Evaluation of clinicopathological abnormalities in sick cats naturally infected by *Leishmania infantum*

Manolis K. Chatzis a,*, Panagiotis G. Xenoulis a, Leonidas Leontides b, Dimitrios Kasabalakis a, Mathios E. Mylonakis c, Margarita Andreadou d, John Ikonomopoulos d, Manolis N. Saridomichelakis a

a Clinic of Medicine, Faculty of Veterinary Science, University of Thessaly, 224 Trikalon Str., GR-43132, Karditsa, Greece

b Laboratory of Epidemiology, Biostatistics and Animal Health Economics, Faculty of Veterinary Science, University of Thessaly, 224 Trikalon Str., GR-43132, Karditsa, Greece

c Companion Animal Clinic, School of Veterinary Medicine, Aristotle University of Thessaloniki, 11 Stavrou Voutyra Str., GR-54627, Thessaloniki, Greece

d Department of Anatomy and Physiology of Farm Animals, Faculty of Animal Science, Agricultural University of Athens, 75 Iera Odos, Votanikos, GR-11855, Athens, Greece

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ABSTRACT

Feline infection by *Leishmania infantum* (syn. *L. chagasi*) has been described in areas where canine leishmaniosis is endemic. A wide variety of clinicopathological abnormalities have been reported in cats presenting clinical signs of leishmaniosis but there is a paucity of information regarding cats infected by *L. infantum* that do not suffer from leishmaniosis but from other diseases. The aim of this study was to compare: a) the frequency of clinicopathological abnormalities and b) the values of hematology, serum biochemistry and urinalysis parameters, between non-infected sick cats and sick cats that were infected by *L. infantum*. A total of 50 cats with cutaneous, ocular and/or systemic clinical signs that lived in an endemic area and had been tested for infection by *L. infantum* using PCR from four different tissues, were included. Based on the results of PCR, 20/50 cats were found to be infected and 30/50 non-infected. The only difference between the two groups of cats was that the concentration of inorganic phosphorus (*P* = 0.043) was higher in infected cats. This finding may suggest an association between infection by *L. infantum* and feline kidney disease.

1. Introduction

Feline infection by *Leishmania infantum* (syn. *L. chagasi*) has been described in areas where canine leishmaniosis is endemic, including several European (Pennisi et al., 2013; Vilhena et al., 2013; Chatzis et al., 2014; Migliazzo et al., 2015; Persichetti et al., 2016; Spada et al., 2016; Otranto et al., 2017), Latin American (Benassi et al., 2017; Metzdorf et al., 2017), and Asian (Hatam et al., 2010; Akhtardanesh et al., 2017) countries. Most infected cats remain healthy, possibly because of their natural resistance to the disease (Solano-Gallego et al., 2007; Pennisi et al., 2013). Only a minority of them develop leishmaniosis and they present a variety of skin lesions, ocular and/or systemic signs (Pennisi et al., 2004; Vila et al., 2005; Marcos et al., 2009; Vides et al., 2011; Sobrino et al., 2012; Pennisi et al., 2013; Metzdorf et al., 2017; Fernandez-Gallego et al., 2020). However, not all clinical signs of cats with leishmaniosis are necessarily due to this disease, some may be caused by comorbidities. Clinical findings and pathologies that could be reliably attributed to feline leishmaniosis, based on the presence of the parasite and/or of compatible histologic lesions in affected tissues and organs, include skin ulcers and nodules, conjunctivitis, keratitis, uveitis, peripheral lymphadenomegaly, splenomegaly, hepatomegaly, rhinitis, ulcerative stomatitis and chronic kidney disease due to glomerulonephritis and/or interstitial nephritis (Marcos et al., 2009; Hatam et al., 2010; Sobrino et al., 2012; Pennisi et al., 2013; Migliazzo et al., 2015; Metzdorf et al., 2017). We have previously reported a positive association between infection by *L. infantum* and the presence of at least one systemic (but not cutaneous or ocular) clinical sign that has been reported in cats with leishmaniosis (Chatzis et al., 2014).

