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**Original Article**

**Serological, molecular and clinicopathological findings associated with *Leishmania infantum* infection in cats in Northern Italy**

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## Abstract

**Objectives** To investigate the prevalence of *Leishmania* species infection in cats in Northern Italy and to evaluate the associations between infection, and signalment and clinicopathological data.

**Methods** The study was carried out in a veterinary university hospital from June to November 2017. Blood, urine, conjunctival swabs and hair were collected from all cats which had been randomly selected. *Leishmania* species infection was evaluated using the indirect fluorescent antibody test (IFAT), setting a cut-off value of 1:80 and real-time PCR on blood, conjunctival and hair samples. A complete blood count, serum chemistry profile, serum electrophoresis and urinalysis were also carried out. The cats were grouped on the basis of the results of the diagnostic criteria adopted in positive, negative and unconfirmed *Leishmania* cases. Non-parametric variables and continuous data were compared among the study groups using the  $\chi^2$  test and the Mann–Whitney U-test, respectively.

**Results** One hundred and fifty-two cats were included. Nineteen of the 152 (12.5%) cats were positive: 18/152 (11.8%) showed IFAT titre  $\geq 1:80$  and 1/152 (0.7%) was real-time PCR-positive in a hair sample; 106/152 (69.7%) cats were negative, and 27/152 (17.8%) cats were unconfirmed for *Leishmania*. Total proteins, beta<sub>2</sub>-globulin and gamma-globulin were significantly increased in the positive *Leishmania* group when compared to the negative group.

54    **Conclusions and relevance** The results of the present study demonstrated the spread of  
55    *L infantum* infection in cats in Northern Italy. Hyperprotidaemia and  
56    hypergammaglobulinaemia appeared to be significant clinicopathological abnormalities  
57    in this population of cats with *L infantum* infection.

- 58    **Keywords:** feline leishmaniosis; indirect immunofluorescence test; real-time PCR;
- 59    clinicopathological abnormalities

## Introduction

To date, in Italy, canine leishmaniosis (CanL) is considered to be endemic throughout most of the national territory having a variable prevalence. Recent studies have detected *Leishmania* species infection in domestic cats as well in areas where CanL is endemic,<sup>1</sup> with seroprevalence ranging from 0 to 68.5% and molecular prevalence ranging from 0 to 60.7%.<sup>2</sup> Cats have a neglected and controversial role in the cycle of *L. infantum*<sup>3</sup> but whether they serve as primary, secondary or accidental hosts is currently unknown.<sup>4</sup> Furthermore, only a few studies have been carried out regarding clinical and clinicopathological abnormalities in cats with feline leishmaniosis (FeL).<sup>2</sup> The majority of the diagnostic techniques for *Leishmania* species infection available for dogs are also employed for cats. The most common serological test used is the indirect fluorescent antibody test (IFAT).<sup>2,4-7</sup> Of the molecular methods, the real-time PCR (qPCR) allows quantitative and fast detection of *Leishmania* species DNA.<sup>8,9</sup> Detection using DNA can be applied to a variety of biological matrices, including bone marrow, skin biopsy and lymph node aspirate. These samples are difficult to obtain and, in some cases, sedation of the animal is required.<sup>2,10</sup> Little is still known regarding the use of non-invasive biological matrices for the molecular detection of *Leishmania* species DNA in cats, such as peripheral blood, conjunctival swabs and hair.<sup>6,11-13</sup> The aims of this study were (a) to investigate the presence and diffusion of *Leishmania* species infection in cats in Northern Italy using IFAT and qPCR assays applied to

different biological samples (serum, blood, conjunctival swabs and hair) and (b) to evaluate possible associations between infection by *L infantum* and signalment and clinicopathological data.

## **Materials and Methods**

### **Study design, inclusion criteria, sampling and groups**

This was a prospective study carried out in a veterinary university hospital in Northern Italy (Department of Veterinary Medical Science, University of Bologna). Client-owned cats were included in the study during the sandfly activity season (from June to November 2017) after random selection and owner consent. Cats were included if they had the following requisites: (1) living in the Emilia-Romagna region and (2) had undergone a blood sample after clinical examination. Signalment, lifestyle (indoor or outdoor) and clinical data were recorded for each cat sampled.

