                                  |                 |                                    | Lymph node            | 3.8 ( <i>L. chagasi</i> )         |
|           |                              |               |                                  |                 |                                    | Spleen                | 1.9 ( <i>L. chagasi</i> )         |
|           | Sobrinho et al. (2012)       | na            | <i>F. catus</i> (shelter/stray)  | 302             | Parasitological (Cytology)         | Bone marrow           | 7.0 ( <i>Leishmania</i> spp.)     |
|           |                              |               |                                  |                 | Serological (ELISA, 0.301)         | Lymph node            | 7.9 ( <i>Leishmania</i> spp.)     |
|           |                              |               |                                  |                 |                                    | Serum                 | 13.0 ( <i>Leishmania</i> spp.)    |
|           |                              |               |                                  | 5 <sup>b</sup>  | Serological (IFAT, 1:40)           | Serum                 | 4.6 ( <i>Leishmania</i> spp.)     |
|           | de Morais et al. (2013)      | na            | <i>F. catus</i> (domestic)       | 5               | Molecular (qPCR, <i>gp63</i> )     | Whole blood           | 100 ( <i>L. infantum</i> )        |
|           |                              |               |                                  |                 | Molecular (qPCR, kDNA)             | Whole blood           | 80.0 ( <i>L. infantum</i> )       |
|           |                              |               |                                  |                 | Molecular (PCR, kDNA)              | Whole blood           | 80.0 ( <i>L. infantum</i> )       |
|           | Braga et al. (2014a)         | na            | <i>F. catus</i> (domestic)       | 50              | Serological (IFAT, 1:40)           | Serum                 | 4.0 ( <i>Leishmania</i> spp.)     |
|           | Braga et al. (2014b)         | na            | <i>F. catus</i>                  | 100             | Parasitological (culture)          | Whole blood           | 2.0 ( <i>Leishmania</i> spp.)     |
|           |                              |               |                                  |                 | Serological (IFAT, 1:40)           | Serum                 | 15.0 ( <i>Leishmania</i> spp.)    |
|           |                              |               |                                  |                 |                                    | Whole blood           | 0                                 |
|           | Oliveira et al. (2015)       | na            | <i>F. catus</i> (domestic)       | 443             | Molecular, PCR, kDNA               | Whole blood           | 0                                 |
|           |                              |               |                                  |                 | Serological (DAT, 1:40)            | Serum                 | 5.6 ( <i>Leishmania</i> spp.)     |
|           |                              |               |                                  |                 | Serological (IFAT, 1:40)           | Serum                 | 4.1 ( <i>Leishmania</i> spp.)     |
|           | Benassi et al. (2017)        | na            | <i>F. catus</i> (domestic/stray) | 108             | Molecular (PCR, kDNA)              | Conjunctival swab     | 1.9 ( <i>Leishmania</i> spp.)     |
|           |                              |               |                                  |                 |                                    | Whole blood           | 0                                 |
|           |                              |               |                                  |                 | Molecular (qPCR, kDNA)             | Conjunctival swab     | 1.9 ( <i>Leishmania</i> spp.)     |
|           |                              |               |                                  |                 |                                    | Whole blood           | 0                                 |
|           |                              |               |                                  | 2 <sup>b</sup>  | Molecular (PCR, ITS1)              | Conjunctival swab     | 50.0 ( <i>L. infantum</i> )       |
|           | Coura et al. (2018)          | na            | <i>F. catus</i> (shelter)        | 100             | Parasitological (cytology)         | Bone marrow           | 0                                 |
|           |                              |               |                                  |                 | Parasitological (culture)          | Bone marrow           | 0                                 |
|           |                              |               |                                  |                 | Serological (IFAT, 1:40)           | Serum                 | 54.0 ( <i>Leishmania</i> spp.)    |
|           |                              |               |                                  | 54 <sup>b</sup> | Molecular (PCR, kDNA)              | Bone marrow/skin      | 0                                 |
|           | da Costa-Val et al. (2020)   | na            | <i>F. catus</i> (domestic)       | 64              | Serological (ELISA, 0.955)         | Serum                 | 29.8 ( <i>Leishmania</i> spp.)    |
|           |                              |               |                                  | 64              | Molecular (PCR, kDNA)              | Conjunctival swab     | 6.3 ( <i>Leishmania</i> spp.)     |
|           |                              |               |                                  | 64              | Molecular (PCR, kDNA)              | Oral swab             | 4.7 ( <i>Leishmania</i> spp.)     |