Numerous clinicopathological abnormalities such as anemia, leukocytosis or neutropenia, neutrophilia or neutropenia, lymphocytosis or lymphopenia, monocytosis, thrombocytopenia, increased total protein and globulin concentrations, azotemia, increased alkaline phosphatase...
(ALT), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) activities, proteinuria, bilirubinuria and hematuria have been reported in cats with leishmaniosis, without always being clear whether they are caused by the disease or by comorbidities (Pennisi et al., 2004; Rufenacht et al., 2005; Vita et al., 2005; Paludo et al., 2008; Marcos et al., 2009; Pennisi et al., 2011; Akhtardanesh et al., 2017; Fernandez-Gallego et al., 2020). The only clinicopathological abnormality that appears to be directly associated with this disease is hyperglobulinemia, which is usually polyclonal and is caused by increased alpha-2, beta- and/or gamma-globulins (Pennisi et al., 2004; Marcos et al., 2009).

Based on the combined results of blood, skin, bone marrow and conjunctiva PCR, we have previously reported that 20/50 (40%) of the cats that lived in an endemic area and presented various cutaneous, ocular and/or systemic clinical signs, were positive for *L. infantum* DNA, although none were diagnosed with leishmaniosis (Chatzis et al., 2014). The aim of the present study was to compare a) the frequency of clinicopathological abnormalities and b) the values of hematology, serum biochemistry and urinalysis parameters between these 20 infected and the 30 non-infected cats.

2. Materials and methods

2.1. Study population and PCR

A total of 50 cats living in Central and Northern, Greece, where canine leishmaniosis due to *L. infantum* is endemic (Leontides et al., 2002; Athanasiou et al., 2012), were included. Handling of these animals was in compliance with European Communities Council Directive 86/609/EEC and state laws. The experimental protocol had been approved by State Authorities (license Nr. 3698/31-10-08).

Details of inclusion and exclusion criteria, sampling and PCR have been previously published (Chatzis et al., 2014). Briefly, all cats were at least 1-year old with no history of leishmaniosis or administration of drugs with known anti-leishmania or immune-modulating activity. Conventional PCR (Andreadou et al., 2012) in blood, skin biopsies, bone marrow and conjunctiva swabs was performed to differentiate infected from non-infected cats (Chatzis et al., 2014).

2.2. Sample collection

For cats less than 8 years-old without systemic clinical signs, chemical restraint was induced by intramuscular administration of a combination of ketamine (5 mg/kg body weight; Ketaset®, Fort Dodge Animal Health, Iowa, USA), midazolam (0.2 mg/kg body weight; Dormicum®, Roche Hellas, Athens, Greece) and dexamethomidine (20 μg/kg body weight; Dexdomitor®, Zoetis, New Jersey, USA). In older cats and/or those with systemic disease, the dose of dexamethomidine was reduced (5 μg/kg body weight) and butorphanol (0.2-0.4 mg/kg body weight; Butomidor®, Richter Pharma, Austria) was added to the drug combination.

Blood samples were collected via jugular venipuncture using a 21 G needle attached to a Monovette® syringe (Sarstedt G mbH & Co Germany). Approximately 10 ml of blood was collected: 2 ml were immediately transferred into EDTA tubes and the remaining was left to clot for 20 min and was centrifuged at 3,000 rpm for 20 min to harvest the serum. Urine samples were collected with cystocentesis: urinalysis, including sediment microscopy after centrifugation at 1,500 rpm for 5 min, was performed within 1 h, and an aliquot was stored at -20 °C until measurement of urine protein/creatinine ratio (UP/C). Urine samples were cultured for the detection of aerobic bacteria if there was evidence of infection.