Blood sampling was carried out by venepuncture, and samples were collected using a vacuum system (Vacutest Kima). The K<sub>3</sub>EDTA samples were used for a complete blood count (CBC) and molecular analyses. Serum samples underwent a chemistry profile and serum protein electrophoresis. The urine samples were collected by spontaneous voiding, cystocentesis or urethral catheterisation, when required, by the attending clinicians, and underwent urinalysis. Conjunctival swabs from both eyes and hair (pulling) samples were also collected from each cat. Clinicopathological evaluation was



carried out within 1 h from the sampling and the samples were stored at  $-80^{\circ}\text{C}$  after examination. *Leishmania* species infection was investigated using IFAT and qPCR on serum and blood, conjunctival swabs and hair samples, respectively. Cats with a positive IFAT titre ( $\geq 1:80$ )<sup>14</sup> and/or positive qPCR on blood and/or conjunctival swabs and/or hair samples were included in the positive *Leishmania* (PL) group; cats with a negative IFAT titre ( $< 1:40$ ) and negative qPCR results were included in the negative *Leishmania* (NL) group; cats with a IFAT titre equal to 1:40 and negative qPCR results were included in the unconfirmed *Leishmania* (UL) group. Moreover, the medical records of the PL group were analysed to evaluate signalment, history, and clinical and clinicopathological data.

#### **Indirect fluorescent antibody test**

Anti-*Leishmania* IgG antibodies were investigated in serum samples using IFAT according to the CanL diagnosis described in the World Organisation for Animal Health (OIE) manual of diagnostic tests and vaccines for terrestrial animals.<sup>15</sup> Slides coated with 30  $\mu\text{l}$  of an in-house antigen consisting of promastigotes of *L. infantum* (strain MHOM/TN/80/IPT1)<sup>16</sup> were probed with sera serially diluted in phosphate-buffered saline (PBS) starting with a concentration of 1:40 until reaching a concentration of 1:5120, incubated for 30 mins at  $37^{\circ}\text{C}$  and washed three times with PBS. Internal feline positive (1:160) and negative sera controls were included on each slide. The slides were probed with 30  $\mu\text{l}$  fluorescein isothiocyanate (FITC) conjugated anti-cat IgG antibody

diluted in PBS at a concentration of 1:32 (Anti-Cat IgG-FITC antibody; Sigma-Aldrich) for 30 mins at 37°C, were washed three times with PBS, and examined under the fluorescent microscope. The highest dilution showing fluorescent promastigotes was the final antibody titre.

#### **Molecular diagnosis**

Genomic DNA was extracted from blood, conjunctival swabs and hair samples using a commercially available kit (NucleoSpin Tissue Kit; Macherey-Nagel) according to the manufacturer's protocol. Before extraction, for each cat sampled, the two conjunctival swab samples (right and left eyes) were resuspended together in 250 µl of PBS. Instead, the hair samples were cut into three segments (proximal, central and distal) and lysed at 56°C overnight with 250 µl of lysis buffer (Buffer T1) and 50 µl of proteinase K supplied in the extraction kit.

*Leishmania* species DNA detection was carried out using SYBR Green qPCR as reported by Ceccarelli and collaborators.<sup>17</sup> A reaction was carried out using a commercially available kit (PowerUp SYBR Green Master Mix Kit, Life Technologies) and the StepOnePlus Real-Time PCR System (Thermo Fisher Scientific, Life Technologies). Serial 10-fold dilutions of a plasmid (pCR4 plasmid; Invitrogen; Life Technologies) containing one copy of the target sequence was used as an external standard for the construction of the assay standard curve. Melting experiments were carried out after the last extension step with a continuous increment from 60 to 95°C;

the specific melting temperature ( $T_m$ ) was 80.7°C. The limit of detection (LOD) of the assay was assessed by testing serial 10-fold dilutions of the recombinant plasmid and was found to be  $1 \times 10^0$  copy/ $\mu$ l. The DNA samples and standards were repeated within each run in duplicate. A no template control underwent analysis simultaneously. The specimens were considered positive if the fluorescence curve in the amplification plot showed an exponential increase, a specific melting peak was observed and the mean of the DNA copy number obtained from the replicates was greater than the LOD.

#### **Clinicopathological investigations**

A CBC was carried out using an automated haematology analyser (ADVIA 2120; Siemens Healthcare Diagnostics). The haematology was completed with a microscopic blood smear examination using May-Grünwald Giemsa staining. A serum chemistry profile, including creatinine, urea, phosphate, total protein, albumin, the albumin to globulin ratio (A:G), alanine transaminase, aspartate transaminase, alkaline phosphatase, gamma( $\gamma$ )-glutamyltransferase, total bilirubin, cholesterol, total calcium, sodium, potassium, chloride, glucose, and serum amyloid A (SAA), as reported previously,<sup>18</sup> was determined. Urinalysis included urine specific gravity (USG), dipstick (Combur<sup>10</sup>TestUX; Roche) and microscopic sediment examination, and the urine protein:creatinine ratio (UPC). Urine samples with a visible red colour and/or >250 red blood cells (RBCs) in a high power field (hpf) were excluded from the UPC analysis.

