PAPER

Clinical leishmaniasis in dogs living in the UK

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Objectives: To investigate the prevalence of leishmaniasis in dogs in the UK and to describe clinical presentation, clinicopathological abnormalities, therapeutic protocols and outcome in this non-endemic country.

Materials and Methods: Medical records of dogs diagnosed with leishmaniasis at seven referral centres in the UK were retrospectively reviewed.

Results: The prevalence was between 0·007 and 0·04% with a higher number of cases in southern England. All dogs had a history of travel to or from an endemic country. Lethargy, dermatological disease, decreased appetite and lameness were the most common reasons for presentation. Allopurinol was used alone for treatment in the majority of cases.

Clinical Significance: Although rare, leishmaniasis should be considered in dogs in the UK if they have compatible clinical signs and history of travel to or from endemic areas.

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INTRODUCTION

Canine leishmaniasis (CanL) is a systemic zoonotic disease caused by the protozoan *Leishmania infantum*. Infected dogs are the main reservoir of the parasite (Baneth *et al.* 2008) and play an important role in the epidemiology of human visceral (HVL) and cutaneous (HCL) leishmaniasis. Approximately 0·2 to 0·4 and 0.7 to 1.2 million HVL and HCL cases, respectively, occur each year. More than 90% of global HVL cases occur in six countries: India, Bangladesh, Sudan, South Sudan, Ethiopia and Brazil. HCL is more widely distributed with about one-third of cases occurring in each of three epidemiological regions, the Americas, the Mediterranean Basin, and western Asia from the Middle East to Central Asia (Alvar *et al.* 2012). CanL is endemic in more than 70 countries worldwide (Solano-Gallego *et al.* 2011) and especially in the Mediterranean areas of Europe (Cyprus, Greece, Albania, Croatia, Italy, Malta, France, Spain and Portugal) (Maia & Cardoso 2015), the Middle East and many tropical and subtropical areas of the world. However, the infection is spreading to non-endemic areas with an increasing number of cases reported in dogs living in North America (Gaskin *et al.* 2002, Duprey *et al.* 2006) and northern Europe (Shaw *et al.* 2009, Maia & Cardoso 2015). Recent studies have documented the disease in Germany (Geisweid *et al.* 2012), UK (Shaw *et al.* 2009) and Netherlands (Teske *et al.* 2002). This is probably due to a wider spread of the vector and, especially, to a larger numbers of dogs being imported from, or having visited, endemic countries. Since the introduction of the UK Pet Travel Scheme (PETS) in 2000, the number of dogs travelling into the UK has increased year after year with a total of 411,582 dogs recorded between 2000 and January 2008 (Mencke 2011). As a result, the disease has gained importance

in the UK, albeit largely limited to the dogs that travel. It is likely that only very little natural transmission occurs in the UK because environmental conditions prevent the viability of the vector (Shaw *et al.* 2009). However, other mechanisms of transmission are possible, including blood transfusion (de Freitas *et al.* 2006), vertical (Rosypal & Lindsay 2005, Pangrazio *et al.* 2009, Boggiatto *et al.* 2011, Naucke & Lorentz 2012, Turchetti *et al.* 2014) and venereal transmission (Diniz *et al.* 2006). Despite the increase in awareness, the prevalence of infected dogs entering the UK is unknown, because no pre-or post-travel testing is required. Furthermore, clinically apparent cases represent the minority of infected dogs in endemic areas (Solano-Gallego *et al.* 2009, Schallig *et al.* 2013), so dogs with subclinical infection that appear healthy may unknowingly be imported.

Only one study (Shaw *et al.* 2009) has previously investigated dogs with positive diagnostic tests for CanL in the UK. The objectives of this study were, firstly, to investigate the prevalence of leishmaniasis in a canine population attending referral centres in the UK and, secondly, to describe clinical presentation, most frequent clinicopathological abnormalities, diagnostic investigations, different therapeutic protocols used and outcome of dogs diagnosed with CanL in this non-endemic country.

