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 ≥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, $beta_2$-globulin and gamma-globulin were significantly increased in the positive *Leishmania* group when compared to the negative group.

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

Keywords: feline leishmaniosis; indirect immunofluorescence test; real-time PCR; 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,[1] with seroprevalence ranging from 0 to 68.5% and molecular prevalence ranging from 0 to 60.7%.[2] Cats have a neglected and controversial role in the cycle of *L infantum*[3] but whether they serve as primary, secondary or accidental hosts is currently unknown.[4] Furthermore, only a few studies have been carried out regarding clinical and clinicopathological abnormalities in cats with feline leishmaniosis (FeL).[2]

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).[2,4-7] Of the molecular methods, the real-time PCR (qPCR) allows quantitative and fast detection of *Leishmania* species DNA.[8,9] 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.[2,10] 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.[6,11-13]

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_3EDTA 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°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 (≥1:80)[14] 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.[15] Slides coated with 30 µl of an in-house antigen consisting of promastigotes of *L infantum* (strain MHOM/TN/80/IPT1)[16] 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°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 µl fluorescein isothiocyanate (FITC) conjugated anti-cat IgG antibody

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

124 **Molecular diagnosis**

125 Genomic DNA was extracted from blood, conjunctival swabs and hair samples using a
126 commercially available kit (NucleoSpin Tissue Kit; Macherey-Nagel) according to the
127 manufacturer's protocol. Before extraction, for each cat sampled, the two conjunctival
128 swab samples (right and left eyes) were resuspended together in 250 µl of PBS. Instead,
129 the hair samples were cut into three segments (proximal, central and distal) and lysed at
130 56°C overnight with 250 µl of lysis buffer (Buffer T1) and 50 µl of proteinase K
131 supplied in the extraction kit.

132 *Leishmania* species DNA detection was carried out using SYBR Green qPCR as
133 reported by Ceccarelli and collaborators.[17] A reaction was carried out using a
134 commercially available kit (PowerUp SYBR Green Master Mix Kit, Life Technologies)
135 and the StepOnePlus Real-Time PCR System (Thermo Fisher Scientific, Life
136 Technologies). Serial 10-fold dilutions of a plasmid (pCR4 plasmid; Invitrogen; Life
137 Technologies) containing one copy of the target sequence was used as an external
138 standard for the construction of the assay standard curve. Melting experiments were
139 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 x 10^0 copy/µl. 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(γ)-glutamyltransferase, total bilirubin, cholesterol, total calcium, sodium, potassium, chloride, glucose, and serum amyloid A (SAA), as reported previously,[18] was determined. Urinalysis included urine specific gravity (USG), dipstick (Combur[10]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 ± 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

179 **Study groups and diagnosis of *L infantum* infection**

180 During the study period, 152 cats met the inclusion criteria. Nineteen of the 152
181 (12.5%) cats were included in the PL group: 18/152 (11.8%) showed IFAT titre ≥1:80
182 and 1/152 (0.7%) was tested qPCR-positive in hair sample. The NL group included
183 106/152 (69.7%) cats and the UL group included 27/152 (17.8%). Table 1 shows the
184 descriptive statistics results and data regarding the prevalence of infection in the study
185 population.
186 Thirteen of the 19 (68.4%) cats included in the PL group were male of which 12/13
187 (92.3%) were castrated, and 6/19 (31.6%) were female of which 5/6 (83.3%) were
188 spayed. Seventeen of the 19 (89.5%) were more than 24 months of age and 2/19
189 (10.5%) were between 6 and 24 months of age, with a median age of 8 years (9 months-
190 15 years). Fourteen of the 19 (73.7%) were European domestic shorthair cats and 5/19
191 (26.3%) were from other various breeds (Maine Coon = 1; Bengal = 1; Birman = 1;
192 Persian = 1; Chartreux = 1); 12/19 cats (63.2%) were indoor/outdoor while 7/19
193 (36.8%) were exclusively indoor. Sixteen of the 19 (84.2%) cats were from the province
194 of Bologna. The highest frequency of positivity was detected in July (5/35; 14.3%) and
195 August (7/22; 31.8%) while the frequency decreased in the other months of sampling.
196 Seroreactivity (IFAT titre ≥1:40) to *L infantum* was found in 45 of the 152 (29.6%) cats
197 tested. Of the 18 seropositive cats (IFAT titre ≥1:80), 11 (61.1%) had an antibody titre
198 equal to 1:80, five (27.8%) 1:160 and two (11.1%) 1:320 (Tables 2 and 3).