2.3. Clinicopathological examinations

Complete blood count was performed using the VetAutoread™ Hematology Analyzer (IDEXX Laboratories, Westbrook, MA) followed by a manual 100-cell leucocyte differential count on a Giemsa-stained blood smear. Serum concentration of total proteins, albumin, blood urea nitrogen, creatinine, glucose, cholesterol, total bilirubin, calcium, inorganic phosphorus, and activities of ALT, AST, gamma-glutamyl transferase (GGT) and creatine kinase were measured using the Vet-Test® Chemistry Analyzer (IDEXX Laboratories, Westbrook, MA). The concentration of globulins was calculated by subtracting albumin from the total protein concentration, and subsequently the albumin/globulin ratio was also calculated. Additionally, serum potassium, sodium and chloride concentrations were measured using the VetStat® Electrolyte and Blood Gas Analyzer (IDEXX Laboratories, Westbrook, MA).

Urinealyses included the measurement of specific gravity with a refractometer (American Optical), dipstick examination (Multistix® 10 SG, Bayer), microscopic examination of sediment and calculation of the UP/C ratio (for those samples without evidence of hematuria or urinary tract infection). Urine protein concentration was determined by the pyrogallol red-molybdate method (Watanabe et al., 1986) with a spectrophotometer (Shimadzu, UV-1601), using commercially available reagents (Urinary Proteins LR®, Cesan). Urine creatinine concentration was determined by the colorimetric kinetic method (Romeo, 1975) with the same spectrophotometer, using commercially available reagents (Creatinine Kinetic®, Flowcytogen Laboratories). Cats were classified as non-proteinuric, borderline-proteinuric, or proteinuric when the UP/C ratio was <0.2, ≥0.2 and <0.4, or ≥0.4, respectively (Elliott, 2007).

2.4. Statistical analysis

In the initial univariable screening, the frequency of clinicopathological abnormalities was compared between infected and non-infected cats, by either Pearson’s χ² or Fisher’s exact test. Subsequently, the normality of the distributions of the concentration or activity of serum biochemistry parameters was tested by the Kolmogorov-Smirnov test. The parameters which did not deviate from normality are presented as means ± standard deviation and were compared between infected and non-infected cats by independent sample t-tests. The parameters whose distributions deviated from normality were presented as medians and range and were compared between infected and non-infected cats by the Mann Whitney U test.

Variables that were significantly different (P ≤ 0.25) between infected and non-infected cats in the univariable screening of the frequency of clinicopathological abnormalities or in the univariable comparison of the distributions of the concentration or activity of serum and urine biochemistry parameters, were selected as initial candidates for two separate logistic regression models using stepwise, forward, and backward selection procedures. Variables identified as being associated with the infection status of the cat (at P < 0.05) were retained in the final models. Odds ratios (OR) derived from the models were interpreted as measures of increased risk of infection. Because infection was not a rare condition in the study population, the OR were not precise measures of increased risk, but the approximation was reasonable and was utilized to clarify the presentation of results. The goodness of fit of the models was evaluated using the Hosmer-Lemeshow goodness-of-fit chi-square statistic with the data divided into 4 groups. The analyses were done in Stata 13 (Stata Corp, College Station, TX).

3. Results

Details on the signalment and the final diagnoses of the cats included in the present study have been previously published (Chatzis et al., 2014).