MATERIALS AND METHODS

Patients and eligibility

Medical records of dogs diagnosed of clinical leishmaniasis in seven different referral centres in the UK (University of Liverpool, University of Bristol, University of Edinburgh, University of Cambridge, Royal Veterinary College of London, Anderson Moores Veterinary Specialists, Animal Health Trust), between January 2005 and January 2014, were retrospectively reviewed. The database of each institution was searched by use of the following terms: leishmaniasis, leishmaniosis, allopurinol, N-methylglucamine antimoniate and miltefosine. Dogs on therapy with allopurinol for diseases other than CanL were excluded. In this way only patients with a final diagnosis of clinical leishmaniasis were selected and then included. The prevalence of the disease was calculated as the ratio between the number of cases diagnosed of CanL in the study period at each referral centre and the total canine population that attended the respective centre in the same time period. The study was approved by the Veterinary Research Ethics Committee of the University of Liverpool.

Data collection

The diagnosis of CanL was made when there were compatible clinical signs and/or laboratory abnormalities together with detection of the parasites by polymerase chain reaction (PCR) or cytology (from lymph node, bone marrow, spleen or skin), and/or detection of antibodies using an immunofluorescence assay (IFAT) or an enzyme-linked immunosorbent assay (ELISA). Where available, information was reviewed regarding travel history (i.e. country to which the dog had travelled or from where it had been imported), reasons for presentation, physical examination findings, results of diagnostic investigations (e.g. haematology, biochemistry, urinalysis, cytology, serology, and PCR), therapeutic protocol used, and outcome. Dogs were tested for other vector-borne diseases (*Ehrlichia canis, Babesia canis*) if a co-infection was suspected. Survival time was defined as time (in days) from first presentation to last re-check or to time of death.

Diagnostic investigations

All routine clinicopathological analyses, serology, and real-time quantitative PCR (qPCR) assays were conducted at the respective university or by commercial laboratories. Clinicopathological abnormalities such as anaemia, azotaemia and hypoalbuminaemia were defined when results were outside the reference intervals established by each corresponding laboratory. Proteinuria was diagnosed by an elevated urine protein-to-creatinine ratio (UPCR>0·5) with inactive urinary sediment. Renal azotaemia was defined as increased creatinine with concurrent isosthenuria (1·008 to 1·012).

For serological investigations, the upper reference interval for IFAT was either 1:80 or 1:128, depending on the laboratory, whilst the positive threshold value for ELISA used by all laboratories was 35 ELISA Units (EU). Serological results were classified as low, medium or high positive if IFAT titres were less than twofold, two to fourfold, or greater than fourfold greater than the threshold positive value indicated by the reference laboratory. ELISA results were classified as mild when less than 80 EU, moderate when between 80 and 150 EU, and high when greater than 150 EU. Detection and quantification of *Leishmania* kinetoplastic DNA was performed on blood, bone marrow, and/or skin samples by qPCR as previously described (Caldin *et al.* 2004, Solano-Gallego *et al.* 2007, Maia & Campino 2008).

Classification of cases

Dogs were classified at the time of diagnosis into different clinical stages according to the Canine Leishmaniasis Working Group (CLWG) guidelines (Paltrinieri *et al.* 2010). Dogs were also classified according to the International Renal Interest Society (IRIS) guidelines, based on measurement of serum creatinine concentration at the first two appointments.

Statistical analysis

Data are reported as median or mean and range (minimum and maximum).

RESULTS

Patient population

Thirty-eight dogs were included in the study: 14 were diagnosed at the Royal Veterinary College, 7 at the University of Liverpool, 7 at the University of Edinburgh, 3 at the University of Bristol, 3 at the University of Cambridge, 3 at the Animal Health Trust and 1 at Anderson Moores Veterinary Specialists. Median age was 4·8 years (range 1·11 to 12·2 years) and median body weight was 26·3 kg (range 5·9 to 49 kg). The prevalence of the disease was 0·007 to 0·04%; the higher values (0·04% at the Royal Veterinary College of London, 0·03% at the University of Bristol and

0·02 % at the Animal Health Trust) were found in southern England.

All dogs had a history of having travelled to, or been imported from, an endemic area for *Leishmania infantum*. No clinical or clinicopathological differences were noted between dogs imported from and dogs that have travelled to an endemic area. No autochthonous cases were found in this population. Details of patient and travel history are presented in Table 1.

