Leishmaniosis causing chronic diarrhoea in a dog

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Abstract

A 10-year-old, male, neutered Podenco Canario was presented for investigation of chronic mixed intestinal diarrhoea and weight loss. The dog was imported from Spain seven years earlier and had not subsequently travelled outside of the UK. Clinical investigations also revealed a lingual mass, right hindlimb lameness, splenomegaly, a non-regenerative anaemia and hyperglobulinaemia. Histopathology of endoscopic gastrointestinal biopsies revealed neutrophilic and histiocytic enteritis and colitis with high numbers of intracytoplasmic organisms suggestive of *Leishmania*. Similar organisms were identified on cytology from the spleen, bone marrow aspirate and lingual mass. *Leishmania* PCR was positive on a bone marrow aspirate. Clinical signs resolved with miltefosine and allopurinol treatment. This case describes an atypical presentation of leishmaniosis with chronic diarrhoea which presented for veterinary investigation and treatment, seven years after moving from a *Leishmania* endemic area to the UK.

Background

Canine leishmaniosis is a zoonotic, vectorborne disease caused by the protozoan parasite *Leishmania infantum*.1 2 Transmitted by biting phlebotomine sandflies, the disease is endemic in the Mediterranean area.1–4 Environmental conditions prevent the viability of the vector in the UK and therefore the disease becoming endemic here; however, increasing
numbers of cases of canine leishmaniosis are being reported, as the number of pets travelling to and being imported from endemic countries continues to rise.\(^5\)–\(^8\)

The incubation period is variable and, in some instances, can be prolonged, with the development of clinical signs reported from one month to seven years after infection.\(^1\) \(^9\)\(^10\) Infection may be asymptomatic but can cause chronic systemic disease, associated with a wide range of clinical signs and degrees of severity, ranging from mild to severe fatal disease.\(^1\) \(^4\) \(^11\) \(^12\) The most common clinical signs include skin lesions, lymphadenomegaly and weight loss.\(^7\) \(^11\) \(^13\) Splenomegaly, \(^4\) lameness,\(^14\)–\(^16\) epistaxis\(^17\) and ocular abnormalities\(^18\) are also frequently reported. Less typical manifestations of the disease include vomiting and diarrhea,\(^19\)–\(^24\) oral mucosal lesions,\(^25\)–\(^30\) haemostatic abnormalities,\(^24\) \(^31\) \(^32\) neurological manifestations,\(^24\) \(^33\) myopathies,\(^34\) and cardiovascular\(^35\) and respiratory\(^24\) disorders.

Practitioners should be aware of the variable incubation period and the potential for disease development many years after leaving endemic areas. In addition, the disease has an insidious onset, and many cases do not present with the classic clinical signs,\(^24\) making it a challenging disease to diagnose in the UK.

**Case presentation**

An approximately 10-year-old, male, neutered Podenco Canario was referred to a referral teaching hospital, in 2016, for further investigation of chronic mixed intestinal diarrhoea and weight loss. The dog was imported into the UK seven years earlier, in 2009, from a rehoming charity in southern Spain. Following importation, the dog never travelled outside Scotland again.

In 2012, the dog developed intermittent episodes of mild, large intestinal-type diarrhoea. These occurred approximately once every two months, resolved spontaneously within a few days, without treatment, and the owner never sought veterinary advice regarding them.
Clinical signs worsened in 2016, with increasingly frequent episodes of mixed intestinal-type diarrhoea. The diarrhoea varied from small volumes of very watery stools, frequently with tenesmus and occasional haematochezia, to large volumes of watery stool. Vomiting was not reported. During this time, the dog experienced significant weight loss (more than 30 per cent of his bodyweight) and became lethargic. Investigations performed by the referring vets revealed a mild non-regenerative anaemia and mild hyperglobulinaemia. Faecal parasitology (sedimentation/flotation and *Giardia* antigen ELISA) was negative. Aerobic culture revealed a mixed faecal flora, and no specific pathogens (including *Salmonella* and *Campylobacter*) were identified. Biochemistry and urinalysis (including urine protein to creatinine ratio) were unremarkable. An adrenocorticotropic hormone stimulation test excluded hypoadrenocorticism. The dog failed to respond to treatments with probiotics, metronidazole, sulphasalazine and a novel protein diet trial.