Serum and urine chemical analysis were carried out using an automated analyser (AU480; Beckman Coulter-Olympus). Serum protein electrophoresis was carried out on agarose gel using a semi-automated system (Hydragel protein kit; Hydrasys; Sebia).

### **Statistical analysis**

The data were evaluated using standard descriptive statistics and reported as mean  $\pm$  standard deviation or median and range (min–max), based on their distribution. The cats included in the UL group were excluded from all analyses. The categorical data were analysed using the  $\chi^2$  test. The upper limits of the reference interval used in the university lab for cats were used as cut-off values for hyperproteinaemia and hypergammaglobulinaemia and were 8.8 g/dl and 1.30 g/dl, respectively. The continuous data (age and clinicopathological results) were compared among the study groups using the Mann–Whitney U-test with compensated post-hoc analysis. A *P* value  $<0.05$  was considered significant. An inter-rater agreement statistic (Cohen’s kappa coefficient) was calculated to compare the results obtained by IFAT and qPCR, and between the qPCR carried out on three different biological samples. Statistical analysis was carried out using commercially available software (MedCalc Statistical Software version 16.8.4).

### **Results**

## **Study groups and diagnosis of *L. infantum* infection**

During the study period, 152 cats met the inclusion criteria. Nineteen of the 152 (12.5%) cats were included in the PL group: 18/152 (11.8%) showed IFAT titre  $\geq 1:80$  and 1/152 (0.7%) was tested qPCR-positive in hair sample. The NL group included 106/152 (69.7%) cats and the UL group included 27/152 (17.8%). Table 1 shows the descriptive statistics results and data regarding the prevalence of infection in the study population.

Thirteen of the 19 (68.4%) cats included in the PL group were male of which 12/13 (92.3%) were castrated, and 6/19 (31.6%) were female of which 5/6 (83.3%) were spayed. Seventeen of the 19 (89.5%) were more than 24 months of age and 2/19 (10.5%) were between 6 and 24 months of age, with a median age of 8 years (9 months-15 years). Fourteen of the 19 (73.7%) were European domestic shorthair cats and 5/19 (26.3%) were from other various breeds (Maine Coon = 1; Bengal = 1; Birman = 1; Persian = 1; Chartreux = 1); 12/19 cats (63.2%) were indoor/outdoor while 7/19 (36.8%) were exclusively indoor. Sixteen of the 19 (84.2%) cats were from the province of Bologna. The highest frequency of positivity was detected in July (5/35; 14.3%) and August (7/22; 31.8%) while the frequency decreased in the other months of sampling.

Seroreactivity (IFAT titre  $\geq 1:40$ ) to *L. infantum* was found in 45 of the 152 (29.6%) cats tested. Of the 18 seropositive cats (IFAT titre  $\geq 1:80$ ), 11 (61.1%) had an antibody titre equal to 1:80, five (27.8%) 1:160 and two (11.1%) 1:320 (Tables 2 and 3).

The cat identified as positive on hair using qPCR showed 8 copies of *Leishmania* kinetoplast DNA/ $\mu$ l of DNA extract and was seronegative to *L. infantum* (Table 2). This cat was a 10-year-old European domestic shorthair castrated male which lived indoors and was sampled in August.

Forty-nine of the 106 cats included in the NL group were male of which 43/49 (87.8%) were castrated while the remaining 57 were female of which 45/57 (78.9%) were spayed. Ninety-two (86.8%) were more than 24 months old, with a median of 7 years (4 months–20 years); 92/106 (86.8%) were European domestic shorthair cats and 93/106 (87.7%) were from the province of Bologna. Forty-nine of the 106 (46.2%) were indoor cats and 47/106 (44.3%) were indoor/outdoor cats.

No statistical association was found among positivity to *L. infantum* and sex, age, breed, geographical origin, access to outdoor environment and month of sampling. Although not significant, an increased frequency in positivity among cats sampled in July and August was observed ( $P = 0.071$ ) (Table 1).

No agreement between the results obtained by IFAT and qPCR and between the qPCR carried out on three different biological samples was shown by the value of Cohen's kappa coefficient ( $P = 0.309$ ).