|           |                              |               |                                  | 8 <sup>b</sup>  | Molecular (PCR-RFLP, ITS1)         | Conjunctival swab     | 12.5 ( <i>L. infantum</i> )       |
|           |                              |               |                                  |                 |                                    | Oral swab             | 37.5 ( <i>L. infantum</i> )       |
|           |                              |               |                                  |                 |                                    | Oral swab             | 12.5 ( <i>L. braziliensis</i> )   |
| Honduras  | Mccown & Grzeszak (2010)     | na            | <i>F. catus</i> (stray)          | 12              | Serological (IFAT, 1:32)           | Serum                 | 25.0 ( <i>L. donovani</i> )       |
| Mexico    | Longoni et al. (2012)        | 2008–2009     | <i>F. catus</i> (stray)          | 95              | Serological (ELISA-H)              | Serum                 | 5.3 ( <i>L. braziliensis</i> )    |
|           |                              |               |                                  |                 |                                    | Serum                 | 13.7 ( <i>L. infantum</i> )       |
|           |                              |               |                                  |                 | Serological (ELISA-SODE)           | Serum                 | 1.1 ( <i>L. mexicana</i> )        |
|           |                              |               |                                  |                 |                                    | Serum                 | 11.6 ( <i>L. braziliensis</i> )   |
|           |                              |               |                                  |                 |                                    | Serum                 | 22.1 ( <i>L. infantum</i> )       |
|           |                              |               |                                  |                 | Serological (WB)                   | Serum                 | 10.5 ( <i>L. mexicana</i> )       |
|           |                              |               |                                  |                 |                                    | Serum                 | 10.5 ( <i>L. braziliensis</i> )   |
|           |                              |               |                                  |                 |                                    | Serum                 | 20.0 ( <i>L. infantum</i> )       |
|           |                              |               |                                  |                 |                                    | Serum                 | 10.5 ( <i>L. mexicana</i> )       |
| Venezuela | Viettri et al. (2018)        | na            | na                               | 5               | Molecular (nested PCR, ITS1)       | Blood on filter paper | 20.0 ( <i>Leishmania</i> spp.)    |
|           |                              |               |                                  |                 | Molecular (nPCR, <i>SSU</i> rDNA)  | Blood on filter paper | 20.0 ( <i>Leishmania</i> spp.)    |
|           | Rivas et al. (2018)          |               | <i>F. catus</i> (domestic/stray) | 6               | Parasitological (cytology)         | Skin lesions          | 66.7 ( <i>Leishmania</i> spp.)    |
|           |                              |               |                                  | 5               | Parastiological (histology)        | Skin lesions          | 80.0 ( <i>Leishmania</i> spp.)    |
|           |                              |               |                                  | 5               | Parasitological (IHC)              | Skin lesions          | 100 ( <i>Leishmania</i> spp.)     |
|           |                              |               |                                  | 30              | Serological (ELISA, 15.3 EU)       | Serum                 | 6.7 ( <i>L. braziliensis</i> )    |
|           |                              |               |                                  |                 | Serological (ELISA, 15.3 EU)       | Serum                 | 6.7 ( <i>L. infantum</i> )        |
|           |                              |               |                                  |                 | Serological (WB)                   | Serum                 | 33.3 ( <i>L. braziliensis</i> )   |
|           |                              |               |                                  |                 | Serological (WB)                   | Serum                 | 33.3 ( <i>L. infantum</i> )       |
|           |                              |               |                                  | 31              | Molecular (qPCR, kDNA)             | Whole blood           | 9.7 ( <i>Leishmania</i> spp.)     |
|           |                              |               |                                  | 5               | Molecular (qPCR, kDNA)             | Skin lesions          | 100 ( <i>Leishmania</i> spp.)     |
|           |                              |               |                                  | 2 <sup>b</sup>  | Molecular (qPCR, ITS1)             | Skin lesions          | 40.0 ( <i>L. mexicana</i> )       |
|           |                              |               |                                  |                 | Molecular (PCR-RFLP, ITS1)         | Skin lesions          | 50.0 ( <i>L. mexicana</i> )       |
|           | Paniz Mondolfi et al. (2019) | na            | na                               | 12              | Molecular (nPCR, <i>cytB</i> )     | Skin lesions          | 83.3 ( <i>L. mexicana</i> )       |
|           |                              |               |                                  |                 |                                    |                       | 16.7 ( <i>Leishmania</i> spp.)    |