Detection of *Leishmania infantum* and concurrent vector-borne diseases

Leishmania infantum infection was demonstrated by serology and/or PCR and/or cytology. Details of the diagnostic tests are shown in Table 2. Only three dogs were tested for other vector-borne diseases, including two dogs tested by serology for *Ehrlichia canis* and one for *Babesia canis;* all tests were negative.

Clinical signs

All dogs had at least one clinical sign compatible with leishmaniasis. The most frequent reasons for presentation were lethargy (20/38, 53%), dermatological lesions (17/38, 45%), decreased appetite and lameness (8/38, 21%). On physical examination the most common signs observed were dermatological signs (24/38, 63%, including localised or multifocal alopecia [10], and crusting dermatitis [8]) and systemic lymphadenopathy (22/38, 58%). Twenty-four percent (9/38) of dogs were diagnosed with polyarthritis.

Table 1. Patient population and travel history

	Number of dogs (%)
Mixed breed	12 (31%)
Labrador retriever	4 (10%)
Lurcher	3 (8%)
Cocker spaniel	2 (5%)
Golden retriever	2 (5%)
Staff bull terrier	2 (5%)
Basset hound	1 (3%)
Border collie	1 (3%)
Boxer	1 (3%)
English pointer	1 (3%)
English setter	1 (3%)
Greek hare hound	1 (3%)
Greyhound	1 (3%)
Siberian husky	1 (3%)
Labradoodle	1 (3%)
Miniature poodle	1 (3%)
Miniature schnauzer	1 (3%)
Rottweiler	1 (3%)
Spanish galgo	1 (3%)
Neutered males	18 (47%)
Neutered females	15 (40%)
Entire males	3 (8%)
Entire female	2 (5%)
Imported from	32 (84%)
Spain	16 (42%)
Greece	7 (18%)
Cyprus	3 (8%)
Italy	2 (5%)
Portugal	2 (5%)
Hungary	1 (3%)
Brazil	1 (3%)
Travelled to	6 (16%)
Spain	3 (8%)
France	2 (5%)
Germany	1 (3%)

Clinicopathological investigations

Table 3 shows the main clinicopathological findings. All dogs had at least one laboratory abnormality compatible with leishmaniasis. In total, 19/32 dogs (60%) were anaemic, with the anaemia being classified as mild (haematocrit [HCT] 30 to 36%) and moderate (HCT 18 to 29%) in 11 (58%) and 8 dogs (42%), respectively, and non-regenerative (reticulocytes<60×10^9/L) in

Table 2. Diagnostic tests used to indentify *Leishmania infantum* infection

	Number of dogs (%)
Serology + PCR	10 (26%)
Serology + PCR + cytology	8 (21%)
Serology	7 (18%)
PCR	7 (18%)
Serology + cytology	3 (8%)
PCR + cytology	2 (5%)
Cytology	1 (2%)
Serology	28 (74%)
ELISA	19 (68%)
IFAT	9 (32%)
Mild	*7 (25%)*
Moderate	*11 (39%)*
High	*10 (36%)*
PCR	27 (71%)
Blood	12 (44%)
Spleen	4 (15%)
Lymph node	3 (11%)
Bone marrow	2 (7%)
Blood + bone marrow	2 (7%)
Blood + spleen	1 (4%)
Spleen + lymph node	1 (4%)
Blood + conjunctiva + skin	1 (4%)
Blood + bone marrow + joint fluid	1 (4%)
Cytology	14 (37%)
Lymph node	8 (57%)
Spleen	3 (22%)
Bone marrow	2 (14%)
Lymph node + spleen	1 (7%)

Table 3. Main clinico-pathological findings

	Number of dogs (%)
Anaemia	19/32 (60%)
Thrombocytopenia	8/23 (35%)
Leukopenia	7/22 (32%)
Pancytopenia	2/22 (9%)
Azotaemia (*increased urea and/or creatinine*)	17/30 (57%)
Renal azotaemia (*increased creatinine and isosthenuria*)	6/25 (24%)
Hyperproteinaemia	20/30 (67%)
Hypoalbuminaemia	28/30 (93%)
Hyperglobulinaemia	28/30 (93%)
Decreased A/G ratio	28/30 (93%)
Hyperphosphataemia	10/23 (43%)
Hyperkalaemia	4/23 (17%)
Isosthenuria (1.008 to 1.012)	16/26 (61%)
Proteinuria (UPC>0.5)	19/28 (68%)