#### **Clinical and clinicopathological findings**

The results of the CBC, serum chemistry, protein electrophoresis, and urinalysis including UPC obtained in the PL and NL groups are shown in Table 4. Total proteins,

beta( $\beta$ )<sub>2</sub>-globulin and gamma( $\gamma$ )-globulin fractions were significantly increased in the PL group (Table 4). Hyperproteinaemia and hypergammaglobulinaemia were detected in 3/19 (15.8%) and 11/19 (25.6%) PL cats, respectively. The frequency of hyperproteinaemia and hypergammaglobulinaemia were significantly increased in the PL group when compared to the NL group ( $P = 0.028$  and  $P = 0.038$ , respectively). No other significant differences among the groups selected were detected.

No clinical signs associated with leishmaniosis were observed in any cat included in this study, and leishmaniosis was never suspected by the attending clinicians. In the PL group, cats with antibody titre equal to 1:80 (11/18) had gastroenteritis ( $n = 6$ ), neurological disorders ( $n = 1$ ) and chronic kidney disease (CKD) ( $n = 2$ ). Moreover, 2/11 in the PL group tested positive for feline *calicivirus* (FCV) and 1/11 for feline immunodeficiency virus. Cats with an antibody titre greater than 1:80 (7/18) had more severe diseases including autoimmune thrombocytopenia ( $n = 1$ ), hypertrophic cardiomyopathy ( $n = 1$ ), hyperthyroidism ( $n = 2$ ), neoplasia ( $n = 2$ ), CKD ( $n = 3$ ) and Addison's disease ( $n = 1$ ). One out of the seven presented coinfection with feline herpesvirus and FCV, and was treated with glucocorticoids. The only cat identified positive by qPCR was affected by CKD.

Six months after serological and molecular positivity, 8/19 (42.1%) cats included in the PL group died from causes not apparently attributable to leishmaniosis. Two of them had an IFAT titre of 1:80 upon admission, four had 1:160 and one had 1:320. The

qPCR-positive cat was euthanised within six months after inclusion in the study due to CKD progression.

## Discussion

The primary aim of this study was to investigate the prevalence of *Leishmania* species infection in cats in Northern Italy, an area which is becoming endemic for CanL. The secondary aim was to determine whether there was an association between *L infantum* positivity, and signalment and clinicopathological data.

Two different diagnostic methods, IFAT and qPCR, were used to evaluate the presence of *Leishmania* species infection in cats, and the overall prevalence detected was 12.5%. Recent studies carried out in Italy have reported a similar (12.2%) or a greater (25.8%) prevalence of infection.<sup>5,6</sup> In the present survey, the use of a molecular test allowed the identification of infected subject which had not developed a detectable antibody response, suggesting that serology may underestimate the real number of infected animals.<sup>19</sup>

No standardised IFAT assay for the detection of antibodies against *Leishmania* species is currently available for cats, and an antibody titre value universally accepted as indicative of active infection has not been identified.<sup>20</sup> In the present study, seroreactivity ( $\geq 1:40$ ) to *L infantum* was found in 29.6% of the cats tested. Other studies have reported seroreactivity ranging from 16.3 to 30% in Italy.<sup>5,21</sup> However, positivity



with a 1:40 titre is not indicative of infection; therefore, the seroprevalence could be overestimated using lower cut-offs.<sup>4,14</sup> Based on the cut-off value  $\geq 1:80$ ,<sup>2,4,14</sup> the seroprevalence detected decreases to 11.8%. Similar results have been reported in Northern Italy by Spada and colleagues,<sup>5</sup> suggesting the spread of feline infection in recent years. In the present study, *L. infantum* DNA was only identified in the hair sample of one cat. *L. infantum* DNA was not detected in any of the blood and conjunctival swab samples, even in seropositive cats. The possible reasons for the inconsistency of DNA detection in blood is the absence of parasitaemia in cats except in case of severe disease or intermittent parasitaemia as described in dogs.<sup>22,23</sup> Nevertheless, higher and variable values of molecular prevalence of *L. infantum* infection assessed by testing feline blood and conjunctival samples have been reported in Italy;<sup>5,6,11,13,24</sup> and in other European countries.<sup>12,19,25-28</sup> These results suggest that future molecular studies should be carried out to evaluate the diagnostic accuracy of non-invasive samples in cats as has already been done in dogs.