<sup>a</sup> Species defined according to the original study.

<sup>b</sup> Previously identified as positive by another test.

<sup>c</sup> 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.

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 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; Metzendorf 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; Metzendorf 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; Metzendorf et al., 2017; Marcondes et al., 2018; Fernandez-Gallego et al., 2020), liver (Vides et al., 2011; Mohebbali

**Table 3**Frequency of clinical signs in domestic cats (*Felis catus*) with clinical leishmaniasis caused by *Leishmania infantum*

| Historical or physical signs    | Frequency (%) <sup>a</sup> | Reference                                                                                                                                                                                                                                                                                                             |
|---------------------------------|----------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Dermatological</b>           |                            |                                                                                                                                                                                                                                                                                                                       |
| 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. (2017a,b); Leal et al. (2018); Brianti et al. (2019); Headley et al. (2019); Pereira Mondolfi 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)                                                                                                                                                                                                                                                                                       |
| <b>General/miscellaneous</b>    |                            |                                                                                                                                                                                                                                                                                                                       |
| 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 Mondolfi et al. (2019)                                                                                                                                                                                                                                                                                        |
| <b>Ocular</b>                   |                            |                                                                                                                                                                                                                                                                                                                       |
| 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 Mondolfi et al. (2019); Fernandez-Gallego et al. (2020)                                                                                                                       |
| Corneal oedema                  | 10                         | Hervás et al. (2001); Pimenta et al. (2015); Fernandez-Gallego et al. (2020)                                                                                                                                                                                                                                          |

**Table 3 (continued)**

| Historical or physical signs      | Frequency (%) <sup>a</sup> | Reference                                                                                                                                                      |
|-----------------------------------|----------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 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)                                                                                                                                           |
| <b>Gastrointestinal/abdominal</b> |                            |                                                                                                                                                                |
| 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)                                                                                                                                |
| <b>Cardiorespiratory</b>          |                            |                                                                                                                                                                |
| 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)                                                                                                                                             |
| <b>Musculoskeletal</b>            |                            |                                                                                                                                                                |
| Muscle atrophy                    | 2                          | da Silva et al. (2010)                                                                                                                                         |
| <b>Neurological</b>               |                            |                                                                                                                                                                |
| Ataxia                            | 2                          | Fernandez-Gallego et al. (2020)                                                                                                                                |
| <b>Urogenital</b>                 |                            |                                                                                                                                                                |
| Vaginal bleeding                  | 2                          | Maia et al. (2015)                                                                                                                                             |

<sup>a</sup> n = 52.

et al., 2017; Fernandez-Gallego et al., 2020), spleen (Vides et al., 2011; Mohebbali 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). Cytological 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),

**Table 4**  
Frequency of clinicopathological abnormalities in domestic cats (*Felis catus*) with leishmaniosis caused by *Leishmania infantum*

| Parameter                          | Frequency (%) <sup>a</sup> | Reference                                                                                                                                                                                                                                                                                                       |
|------------------------------------|----------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Hemogram                           |                            |                                                                                                                                                                                                                                                                                                                 |
| Anaemia                            | 31                         | Hervás et al. (1999); Pennisi et al. (2004); Marcos et al. (2009); Richter et al. (2014); Pereira Mondolfi 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)                                                                                                                                                                          |
| Thrombocytopenia                   | 17                         | Pennisi et al. (2004); Marcos et al. (2009); Richter et al. (2014); Pimenta et al. (2015); Basso et al. (2016); Pereira Mondolfi et al. (2019)                                                                                                                                                                  |
| Leukocytosis                       | 10                         | Ozon et al. (1998); da Silva et al. (2010); Fernandez-Gallego et al. (2020)                                                                                                                                                                                                                                     |
| Leukopenia                         | 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)                                                                                                                                                                                                                         |
| Neutropenia                        | 5                          | Fernandez-Gallego et al. (2020)                                                                                                                                                                                                                                                                                 |
| Lymphopenia                        | 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. (2017a,b); Leal et al. (2018); Brianti et al. (2019); Pereira Mondolfi 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 Mondolfi et al. (2019); Altuzarra et al. (2020); Fernandez-Gallego et al. (2020) |

**Table 4 (continued)**

| Parameter                      | Frequency (%) <sup>a</sup> | Reference                                                                 |
|--------------------------------|----------------------------|---------------------------------------------------------------------------|
| Increased $\alpha_2$ 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)                                                       |

<sup>a</sup> Hemogram,  $n = 42$ ; Blood chemistry,  $n = 39$ ; Serum protein electrophoresis,  $n = 32$ ; Urinalysis,  $n = 24$ .