USG Urine specific gravity, UPC urine protein/urine creatinine ratio

four of the six cases for which a reticulocyte count was available. Eight dogs (8/23, 35%) had thrombocytopoenia (median: 94×10^9/L, range: 30 to 150; laboratory reference interval: 155 to 400) and 2 (2/22, 9%) were pancytopoenic. Renal azotaemia was detected in six dogs (6/25, 24%), and 20 dogs (20/30, 67%) were classified in IRIS stage 1 CKD (creatinine<125 µmol/L), 4 (4/30, 13%) in IRIS stage 2 (creatinine between 125 to 180 µmol/L) and 6 (6/30, 20%) in IRIS stage 3 (creatinine between 181 to 440 µmol/L). None of the dogs were classified in IRIS stage 4 CKD (creatinine>440 µmol/L). Nineteen (19/28, 78%) of dogs were proteinuric based on increased urine protein-to-creatinine ratio (median: 5·6, range: 0·7 to 18·8; normal values<0.5). Finally, 28 dogs (28/30, 93%) were hypoalbuminaemic (median: 16 g/L, range: 11 to 20, laboratory reference interval: 23 to 31), hyperglobulinaemic (median: 58 g/L, range: 52·1 to 70; laboratory reference interval: 25 to 45) and had a low (<0·6) albumin/globulin ratio. Serum protein electrophoresis was rarely used in the diagnostic work-up and/or in the follow-up rechecks.

Treatment

Of the 38 cases, 35 (92%) were given a specific treatment for CanL. In 17 of the 35 cases (48%) allopurinol was used alone, allopurinol and miltefosine were used in 15 (43%) and allopurinol and N-methylglucamine antimoniate used in 3 (9%) dogs. A variety of other drugs were used in addition to the anti-*Leishmania* therapy, depending upon the specific case and attending clinician's judgement, including ace-inhibitors (benazepril, enalapril), anti-hypertensive drugs (amlodipine), anti-thrombotics (clopidogrel, aspirin) analgesics and anti-inflammatory drugs (tramadol, meloxicam), gastro-protectants (sucralfate, famotidine), anti-emetics (maropitant, ondansetron, metoclopramide), immune-suppressive drugs (prednisolone, azathioprine), diuretics (spironolactone) and antibiotics (doxycycline, amoxicillin-clavulanate, enrofloxacin and marbofloxacin).

Staging and survival

Based on CLWG clinical staging, 32/38 dogs (84%) were classified as stage C (sick dogs with clinically evident leishmaniasis), and 6 (16%) as stage D (severely sick dogs often unresponsive to repeated courses of anti-*Leishmania* drugs). Twenty-eight (74%) dogs were alive at the end of the study period and 10 (26%) had died or had been euthanased. Six of the 10 non-surviving dogs (60%) were classified in stage D and four (40%) in stage C. Median survival time was 400 days (range 2 to 2160 days). Reasons for death and/or euthanasia included worsening of kidney disease (3/10), lack of response to therapy (3/10), acute thromboembolism (1/10), neurological signs due to myelomalacia likely secondary to severe systemic vasculitis (1/10) and development of lymphoma (1/10) and osteosarcoma (1/10).

DISCUSSION

This is the first time that clinical CanL has been described in all its aspects in a UK dog population. The prevalence of the disease was low, although the real prevalence of the disease is likely higher because no cases from primary practices were included. Furthermore, only dogs with clinical leishmaniasis were considered, with either exposed or infected animals (those having positive results to the diagnostic tests but not showing any clinical and clinicopathological abnormalities of the disease) excluded. It is unpredictable whether those dogs will develop clinical signs in the future. Moreover, due to the low familiarity of the veterinary surgeons in the UK with this disease, it is possible that some cases could have been missed because CanL was not considered among the possible differentials or clients could have declined serology testing.