In the present study, *L. infantum* DNA was detected in the hair of one cat, confirming the possible utility of this biological sample for the diagnosis of FeL as has been reported for CanL.<sup>29</sup> The negative result obtained in the IFAT carried out on this cat could reflect an ineffective immune system response or could be explained by the absence of antibody production during an early stage of the infection.<sup>25</sup> Discordant

findings between the serological testing and molecular methods have also been reported previously.<sup>20,30-33</sup>

No significant association was found between *L. infantum* infection and signalment data as has been reported in previous studies.<sup>5,25,33,34</sup> An interesting finding of the present study, although not sustained by statistical significance, is the increased prevalence of infection in the cats sampled in July and August, probably due to the increased phlebotomus activity in Southern Europe during these months.<sup>35</sup>

Positivity to *L. infantum* in cats was associated with a significant increase in total proteins,  $\beta_2$  and  $\gamma$  globulins and with a decrease in the A:G, although not significant when compared to the negative cases. Interestingly, 15.8% and 25.6% of the PL group had hyperproteinaemia and hypergammaglobulinaemia, respectively, and the frequency of these abnormalities was significantly increased when compared to the NL group.

Hyperproteinaemia associated with hyperglobulinaemia has been commonly found in FeL,<sup>4,36,37,38</sup> and, in a study carried out on four cats, hyperglobulinaemia was classified as hypergammaglobulinaemia.<sup>36</sup> However, the presence of hyperproteinaemia, decreased the A:G, and polyclonal  $\beta$  and  $\gamma$  hyperglobulinaemia have long been reported in the course of CanL.<sup>39</sup> Therefore, it can be hypothesised that in cats, as in dogs, the humoral immune response could also be activated by the production of immunoglobulins after infection. When FeL is suspected in a feline patient, performing

serum electrophoresis can be recommended to support the diagnosis by means of evidence of  $\beta_2$  and  $\gamma$  polyclonal hyperglobulinaemia.

No other significant difference was found in the haematological and serum chemistry variables between the two groups studied, although mild to severe non-regenerative normocytic anaemia, thrombocytopenia, leukopenia, pancytopenia and hypoalbuminemia increased liver enzyme activity, and serum creatinine and urea concentrations have been the most frequent abnormalities previously reported in clinical cases of FeL.<sup>2,4,36-38,40</sup> Proteinuria is often the only abnormal clinicopathological finding in dogs with CanL<sup>39</sup> whereas it has been described only occasionally in cats with FeL.<sup>4,36,41</sup> In this study, an increase in the UPC in the PL group was not observed as compared to the NL group. These results could be explained by the composition of the NL group. In fact, the NL group was not composed of healthy cats, but also included patients suffering from other diseases not related to *L. infantum* infection, including numerous cats suffering from renal disease which can be accompanied by several laboratory abnormalities.

The clinical cases of feline leishmaniosis reported have frequently been associated with skin lesions as well as other less specific clinical signs.<sup>30,34</sup> None of the positive cats included in this study showed clinical signs of leishmaniosis, and the disease had never been suspected by the attending clinicians. This suggests that subclinical feline

infections could be common in areas which are becoming endemic for CanL and that clinical illness due to *L infantum* in cats is currently rare.<sup>2</sup> Some limitations should be acknowledged when interpreting the present results. Sampling should have lasted for more than a year, and the number of cats sampled within the inclusion period should have been more homogeneous in each month. A second staggered sampling would have been necessary for the cats included in the UL group to assess the changes in the antibody response. Moreover, the study population consisted mainly of cats referred to a veterinary hospital and, consequently, the animals included in the study could have presented clinicopathological abnormalities not related to *L infantum* infection, affecting the comparison between the PL and NL groups.

## Conclusions

The results of the present study suggested the spread of *L infantum* infection in cats in Northern Italy, although in the majority of cases, the infection did not seem to lead to active disease. The use of a molecular technique for the detection of the *Leishmania* species does not replace the serology but may support the final diagnosis. Hyperproteinaemia and hypergammaglobulinaemia appeared to be significant clinicopathological abnormalities in cats with *Leishmania* species infection. Additional studies are needed to better assess the role of cats in the epidemiological cycle of *Leishmania* species and the pathogenic role of *L. infantum* in this host.

336

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343

344 **Ethical approval** This work involved the use of non-experimental animals only (owned  
345 or unowned) and followed established internationally recognised high standards ('best  
346 practice') of individual veterinary clinical patient care. Ethical approval from a  
347 committee was not necessarily required.

348

349 **Informed consent** Informed consent (either verbal or written) was obtained from the  
350 owner or legal custodian of all animals described in this work for the procedures  
351 undertaken.