In February 2016, the dog also developed intermittent lameness of the right stifle, which was occasionally non-weightbearing. Examinations also revealed mild left elbow discomfort. Radiographs and examination under anaesthesia failed to reveal a definitive cause. Medical management with rest and acupuncture was ineffective, and worsening of the gastrointestinal signs occurred with NSAIDs, so these were discontinued.

A *Leishmania* antibody ELISA test (NationWide Laboratories) was performed one month before referral, with a result of 540 enzyme units (values <70 *Leishmania* antibodies not detected; 70–700 low-moderate levels of *Leishmania* antibodies present; values >700 high levels of *Leishmania* antibodies present). This result signified exposure to *Leishmania* but did not confirm that *Leishmania* was the cause of the atypical clinical signs and further investigation was required. The dog was therefore referred.
On presentation the dog was in poor body condition (body condition score 3/9) and weighed 10.2 kg. A small, non-ulcerated, nodular mass was noted on the ventral aspect of the tongue. Splenomegaly was evident on abdominal palpation. Moderate weightbearing lameness of the right hind was present. Pain was detected on manipulation of the right stifle. Peripheral lymph nodes were normal in size. There was no evidence of skin lesions.

**Investigations**

A haematology profile revealed a mild, normocytic, normochromic, non-regenerative anaemia, with a haematocrit of 32.5 per cent (reference 37–55) and mild lymphopenia of 0.339×10⁹ (reference 1–4.8). A biochemistry profile revealed an increased total protein of 88 g/l (reference 55–78) due to a hyperglobulinaemia of 58 g/l (reference 28–42) and a mild increase in aspartate aminotransferase of 52 u/l (reference <40). The rest of the profile was unremarkable. Serum folate was 9.3 ng/ml (reference 3–13) and cobalamin was 489 ng/l (reference >200). Serum trypsin-like immunoreactivity was 23.7 ng/ml (reference >5), excluding exocrine pancreatic insufficiency. Urinalysis revealed a specific gravity of 1.042, pH of 6 and a urine protein to creatinine ratio of 0.66 (reference <0.5). There were no red or white blood cells on sediment examination.

Abdominal ultrasonography revealed an enlarged, diffusely hypoechoic spleen, with multiple, ill-defined, hypoechoic patches. The surrounding mesentery was reactive. Normal splenic vascularisation was evident. The right medial iliac lymph node and the mesenteric lymph nodes were enlarged (measuring 9.57 mm and 6.49 mm, respectively). There was a trace of anechoic abdominal free fluid. The mucosa of the jejunal loops was mildly speckled. Normal gastrointestinal wall layering was preserved. Other abdominal organs including the duodenum, ileum and colon were unremarkable.
The dog underwent general anaesthesia for fine needle aspirations of the tongue mass and spleen, collection of a bone marrow aspirate, gastroduodenoscopy and ileocolonoscopy. Cytology of the aspirates from the tongue mass revealed a mixed inflammatory cell population with many plasma cells and smaller numbers of neutrophils, small mature lymphocytes and occasional lymphoblasts. Several macrophages contained intracytoplasmic inclusions compatible with *Leishmania* amastigotes. Cytology samples from the spleen and bone marrow aspirate revealed similar inclusions. Real-time quantitative PCR from the bone marrow aspirate tested positive with a $C_T$ (cycle threshold) value of 17 (Langford Diagnostic Services). ELISA serology testing was greater than 1/800 (>1/700 indicates high levels of *Leishmania* antibodies; Langford Diagnostic Services), confirming infection with *Leishmania*.

On endoscopic evaluation, the gastric mucosa appeared grossly normal. The duodenal, ileal and colonic mucosa exhibited mild oedema. Endoscopic pinch biopsies were taken from the stomach, duodenum, ileum and colon.

Histopathology revealed mixed moderate neutrophilic, and histiocytic enteritis and colitis which were most severe in the ileum and colon. There was moderate stunting of villi in the ileum. Macrophages in the inflammatory infiltrate contained numerous intracytoplasmic, oval to round basophilic organisms approximately 2–4 μm in length. Although a kinetoplast could not be clearly seen histologically, the size and appearance of these organisms, which stained positive with Giemsa staining, were considered consistent with amastigotes of *Leishmania* species (figures 1–2).