Unfortunately, the time between the travel from/to endemic areas and the development of clinical signs was not available for many cases. On the other hand, it is well known that the time between infection and development of clinical signs (incubation period) can be very variable and mainly dependent on the host immunologic response (Fisa *et al.* 1999, Cardoso *et al.* 2007).

Similar to previous reports (Shaw *et al.* 2009), most cases were found in southern England, although this was not a systematic nationwide study and not all geographical regions across the UK were included. If cases from the south are genuinely overrepresented, it might be because of the easier connections to Europe and warmer weather. The climate has recently changed enough to support the transmission and diffusion of other vector-borne diseases in the southern part of the UK (Medlock *et al.* 2007, Wilson *et al.* 2013). However, to date a vector of *Leishmania infantum* has not been found in the UK and sand flies that are introduced into the country by car or plane likely die soon after arrival because of the weather. The sand fly's range of activity is between 15 and 28°C in association with high relative humidity and absence of strong rain and winds (Killick-Kendrick 1999, Bogdan *et al.* 2001, Maroli *et al.* 2013). To date, there is little published information regarding the distribution of the competent sand fly in northern Europe and in the UK and how, or if, it is changing. Furthermore, other modes of transmission including blood transfusion (de Freitas *et al.* 2006), vertical transmission from bitches to puppies (Rosypal & Lindsay 2005, Pangrazio *et al.* 2009, Boggiatto *et al.* 2011, Naucke & Lorentz 2012, Turchetti *et al.* 2014) and venereal transmission (Diniz *et al.* 2006) are known. Dog-to-dog mechanisms have also been suggested to explain leishmaniasis outbreaks among foxhounds in the USA and Canada (Duprey *et al.* 2006).

All dogs included in this study had a history of having travelled to, or been imported from, a region endemic for *Leishmania infantum*. Thirty-two of the 38 dogs were imported and the remaining six had travelled to an endemic country, suggesting a higher risk in adopting a dog from an endemic area compared to travelling to those countries. Travelling dogs usually stay for only a short period time and the overall risk they get infected with *Leishmania infantum* is likely low (Hamel *et al.* 2011). Nevertheless, veterinarians in non-endemic regions should be aware of CanL, including its non-vectorial transmission modes, and advise dog owners on preventive measures (Shaw *et al.* 2009, Menn *et al.* 2010). The majority of dogs had been in Spain, which has a high prevalence of leishmaniasis (Mattin *et al.* 2014) and is a popular holiday destination. Imported shelter and stray

dogs have higher risk to be infected because they undergo fewer preventive measures and have greater exposure to sand flies during the period of peak of activity (evening) (Manzillo *et al.* 2006). No autochthonous cases were recognised in this study, which contrasts the findings of Shaw *et al.* (2009) who identified three positive dogs obtained from UK re-homing centres with no history of travel abroad. It remains questionable if transmission was due to vectors, transplacental or even by direct contact.

The spectrum of clinical signs and laboratory abnormalities in the study group of dogs was similar to that reported in endemic areas (Ciaramella *et al.* 1997, Koutinas *et al.* 1999, Paltrinieri *et al.* 2010, Solano-Gallego *et al.* 2011). Dermatological signs and lymphadenopathy were the most frequent clinical findings. Polyarthritis was diagnosed in nine dogs (24%), similar to previous UK reports (17%, Shaw *et al.* 2009), and should be considered a possible indication of leishmaniasis. The most frequent clinico-pathological abnormalities found in the study group included mild-to-moderate anaemia, renal azotaemia, hyperglobulinaemia, hypoalbuminaemia, decreased albumin/globulin ratio and proteinuria, which are also hallmarks of CanL in endemic areas (Ciaramella *et al.* 1997, Koutinas *et al.* 1999, Paltrinieri *et al.* 2010, Solano-Gallego *et al.* 2011).