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 an 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 have allowed the detection 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., 2015; Brianti 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 when used to amplify stretches of multicopy genes has increased the sensitivity of detection, and should be preferred for sample testing under suboptimal conditions (i.e. where the parasite load tends to be low) such as when whole blood is used (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 subsequently analysed by restriction enzyme

**Table 5**  
Common laboratory tests performed for diagnostics 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           | +++                              | +                                   | <ul style="list-style-type: none"> <li>• Bone-marrow (FNB);</li> <li>• Lymph node (FNB);</li> <li>• Nodular lesions (FNB);</li> <li>• Erosive/ulcerative skin lesions (scraping)</li> </ul> | <ul style="list-style-type: none"> <li>• Does not require specific laboratory equipment;</li> <li>• Low cost;</li> <li>• Rapid;</li> <li>• High specificity</li> </ul>                                                                                                                                                        | <ul style="list-style-type: none"> <li>• Requires experienced observers;</li> <li>• Strictly qualitative;</li> <li>• Not suitable for identification at the species level</li> </ul>                                                                                                                                                           | <ul style="list-style-type: none"> <li>• Amastigotes can be found in both intracellular and extracellular areas</li> </ul>                                                                |
| Histopathology           | Detection of parasites           | +++                              | +                                   | <ul style="list-style-type: none"> <li>• Skin/ocular lesions;</li> <li>• Bone marrow;</li> <li>• Lymph-nodes;</li> <li>• Spleen</li> </ul>                                                  | <ul style="list-style-type: none"> <li>• Preserves structure and maintains tissue pathology;</li> <li>• High specificity;</li> <li>• Good sensitivity using IHC</li> </ul>                                                                                                                                                    | <ul style="list-style-type: none"> <li>• Invasive;</li> <li>• Requires experienced observers;</li> <li>• Requires specific laboratory equipment;</li> <li>• More laborious and time-consuming;</li> <li>• IHC is not widely available;</li> <li>• Only qualitative;</li> <li>• Not suitable for identification at the species level</li> </ul> |                                                                                                                                                                                           |
| Parasite culture         | Isolation of viable parasites    | ++                               | +                                   | <ul style="list-style-type: none"> <li>• Biopsy lesions;</li> <li>• Bone marrow;</li> <li>• Lymph nodes</li> </ul>                                                                          | <ul style="list-style-type: none"> <li>• Provides parasites for further analysis;</li> <li>• Confirms active infection;</li> <li>• High specificity</li> </ul>                                                                                                                                                                | <ul style="list-style-type: none"> <li>• Labour-intensive;</li> <li>• Restricted to specialised reference laboratories;</li> <li>• Up to more than 30 days to provide a result;</li> <li>• Only qualitative;</li> <li>• Not suitable for identification at the species level</li> </ul>                                                        | <ul style="list-style-type: none"> <li>• Aseptic sampling should be ensured;</li> <li>• Biopsy sample must be homogenised in saline or culture medium under sterile conditions</li> </ul> |
| Molecular PCR            | Detection of parasite DNA        | +++                              | +++                                 | <ul style="list-style-type: none"> <li>• Biopsy lesions;</li> <li>• Bone marrow;</li> <li>• Lymph nodes</li> </ul>                                                                          | <ul style="list-style-type: none"> <li>• Allows identification at the species level;</li> <li>• High sensitivity and specificity</li> </ul>                                                                                                                                                                                   | <ul style="list-style-type: none"> <li>• Transient infection cannot be excluded;</li> <li>• Requires specific laboratory equipment;</li> <li>• Requires vigilance against false-positive results;</li> <li>• Only qualitative;</li> <li>• Expensive</li> </ul>                                                                                 | <ul style="list-style-type: none"> <li>• Protocols targeting multicopy genes are preferable for diagnosis;</li> <li>• Nested PCR has more sensitivity than conventional PCR</li> </ul>    |
| qPCR                     | Detection of parasite DNA        | +++                              | +++                                 | <ul style="list-style-type: none"> <li>• Biopsy lesions</li> <li>• Bone marrow</li> <li>• Lymph nodes</li> </ul>                                                                            | <ul style="list-style-type: none"> <li>• Allows identification at the species level;</li> <li>• High sensitivity and specificity;</li> <li>• Quantification of parasite load;</li> <li>• Reduced cross-contamination probability;</li> <li>• Valuable for treatment follow-up;</li> <li>• Qualitative/quantitative</li> </ul> | <ul style="list-style-type: none"> <li>• Transient infection cannot be excluded;</li> <li>• Standardised methods to parasite load quantification may not be offered by some laboratories;</li> <li>• Expensive</li> </ul>                                                                                                                      | <ul style="list-style-type: none"> <li>• Protocols targeting multicopy genes are preferable for diagnosis</li> </ul>                                                                      |
| Serological ELISA        | Detection of specific antibodies | +++                              | ++                                  | <ul style="list-style-type: none"> <li>• Serum;</li> <li>• Plasma</li> </ul>                                                                                                                | <ul style="list-style-type: none"> <li>• Valuable for treatment follow-up;</li> <li>• Relatively low cost;</li> <li>• Qualitative/quantitative</li> </ul>                                                                                                                                                                     | <ul style="list-style-type: none"> <li>• Possible cross-reactivity;</li> <li>• Difficult to assess results at threshold of positivity;</li> <li>• Not suitable for unambiguous</li> </ul>                                                                                                                                                      | <ul style="list-style-type: none"> <li>• Established cut-off (40 EU)</li> </ul>                                                                                                           |