352

353 **Informed consent for publications** No animals or humans are identifiable within this  
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355

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473 Table 1. Descriptive statistics and prevalence (%) of infection among the cats included  
 474 in the study groups

Variables	Total	Positive <i>Leishmania</i> (PL) group	Negative <i>Leishmania</i> (NL) group	<i>P</i> value	Unconfirmed <i>Leishmania</i> (UL) group
n	152	19	106		27
Sex					
Male	75 (49.3)	13 (68.4)	49 (46.2)	0.125*	13 (48.1)
Intact	10 (6.5)	1 (5.3)	6 (5.7)	0.322 <sup>†</sup>	3 (11.1)
Castrated	65 (42.8)	12 (63.1)	43 (40.5)		10 (37)
Female	77 (50.7)	6 (31.6)	57 (53.8)		14 (51.9)
Intact	16 (10.6)	1 (5.3)	12 (11.3)		3 (11.1)
Spayed	61 (40.1)	5 (26.3)	45 (42.5)		11 (40.8)
Age (months)					
Kittens <6	4 (2.6)	0 (0)	3 (2.8)	0.755	1 (3.7)
Young 6–24	13 (8.6)	2 (10.5)	11 (10.4)		0 (0)
Adult >24	135 (88.8)	17 (89.5)	92 (86.8)		26 (96.3)
Breed					
European	128 (84.2)	14 (73.7)	92 (86.8)	0.263	22 (81.5)
Pedigree	24 (15.8)	5 (26.3)	14 (13.2)		5 (18.5)
Geographical origin					
Bologna	133 (87.5)	16 (84.2)	93 (87.7)	0.570	24 (88.9)
Modena	5 (3.3)	0 (0)	4 (3.8)		1 (3.7)
Ravenna	5 (3.3)	1 (5.3)	3 (2.8)		1 (3.7)
Ferrara	6 (3.9)	2 (10.5)	4 (3.8)		0 (0)
Forli-Cesena	3 (2)	0 (0)	2 (1.9)		1 (3.7)
Lifestyle					
Indoor/outdoor	74 (48.7)	12 (63.29)	47 (44.3)	0.190	15 (55.6)
Indoor	63 (41.4)	7 (36.8)	49 (46.2)		7 (25.9)
NA	15 (9.9)	0 (0)	10 (9.5)		5 (18.5)
Month of sampling					
June	13 (8.6)	1 (5.3)	10 (9.4)	0.071	2 (7.4)
July	35 (23)	5 (26.3)	26 (24.5)		4 (14.8)
August	22 (14.5)	7 (36.8)	11 (10.4)		4 (14.8)
September	24 (15.8)	2 (10.5)	14 (13.2)		8 (29.6)
October	16 (10.5)	1 (5.3)	13 (12.3)		2 (7.4)

	November	42 (27.6)	3 (15.8)	32 (30.2)	7 (26)
475	The $\chi^2$ test was carried out on the PL and NL groups				
476	* <i>P</i> value referred to male/female				
477	† <i>P</i> value referred to male (intact or castrated)/female (intact or spayed)				

Table 2. Results obtained using the indirect immunofluorescence antibody test (IFAT) and qPCR in blood, conjunctival swabs and hair samples

<b>IFAT</b>	<b>Sample tested (n)</b>	<b>Positive</b>	<b>Negative</b>	<b>Unconfirmed</b>
n	152	18	107	27
Median and range		1:80 (1:80–1:320)	0 (0)	1:40 (1:40)
<b>qPCR</b>	<b>Sample tested (n)</b>	<b>Positive</b>	<b>Negative</b>	<b>Quantity</b>
Blood	146	0	146	–
Conjunctival swabs	150	0	150	–
Hair	150	1	149	8 copies DNA/μl

IFAT = indirect immunofluorescence antibody test; qPCR = real-time polymerase chain reaction