199 The cat identified as positive on hair using qPCR showed 8 copies of *Leishmania*
200 kinetoplast DNA/µl of DNA extract and was seronegative to *L infantum* (Table 2). This
201 cat was a 10-year-old European domestic shorthair castrated male which lived indoors
202 and was sampled in August.
203 Forty-nine of the 106 cats included in the NL group were male of which 43/49 (87.8%)
204 were castrated while the remaining 57 were female of which 45/57 (78.9%) were
205 spayed. Ninety-two (86.8%) were more than 24 months old, with a median of 7 years (4
206 months–20 years); 92/106 (86.8%) were European domestic shorthair cats and 93/106
207 (87.7%) were from the province of Bologna. Forty-nine of the 106 (46.2%) were indoor
208 cats and 47/106 (44.3%) were indoor/outdoor cats.
209 No statistical association was found among positivity to *L infantum* and sex, age, breed,
210 geographical origin, access to outdoor environment and month of sampling. Although
211 not significant, an increased frequency in positivity among cats sampled in July and
212 August was observed ($P = 0.071$) (Table 1).
213 No agreement between the results obtained by IFAT and qPCR and between the qPCR
214 carried out on three different biological samples was shown by the value of Cohen's
215 kappa coefficient ($P = 0.309$).
216 **Clinical and clinicopathological findings**
217 The results of the CBC, serum chemistry, protein electrophoresis, and urinalysis
218 including UPC obtained in the PL and NL groups are shown in Table 4. Total proteins,

beta(β)$_2$-globulin and gamma(γ)-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), neoplastia (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

239 qPCR-positive cat was euthanised within six months after inclusion in the study due to
240 CKD progression.
241
242 **Discussion**
243 The primary aim of this study was to investigate the prevalence of *Leishmania* species
244 infection in cats in Northern Italy, an area which is becoming endemic for CanL. The
245 secondary aim was to determine whether there was an association between *L infantum*
246 positivity, and signalment and clinicopathological data.
247 Two different diagnostic methods, IFAT and qPCR, were used to evaluate the presence
248 of *Leishmania* species infection in cats, and the overall prevalence detected was 12.5%.
249 Recent studies carried out in Italy have reported a similar (12.2%) or a greater (25.8%)
250 prevalence of infection.[5,6] In the present survey, the use of a molecular test allowed the
251 identification of infected subject which had not developed a detectable antibody
252 response, suggesting that serology may underestimate the real number of infected
253 animals.[19]
254 No standardised IFAT assay for the detection of antibodies against *Leishmania* species
255 is currently available for cats, and an antibody titre value universally accepted as
256 indicative of active infection has not been identified.[20] In the present study,
257 seroreactivity (≥1:40) to *L infantum* was found in 29.6% of the cats tested. Other studies
258 have reported seroreactivity ranging from 16.3 to 30% in Italy.[5,21] However, positivity

with a 1:40 titre is not indicative of infection; therefore, the seroprevalence could be overestimated using lower cut-offs.[4,14] Based on the cut-off value ≥1:80,[2,4,14] the seroprevalence detected decreases to 11.8%. Similar results have been reported in Northern Italy by Spada and colleagues,[5] 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.[22,23] 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;[5,6,11,13,24] and in other European countries.[12,19,25-28] 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.[29] 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.[25] Discordant

findings between the serological testing and molecular methods have also been reported previously.[20,30-33]

No significant association was found between *L infantum* infection and signalment data as has been reported in previous studies.[5,25,33,34] 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.[35]

Positivity to *L infantum* in cats was associated with a significant increase in total proteins, β_2 and γ 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,[4,36,37,38] and, in a study carried out on four cats, hyperglobulinaemia was classified as hypergammaglobulinaemia.[36] However, the presence of hyperproteinaemia, decreased the A:G, and polyclonal β and γ hyperglobulinaemia have long been reported in the course of CanL.[39] 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