(continued on next page)

Table 5 (continued)

| Type/test    | Aim                              | Confirmation of clinical disease | Confirmation of subclinical disease | Preferential sample                                                      | Advantages                                                                                                                                          | Disadvantages                                                                                                                                                                                                                       | Observations                                                                                                                                                    |
|--------------|----------------------------------|----------------------------------|-------------------------------------|--------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------|
| IFAT         | Detection of specific antibodies | ++                               | +++                                 | <ul style="list-style-type: none"> <li>Serum;</li> <li>Plasma</li> </ul> | <ul style="list-style-type: none"> <li>Valuable for treatment follow-up;</li> <li>Relatively low cost;</li> <li>Qualitative/quantitative</li> </ul> | <ul style="list-style-type: none"> <li>Requires experienced observers;</li> <li>Subjective interpretation;</li> <li>Possible cross-reactivity;</li> <li>Not suitable for unambiguous identification at the species level</li> </ul> | <ul style="list-style-type: none"> <li>Reference method for the serodiagnosis of human and canine leishmanioses;</li> <li>Established cut-off (1:80)</li> </ul> |
| Western blot | Detection of specific antibodies | +++                              | +++                                 | <ul style="list-style-type: none"> <li>Serum;</li> <li>Plasma</li> </ul> | <ul style="list-style-type: none"> <li>High sensitivity and specificity</li> </ul>                                                                  | <ul style="list-style-type: none"> <li>Labour-intensive;</li> <li>Expensive;</li> <li>Not available in routine practice</li> </ul>                                                                                                  | <ul style="list-style-type: none"> <li>Marker for positivity: 18 kDa band</li> </ul>                                                                            |

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.

digestion (i.e. restriction fragment length polymorphism) and/or DNA 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, Jatta 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 and is rarely available in routine practice. However, this test seems to offer the best diagnostic performance (considering an 18 kDa band as a marker for