482 Table 3. Prevalence (%) of *Leishmania* species infection in cats detected by IFAT

Variables	IFAT			
	1:80 (n = 11)	1:160 (n = 5)	1:320 (n = 2)	Total (n = 18)
Sex				
Male	8 (72.7)	3 (60)	1 (50)	12 (66.7)
Intact	1 (9.1)	0 (0)	0 (0)	1 (5.6)
Castrated	7 (63.6)	3 (60)	1 (50)	11 (61.1)
Female	3 (27.3)	2 (40)	1 (50)	6 (33.3)
Intact	0 (0)	0 (0)	1 (50)	1 (5.6)
Spayed	3 (27.3)	2 (40)	0 (0)	5 (27.7)
Age (months)				
Kitten <6	0 (0)	0 (0)	0 (0)	0 (0)
Young 6–24	0 (0)	1 (20)	1 (50)	2 (11.1)
Adult >24	11 (100)	4 (80)	1 (50)	16 (88.9)
Breed				
European	10 (90.9)	3 (60)	0 (0)	13 (72.2)
Pedigree	1 (9.1)	2 (40)	2 (100)	5 (27.8)
Geographical origin				
Bologna	10 (90.9)	5 (100)	1 (50)	16 (88.9)
Modena	0 (0)	0 (0)	0 (0)	0 (0)
Ravenna	0 (0)	0 (0)	0 (0)	0 (0)
Ferrara	1 (9.1)	0 (0)	1 (50)	2 (11.1)
Forli-Cesena	0 (0)	0 (0)	0 (0)	0 (0)
Lifestyle				
Indoor/Outdoor	7 (63.6)	4 (80)	1 (50)	12 (66.7)
Indoor	4 (36.4)	1 (20)	1 (50)	6 (33.3)
NA	0 (0)	0 (0)	0 (0)	0 (0)
Month of sampling				
June	1 (9.1)	0 (0)	0 (0)	1 (5.6)
July	2 (18.2)	1 (20)	2 (100)	5 (27.7)
August	3 (27.2)	3 (60)	0 (0)	6 (33.3)
September	2 (18.2)	0 (0)	0 (0)	2 (11.1)
October	1 (9.1)	0 (0)	0 (0)	1 (5.6)
November	2 (18.2)	1 (20)	0 (0)	3 (16.7)

483 IFAT = indirect immunofluorescence antibody test



484 Table 4. Clinicopathological results of the cats included in the PL and NL groups

Variables	Negative <i>Leishmania</i> (NL) group (n = 106)	Positive <i>Leishmania</i> (PL) group (n = 19)	Reference interval	<i>P</i> value
Haematology				
RBCs (cells/mm <sup>3</sup> )	7,449,504.95 ± 2,268,986.283	7,473,333.333 ± 2,055,798.113	7,000,000–11,000,000	0.962
WBCs (cells/mm <sup>3</sup> )	10,200 (950–77,480)	10,545 ± 4195.617	4800–14,930	0.705
Hb (fl)	10.974 ± 3.288	11.3 (7–15.2)	10–16	0.879
Hct (g%)	32.202 ± 9.517	34.6 (32.35–44.1)	32–48	0.982
MCV (fl)	43.1 (32.5–56.9)	43.667 ± 4.047	36–55	0.810
MCH (pg)	14.7 (10.8–19.7)	14.689 ± 1.578	12.3–16.2	0.672
MCHC (g%)	34.1 (31.8–46.9)	33.606 ± 1.143	31–36	0.259
MPV (fl)	16.8 (9.2–32.1)	17.683 ± 5.385	8–26	0.727
RDW (%)	15 (12.7–27.1)	15.367 ± 1.843	13.17–17	0.781
Platelets (cells/mm <sup>3</sup> )	217,000 (1000–644,000)	252,277.8 ± 148,734.6	150,000–500,000	0.609
Reticulocytes (cells/mm <sup>3</sup> )	33,000 (2200–162,100)	28,077.78 ± 14,184.48	0–80,000	0.106
Neutrophils (cells/mm <sup>3</sup> )	7110 (390–76,050)	7943.333 ± 4175.889	1600–10,000	0.807
Monocytes (cells/mm <sup>3</sup> )	230 (30–7670)	255 (60–920)	0–650	0.940
Lymphocytes (cells/mm <sup>3</sup> )	1560 (40–17,270)	1817.222 ± 820.701	900–5600	0.750
Basophils (cells/mm <sup>3</sup> )	10 (0–900)	10 (0–40)	0–60	0.096