Given the non-pathognomonic clinical signs and laboratory abnormalities and relative unfamiliarity with the disease in the UK, more than one test was used to confirm the final diagnosis in most cases. PCR on peripheral blood lacks sensitivity and lymph nodes, spleen and bone marrow harbour a higher number of *Leishmania* amastigotes (Caldin *et al.* 2004). Although serum protein electrophoresis was included in the initial diagnostic investigation only a very low number of cases were re-checked using this modality. This test can provide important information, especially during reassessment, because improvement or normalisation of the protein electrophoresis trace generally happens before a negative serology titre occurs (Torres *et al.* 2011). At time of diagnosis, we recommend haematology, biochemistry profile, urinalysis including urine protein-to-creatinine ratio, serology titre and serum protein electrophoresis. In case of peripheral lymphadenopathy and/or skin lesions, fine-needle aspiration for cytology and/or PCR can be also useful. After the first month of therapy, previous abnormal parameters can be re-checked together with serum protein electrophoresis which will be the first to show an improvement of previous hypoalbuminaemia and hyperglobulinaemia (usually polyclonal gammopathy). At this stage, a significant reduction of the serology titre is unlikely. The latter is generally re-evaluated at 3 and 6 months from diagnosis together with quantitative PCR on lymph nodes, spleen and/or bone marrow that can demonstrate a progressive reduction of the number of amastigotes.

The majority of dogs were treated with allopurinol alone, most likely because N-methylglucamine antimoniate is not available in the UK and must be imported and miltefosine requires a special treatment certificate. Where additional anti-*Leishmania* drugs were used, miltefosine was more frequently used than N-methylglucamine antimoniate, probably because it is an oral solution and easier to administer. In contrast, N-methylglucamine antimoniate must be injected subcutaneously, and can often be associated with localised pain and inflammation. Both drugs have been shown to be similarly effective (Miró *et al.* 2009). Currently, N-methylglucamine antimoniate or miltefosine in association with allopurinol are recommended as standard therapy for CanL (Oliva *et al.* 2010, Solano-Gallego *et al.* 2011, Roura *et al.* 2013, Noli & Saridomichelakis 2014).

Some dogs also received other drugs according to the attending clinician's decision. The influence of these drugs on the anti-*Leishmania* therapy and outcome is unknown.

Considering that most dogs were treated only with allopurinol, it is noteworthy that the overall outcome was good, especially since only dogs with moderate-to-severe disease (stages C and D) were included and that these animals generally have a guarded-to-poor prognosis in endemic areas (Solano-Gallego *et al.* 2011, Roura *et al.* 2013). This finding can, perhaps, be explained by the minimal chance of re-infection given the geographical location, and low risk of having another vector-borne disease (Shaw *et al.* 2009), although this cannot be completely ruled out in this study population because only three dogs were tested for these. Response to CanL is known to be influenced by both concurrent disease and immunological stimulation or suppression by shifting the balance from a protective Th1 response to a Th2 immune response that favours the development of a non-protective and possibly detrimental humoral reaction (Koutinas & Koutinas 2014).

Most non-surviving dogs showed progressive kidney disease and advanced renal failure is generally the major cause of death in CanL (Plannelas *et al.* 2009). Further studies evaluating IRIS staging in a larger population, and also in patients already on therapy, could provide more useful information regarding its possible prognostic value. Finally, all dogs in clinical stage D died or were euthanased. Currently, clinical staging at time of diagnosis and periodic re-classification in line with disease progression and regression is considered a useful way to predict outcome (Solano-Gallego *et al.* 2009, Oliva *et al.* 2010, Paltrinieri *et al.* 2010).

In conclusion, although it is a rare disease, veterinary surgeons in the UK should consider the possibility of leishmaniasis in dogs in which there are compatible clinical signs and clinicopathological abnormalities and which have a history of travel to or from endemic areas. Early diagnosis and appropriate therapy can be associated with a relatively good control of the disease (Roura *et al.* 2013; Torres *et al.* 2011). Because *Leishmania* infection has a long incubation period practitioners should instruct owners of imported dogs to retest them for *Leishmania* for at least 2 years after importation or should clinical suspicion increase (Paltrinieri *et al.* 2010). Moreover, veterinarians should be aware of non-vectorial transmission and advise clients on preventative measures before travelling to endemic countries.

Conflict of interest

None of the authors of this article has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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