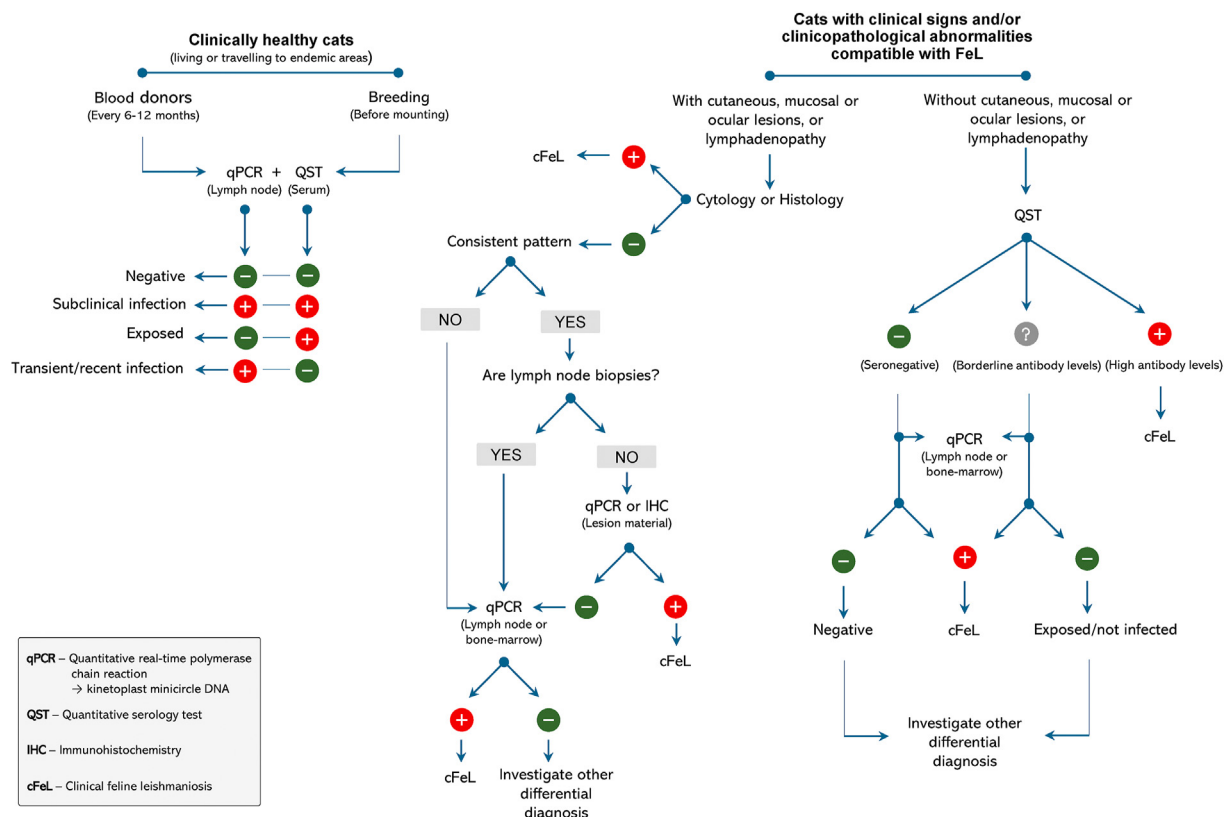


Fig. 3 Proposed diagnostic algorithm for clinically healthy cats used as blood donors, and cats with suspected leishmaniosis

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 on their efficacy and safety have yet 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), coprostitis (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 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, panleukopaemia, 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 from dusk to dawn during the period of vector activity (April to November in Mediterranean areas, see Alten et al., 2016), as well as using physical barriers such as nets (i.e. mesh size 1,240 holes/in<sup>2</sup>) 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

**Table 6**  
Treatment regimens used for feline leishmaniosis

| Type                       | Drug (regimen and dose)                                                                                         | Outcome                                                          | Adverse reactions <sup>a</sup>                                 | Issues to consider                                                                                                                                                        | Reference                                                                                                                                                                                                                                                                                                                                                                                     |
|----------------------------|-----------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------|----------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Monotherapy</b>         |                                                                                                                 |                                                                  |                                                                |                                                                                                                                                                           |                                                                                                                                                                                                                                                                                                                                                                                               |
|                            | Allopurinol (10–30 mg/kg or 100 mg/cat PO q12–24 h; for long-term)                                              | Variable (no response to clinical cure)                          | Increased liver enzymes; coprostitis <sup>b</sup> ; 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. (2017a,b); Brianti et al. (2019); Pereira Mondolfi et al. (2019); Altuzarra et al. (2020); Fernandez-Gallego et al. (2020) Maia et al. (2015) |
|                            | Domperidone (0.5 mg/kg PO q24 h for 1 month)                                                                    | No improvement                                                   | Not reported                                                   | Immunomodulatory drug used on prevention and treatment of CanL. May be hepatotoxic                                                                                        | Pennisi et al. (2004)                                                                                                                                                                                                                                                                                                                                                                         |
|                            | Fluconazole (5 mg/kg PO q24 h for 2 months)                                                                     | No response                                                      | Not reported                                                   |                                                                                                                                                                           | Pennisi et al. (2004)                                                                                                                                                                                                                                                                                                                                                                         |
|                            | Itraconazole (50 mg/cat PO q24 h 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 q24 h 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 q24 h for 4 months)                                                        | Resolution of clinical signs                                     | See previous line                                              | See previous line                                                                                                                                                         | Fernandez-Gallego et al. (2020)                                                                                                                                                                                                                                                                                                                                                               |
| <b>Combination therapy</b> |                                                                                                                 |                                                                  |                                                                |                                                                                                                                                                           |                                                                                                                                                                                                                                                                                                                                                                                               |
|                            | Meglumine antimoniate (50 mg/kg SC q24 h for 30 days) plus allopurinol (10 mg/kg PO q12–24 h 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 Mondolfi et al. (2019); Fernandez-Gallego et al. (2020)                                                                                                                                                                                                                                                                                   |
|                            | Meglumine antimoniate (5 mg/kg SC q24 h) plus ketoconazole (10 mg/kg q24 h); 3 cycles of 4 weeks, 10 days apart | Resolution of lesions                                            | Not reported; see meglumine antimoniate monotherapy            | According to BSAVA (2020) ketoconazole is not recommended for cats                                                                                                        | Hervás et al. (1999)                                                                                                                                                                                                                                                                                                                                                                          |
|                            | Metronidazole (25 mg/kg PO q24 h for 35 days) plus spiramycin (150,000 IU/kg PO q24 h for 35 days)              | No response                                                      | Not reported                                                   |                                                                                                                                                                           | Pennisi et al. (2004)                                                                                                                                                                                                                                                                                                                                                                         |
|                            | Miltefosine (2 mg/kg PO q24 h 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 and Persichetti, 2018) | Leal et al. (2018)                                                                                                                                                                                                                                                                                                                                                                            |
|                            | Miltefosine (2 mg/kg PO q24 h 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)                                                                                                                                                                                                                                                                                                                                                               |