The frequency of the abnormalities found in hematology, serum biochemistry and urinalysis are presented in Table 1 and the values of the clinicopathological parameters in Table 2. The most common abnormalities were hyperglycemia (24/50-48%) and increased creatine kinase activity (30/50–60%), but, in the majority of the cases, they were attributed to the stress plus dexamethomidine administration and to the intramuscular injection of the sedative agents, respectively. Urine samples were obtained from 42/50 (84%) cats. Specific gravity and pH
ranged from 1.001 to 1.040 (median: 1.031) and from 6 to 8 (median: 6), respectively. Glycosuria was present in 7/42 (16.7%) cats and was accompanied by hyperglycemia in six of them. Ketonuria was found in one (2.4%) cat diagnosed with diabetes mellitus and bilirubinuria in 2/42 (4.8%) cats and was accompanied by increased serum total bilirubin concentration. Also, 5/42 (11.9%) cats had hematuria due to idiopathic cystitis (2/5–40%) or urinary tract infection (3/5–60%). Three additional cats had urinary tract infection without hematuria and the UP/C ratio was determined in 34/50 (68%) cats. It ranged from 0.09 to 1.5 (median: 0.23) and proteinuria (UP/C ratio >0.4) was detected in 8/34 (23.5%) cats.

In the univariable screening, the frequency of increased total bilirubin concentration and increased ALP activity was significantly higher in infected cats (Table 1) but in the final logistic regression model, none was associated with the cat’s infection status. Absolute neutrophil count, GGT activity and inorganic phosphorus concentration were significantly higher in infected compared to non-infected cats (Table 2) but in the final logistic model only increased inorganic phosphorus concentration retained significance (P = 0.043; OR = 1.82, 95% CI: 1.02–3.23). The Hosmer-Lemeshow goodness-of-fit χ² statistic for this model, did not show evidence of lack-of-fit (Hosmer-Lemeshow χ² = 1.70, P = 0.43).

4. Discussion

In general, cats are considered resistant to infection by *L. infantum* (Pennisi et al., 2013). However, some infected cats develop clinical signs and/or clinicopathological abnormalities of leishmaniosis (Pennisi et al., 2013). The lack of significant differences in the frequency of

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### Table 1. Comparison of the prevalence of abnormalities found in hematology, serum biochemistry and urinalysis in 50 cats with cutaneous, ocular and/or systemic clinical signs, including 20 cats naturally infected by *L. infantum* and 30 non-infected cats.