297 serum electrophoresis can be recommended to support the diagnosis by means of
298 evidence of β_2 and γ polyclonal hyperglobulinaemia.
299 No other significant difference was found in the haematological and serum chemistry
300 variables between the two groups studied, although mild to severe non-regenerative
301 normocytic anaemia, thrombocytopenia, leukopenia, pancytopenia and
302 hypoalbuminemia increased liver enzyme activity, and serum creatinine and urea
303 concentrations have been the most frequent abnormalities previously reported in clinical
304 cases of FeL.[2,4,36-38,40] Proteinuria is often the only abnormal clinicopathological finding
305 in dogs with CanL[39] whereas it has been described only occasionally in cats with
306 FeL.[4,36,41] In this study, an increase in the UPC in the PL group was not observed as
307 compared to the NL group. These results could be explained by the composition of the
308 NL group. In fact, the NL group was not composed of healthy cats, but also included
309 patients suffering from other diseases not related to *L infantum* infection, including
310 numerous cats suffering from renal disease which can be accompanied by several
311 laboratory abnormalities.
312 The clinical cases of feline leishmaniosis reported have frequently been associated with
313 skin lesions as well as other less specific clinical signs.[30,34] None of the positive cats
314 included in this study showed clinical signs of leishmaniosis, and the disease had never
315 been suspected by the attending clinicians. This suggests that subclinical feline

316 infections could be common in areas which are becoming endemic for CanL and that
317 clinical illness due to *L infantum* in cats is currently rare.[2]
318 Some limitations should be acknowledged when interpreting the present results.
319 Sampling should have lasted for more than a year, and the number of cats sampled
320 within the inclusion period should have been more homogeneous in each month. A
321 second staggered sampling would have been necessary for the cats included in the UL
322 group to assess the changes in the antibody response. Moreover, the study population
323 consisted mainly of cats referred to a veterinary hospital and, consequently, the animals
324 included in the study could have presented clinicopathological abnormalities not related
325 to *L infantum* infection, affecting the comparison between the PL and NL groups.
326

327 **Conclusions**

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

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

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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 *Leishmania* (PL) group**	**Negative *Leishmania* (NL) group**	***P* value**	**Unconfirmed *Leishmania* (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†	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)
Forlì-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 *P value referred to male/female

477 †P value referred to male (intact or castrated)/female (intact or spayed)

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

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

480 IFAT = indirect immunofluorescence antibody test; qPCR = real-time polymerase chain
481 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)
Forlì-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)

IFAT = indirect immunofluorescence antibody test

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

Variables	**Negative *Leishmania* (NL) group (n = 106)**	**Positive *Leishmania* (PL) group (n = 19)**	**Reference interval**	***P* value**
Haematology				
RBCs (cells/mm^3)	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^3)	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^3)	217,000 (1000–644,000)	252,277.8 ± 148,734.6	150,000–500,000	0.609
Reticulocytes (cells/mm^3)	33,000 (2200–162,100)	28,077.78 ± 14,184.48	0–80,000	0.106
Neutrophils (cells/mm^3)	7110 (390–76,050)	7943.333 ± 4175.889	1600–10,000	0.807
Monocytes (cells/mm^3)	230 (30–7670)	255 (60–920)	0–650	0.940
Lymphocytes (cells/mm^3)	1560 (40–17,270)	1817.222 ± 820.701	900–5600	0.750
Basophils (cells/mm^3)	10 (0–900)	10 (0–40)	0–60	0.096

Eosinophil (cells/mm^3)	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)$_1$-globulin (g/dl)	1.180 (0.02–3.62)	1.16 (0.09–1.63)	0.1–0.8	0.710
α_1-globulin (%)	16.3 (0.2–53.6)	14.9 (1.1–20)	–	0.057
α_2-globulin (g/dl)	0.825 (0.33–2.87)	0.81 (0.61–3.3)	0.58–1.05	0.573
α_2-globulin (%)	11.65 (5.6–39)	10.3 (7.4–39.9)	–	0.557
Beta(β)$_1$-globulin (g/dl)	0.560 (0.21–1.29)	0.587 ± 0.122	0.45–0.80	0.507
β_1-globulin (%)	8 (2.9–20.2)	7.458 ± 1.636	–	0.183
β_2-globulin (g/dl)	0.43 (0.14–1.66)	0.55 (0.32–1.21)	0.35–0.75	0.013
β_2-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

A:G = albumin to globulin ratio; ALP = alkaline phosphatase level; ALT = alanine aminotransferase; AST = aspartate aminotransferase; GGT = gamma(γ)-glutamyl transferase; Hb = hemoglobin; Hct = hematocrit value; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; MCV = mean corpuscular volume; MPV = mean platelet volume; RBCs = red blood cells; RDW = red cell distribution width; SAA = serum amyloid A; UPC = urine protein:creatinine ratio; USG = urine specific gravity; WBCs = white blood cells