<sup>a</sup> Reported during treatment of cats with clinical leishmaniosis.

<sup>b</sup> Associated with high doses (50 mg/kg q24 h).

**Abbreviations:** AHCC, active hexose correlated compounds; AKI, acute kidney injury; CanL, canine leishmaniosis; FeL, feline leishmaniosis; IU, international unit; PO, *per os*; SC, subcutaneous.

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 leishmaniasis 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.

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## 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>.

Akhtardaneh, 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>.

Akhtardaneh, 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. Rep.* 20, 100387. <https://doi.org/10.1016/j.vprsr.2020.100387>.

Akhtardaneh, B., Sharifi, I., Mohammadi, A., Mostafavi, M., Hakimipour, 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., Fakhari, 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 leishmaniasis and coinfections with *Hepatozoon felis* and “*Candidatus* Mycoplasma haemominutum”. *J. Feline Med. Surg. Open Rep.* 3. <https://doi.org/10.1177/2055116917740454>, 2055116917740454.

Attipa, C., Papasoulotiis, 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., Amusatogui, I., Villaescusa, A., Rodríguez-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., 2016. Successful treatment of feline leishmaniasis using a combination of allopurinol and N-methyl-glucamine antimoniate. *J. Feline Med. Surg. Open Rep.* 2. <https://doi.org/10.1177/2055116916630002>, 2055116916630002.

Benassi, J.C., Benvenega, G.U., Ferreira, H.L., Pereira, V.F., Keid, L.B., Soares, R., de S. Oliveira, T.M.F., 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., de A. Gomes, C.F.C., de O. Nascimento, J., 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.* 66, 673–676. <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 leishmaniasis in Barquisimeto, Venezuela. *Trans. R. Soc. Trop. Med. Hyg.* 85, 53. [https://doi.org/10.1016/0035-9203\(91\)90153-](https://doi.org/10.1016/0035-9203(91)90153-).

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. *J. Venom. Anim. Toxins Incl. Trop. Dis.* 20, 5. <https://doi.org/10.1186/1678-9199-20-5>.

Bresciani, K.D.S., Serrano, A.C.M., de Matos, L.V.S., 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. Vet.* 19, 127–129. <https://doi.org/10.4322/rbvp.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 leishmaniasis. *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 leishmaniasis 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.







- 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 leishmaniasis in Brazil. *Vet. Parasitol.* 178, 22–28. <https://doi.org/10.1016/j.vetpar.2010.12.042>.
- Vietri, 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. Rep.* 14, 123–130. <https://doi.org/10.1016/j.vprsr.2018.10.002>.
- Vilhena, H., Martínez-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.* <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](https://apps.who.int/iris/bitstream/handle/10665/44412/WHO_TRS_949_eng.pdf?sequence=1&isAllowed=y).