Eosinophil (cells/mm <sup>3</sup> )	290 (0–2430)	499.412 ± 399.585	60–1470	0.439
Serum Chemistry				
ALT (U/l)	64 (3–743)	60.211 ± 38.125	20–72	0.060
AST (U/l)	39 (16–828)	29 (16–115)	9–40	0.050
ALP (U/l)	25 (5–97,772)	15 (4–133)	0–140	0.009
GGT (U/l)	0.1 (0–5.2)	0.1 (0–1.4)	0–4	0.256
Total Bilirubin (mg/dl)	0.18 (0.01–20.85)	0.195 (0.04–3.23)	0–0.3	0.544
Cholesterol (mg/dl)	159.786 ± 52.549	150.5 (72–325)	64–229	0.775
Glucose (mg/dl)	127 (57–591)	121.316 ± 34.041	63–148	0.114
Albumin (g/dl)	3.023 ± 0.502	3.012 ± 0.537	2.6–4	0.997
Total Protein (g/dl)	7.171 ± 0.897	7.933 ± 0.946	6.5–8.8	0.005
A:G (g:g)	0.758 ± 0.191	0.654 ± 0.206	0.52–1.19	0.051
Creatinine (mg/dl)	1.54 (0.41–11.77)	1.71 (0.78–31.46)	0.8–1.8	0.375
Urea (mg/dl)	62.95 (12.6–533.05)	58.52 (26.3–599.85)	30–65	0.773
Phosphate (mg/dl)	4.835 (1.77–15.76)	5.05 (3.37–15.22)	2.5–6.2	0.547
Potassium (mEq/l)	4.25 (2.6–7.4)	4.363 ± 0.585	3.4–5.1	0.418
Sodium (mEq/l)	151.5 (136–168)	151.211 ± 4.454	145–155	0.720
Chloride (mEq/l)	117.000 (96.500–126.800)	116.050 ± 4.119	110–123	0.561
Magnesium (mg/dl)	2.31 (1.52–4.63)	2.466 ± 0.332	1.9–2.8	0.259
Total Calcium (mg/dl)	9.8 (6.7–13.14)	9.822 ± 0.734	8.5–10.5	0.948

SAA (μg/dl)	8 (1–308)	12 (1–202)	0–10	0.724
Serum protein electrophoresis				
Albumin (g/dl)	2.802 ± 0.607	2.912 ± 0.655	2.10–3.30	0.443
Albumin (%)	39.274 ± 7.635	37.205 ± 9.395	–	0.492
Alfa(α) <sub>1</sub> -globulin (g/dl)	1.180 (0.02–3.62)	1.16 (0.09–1.63)	0.1–0.8	0.710
α <sub>1</sub> -globulin (%)	16.3 (0.2–53.6)	14.9 (1.1–20)	–	0.057
α <sub>2</sub> -globulin (g/dl)	0.825 (0.33–2.87)	0.81 (0.61–3.3)	0.58–1.05	0.573
α <sub>2</sub> -globulin (%)	11.65 (5.6–39)	10.3 (7.4–39.9)	–	0.557
Beta(β) <sub>1</sub> -globulin (g/dl)	0.560 (0.21–1.29)	0.587 ± 0.122	0.45–0.80	0.507
β <sub>1</sub> -globulin (%)	8 (2.9–20.2)	7.458 ± 1.636	–	0.183
β <sub>2</sub> -globulin (g/dl)	0.43 (0.14–1.66)	0.55 (0.32–1.21)	0.35–0.75	0.013
β <sub>2</sub> -globulin (%)	6.25 (2.1–24.8)	7.132 ± 1.988	–	0.143
Gamma(γ)-globulin (g/dl)	1.03 (0.02–2.79)	1.37 (0.82–3.44)	0.60–1.30	0.003
γ-globulin (%)	14.85 (0.4–33.1)	20.132 ± 7.221	–	0.014
A:G (g:g)	0.672 ± 0.204	0.625 ± 0.230	–	0.489
Urinalysis				
USG	1026 (1008–1084)	1038.75 ± 23.344	>1040	0.428
Urine creatinine (mg/dl)	105.26 (13.7–436.98)	246.207 ± 186.088	–	0.042
Urine protein (mg/dl)	45.94 (2.28–2188)	93.61 (8.19–564.52)	0–0.4	0.114

	UPC (mg:mg)	0.354 (0.041–38.582)	0.281 (0.115–10.631)	<0.2	0.750
485	A:G = albumin to globulin ratio; ALP = alkaline phosphatase level; ALT = alanine aminotransferase; AST = aspartate aminotransferase;				
486	GGT = gamma(γ)-glutamyl transferase; Hb = hemoglobin; Hct = hematocrit value; MCH = mean corpuscular haemoglobin; MCHC =				
487	mean corpuscular haemoglobin concentration; MCV = mean corpuscular volume; MPV = mean platelet volume; RBCs = red blood cells;				
488	RDW = red cell distribution width; SAA = serum amyloid A; UPC = urine protein:creatinine ratio; USG = urine specific gravity; WBCs =				
489	white blood cells				