| Laboratory abnormality | Infected; n = 20 (%) | Non-infected; n = 30 (%) | P value |
|------------------------|----------------------|--------------------------|--------|
| **Hematology**         |                      |                          |        |
| ↓ PCV                  | 4 (20%)              | 7 (23.3%)                | 1      |
| ↓ Hemoglobin           | 4 (20%)              | 7 (23.3%)                | 1      |
| ↓ White blood cells    | 0 (0%)               | 2 (6.7%)                 | 0.51   |
| ↑ White blood cells    | 7 (35%)              | 6 (20%)                  | 0.236  |
| ↑ Neutrophils          | 7 (35%)              | 8 (26.7%)                | 0.529  |
| ↑ Band neutrophils     | 6 (30%)              | 4 (13.3%)                | 0.171  |
| ↓ Lymphocytes          | 6 (30%)              | 12 (40%)                 | 0.47   |
| ↑ Lymphocytes          | 1 (5%)               | 3 (10%)                  | 0.641  |
| ↑ Monocytes            | 2 (10%)              | 2 (6.7%)                 | 1      |
| ↑ Eosinophils          | 0 (0%)               | 1 (3.3%)                 | 1      |
| ↑ Platelets            | 2 (10%)              | 1 (3.3%)                 | 0.556  |
| **Serum biochemistry** |                      |                          |        |
| ↓ Total proteins       | 3 (15%)              | 5 (16.7%)                | 1      |
| ↓ Albumins             | 2 (10%)              | 3 (10%)                  | 1      |
| ↓ Globulins            | 2 (10%)              | 1 (3.3%)                 | 0.556  |
| ↑ Globulins            | 1 (5%)               | 1 (3.3%)                 | 1      |
| ↓ A/G ratio            | 3 (15%)              | 2 (6.7%)                 | 0.377  |
| ↑ A/G ratio            | 0 (0%)               | 1 (3.3%)                 | 1      |
| ↑ Urea nitrogen        | 5 (25%)              | 4 (13.3%)                | 0.454  |
| ↑ Creatinine           | 3 (15%)              | 0 (0%)                   | 0.058  |
| ↓ Glucose              | 1 (5%)               | 2 (6.7%)                 | 1      |
| ↓ Glucose              | 9 (45%)              | 15 (50%)                 | 0.729  |
| ↓ Cholesterol          | 1 (5%)               | 1 (3.3%)                 | 1      |
| ↓ Cholesterol          | 1 (5%)               | 0 (0%)                   | 0.4    |
| ↑ Total bilirubin      | 5 (25%)              | 1 (3.3%)                 | 0.032  |
| ↑ ALP                  | 5 (25%)              | 1 (3.3%)                 | 0.032  |
| ↑ ALT                  | 4 (20%)              | 2 (6.7%)                 | 0.202  |
| ↑ AST                  | 5 (25%)              | 4 (13.3%)                | 0.454  |
| ↑ GGT                  | 3 (15%)              | 0 (0%)                   | 0.058  |
| ↑ Creatine kinase      | 12 (60%)             | 18 (60%)                 | 1      |
| ↓ Calcium              | 3 (15%)              | 2 (6.7%)                 | 0.377  |
| ↓ Inorganic phosphorus | 0 (0%)               | 2 (6.7%)                 | 0.51   |
| ↓ Inorganic phosphorus | 3 (15%)              | 0 (0%)                   | 0.058  |
| ↓ Potassium            | 1 (5%)               | 1 (3.3%)                 | 1      |
| ↓ Potassium            | 3 (15%)              | 2 (6.7%)                 | 0.377  |
| ↓ Sodium               | 3 (15%)              | 2 (6.7%)                 | 0.377  |
| ↓ Sodium               | 0 (0%)               | 1 (3.3%)                 | 1      |
| ↓ Chloride             | 1 (5%)               | 1 (3.3%)                 | 1      |
| ↓ Chloride             | 2 (10%)              | 2 (6.7%)                 | 1      |
| **Urinalysis**         |                      |                          |        |
| ↑ UP/C ratio           | 3/12 (25%)           | 5/22 (22.7%)             | 1      |

A/G: albumins/globulins; ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transpeptidase; PCV: packed cell volume; UP/C: urine protein/creatinine.
hematological abnormalities between infected and non-infected cats, along with the results of a previous study (Paludo et al., 2008), suggests that infection by *L. infantum* is not accompanied by hematological abnormalities commonly reported in cats with leishmaniosis, such as anemia, leukocytosis, leukopenia, neutrophilia, neutropenia, lymphopenia, thrombocytopenia and pancytopenia (Pennisi et al., 2004; Marcos et al., 2009). Subsequently, in few infected cats, *L. infantum* may contribute to the pathogenesis of kidney disease. However, this finding should be interpreted with caution, since other parameters of kidney function (especially serum creatinine concentration and UP/C ratio) did not differ between the infected and non-infected cats.

5. Conclusions

In conclusion, *L. infantum* infection, not only did not lead to symptoms of the disease (Chatzis et al., 2014), but also was not associated with laboratory abnormalities other than increased serum inorganic phosphorus concentration. Further studies are needed to determine the biological significance of this finding by investigating if parasite antigen, immune complexes and mononuclear inflammatory cells are present in the kidneys of infected cats, similarly to what is observed in asymptomatic dogs infected by *L. infantum* (Esch et al., 2015).

**Declarations**

**Author contribution statement**

Manolis K. Chatzis: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.
Panagiotis G. Xenoulis, Margarita Andreoudou, John Ikonomopoulos: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Leonidas Leontides: Analyzed and interpreted the data.

Dimitrios Kasabalis: Contributed reagents, materials, analysis tools or data.

Mathios E. Mylonakis: Performed the experiments. Manolis N. Saridomichelakis: Conceived and designed the experiments; Analyzed and interpreted the data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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