**Differential diagnosis**

High levels of antibodies in the presence of compatible clinical signs and/or clinicopathological abnormalities are conclusive of the diagnosis of clinical leishmaniosis.²
Although this dog had a positive serology for *Leishmania*, its presentation with chronic diarrhoea and weight loss warranted further investigation to identify its cause and confirm or exclude clinical leishmaniosis.

The main differential diagnoses for chronic mixed intestinal diarrhoea with weight loss were diet-responsive enteropathy, idiopathic inflammatory bowel disease (IBD), neoplastic disease (eg, lymphoma) and chronic idiopathic large bowel diarrhoea. Other possible aetiologies of chronic diarrhoea, such as endoparasitic infection (eg, helminths, tapeworms, *Giardia*), bacterial infection, intestinal exocrine pancreatic insufficiency, antibiotic responsive enteropathy and systemic diseases (eg, chronic kidney disease, chronic liver disease, atypical hypoadrenocorticism), had been excluded by means of previous investigations. Other rarer causes including leishmaniosis, protothecosis and pythiosis remained possible. Endoscopic biopsies in this dog showed the presence of histiocytic inflammation and parasitic organisms in the sampled tissues. Given that the dog had never left Europe and the uniform small size of the organisms, these were most likely to be *Leishmania*. Additional investigation with cytology and PCR confirmed a diagnosis of *Leishmania*.

Differential diagnoses for diffuse splenomegaly included an infectious infiltration (eg, ehrlichiosis, haemobartonellosis, leishmaniosis), extramedullary haematopoiesis, congestion (portal hypertension, drug-induced), primary or secondary hypersplenism, or neoplasia (eg, lymphoma, leukaemia, systemic mastocytosis, multiple myeloma, malignant histiocytosis). Cytology of the spleen revealed *Leishmania* amastigotes inside macrophages.

Differential diagnoses for the tongue mass included neoplasia, eosinophilic granulomas *calcinosi circumscripita*, solar glossitis, vasculitis, amyloidosis, uraemic glossitis and autoimmune diseases (eg, systemic lupus erythematosus, pemphigus vulgaris). Cytology of the tongue mass also revealed *Leishmania* amastigotes.
Treatment

Treatment with meglumine antimoniate (Glucantime; Merial) was recommended, but this was declined by the client due to concern over the possibility of pain on injection (a reported adverse effect). The dog was instead prescribed oral treatment with miltefosine (Milteforan; Virbac) at a dosage of 2 mg/kg once a day for 28 days, alongside allopurinol 10 mg/kg twice daily. Allopurinol was then continued alone as maintenance.

Outcome and follow-up

Ongoing treatment and management were performed by the referring veterinary surgeons. The dog completed the prescribed course of miltefosine with no noted adverse effects. By this time, the gastrointestinal signs had resolved and the right hindlimb lameness had significantly improved. Six months after treatment commencement, the dog was continuing to do well and had gained weight. Repeat urinalysis, haematology and biochemistry were unremarkable. Repeat *Leishmania* antibody ELISA testing was performed with a decrease in value to 91 enzyme units (NationWide Laboratories; reference interval 70–700 indicates *Leishmania* antibodies present at a low/moderate level).

The dog remained on long-term allopurinol treatment and was monitored for adverse effects on the urinary system, a reported side effect of prolonged therapy with this drug. Xanthinuria was not identified on urinalysis. Treatment for *L. infantum* leads to temporary or permanent remission of clinical signs, but does not aim to completely eliminate the parasite. The owner chose to not to discontinue maintenance treatment because of concerns of relapse.

Discussion

The diagnosis of leishmaniosis can be easily overlooked particularly in the UK where it is uncommon.
This dog had lived in the UK for over three years before the development of mild, self-limiting, non-pathognomonic clinical signs, and seven years before the owner sought veterinary advice. Prolonged incubation periods of up to seven years are reported in dogs, and in one report the disease manifested in a person 19 years after travelling from an endemic country.

A further complication in its diagnosis is that not all dogs infected with *L. infantum* will develop clinical disease. The majority remain asymptomatic, while others develop either mild chronic infection or potentially severe and fatal organ failure. Both the outcome of infection and the variable incubation period are thought to be due to the variable immune response of the individual dog to infection. In dogs with subclinical leishmaniosis, immunosuppression, concurrent disease or coinfection with other vectorborne pathogens may trigger the development of clinical signs.

Another unusual aspect of this case was the presentation (diarrhoea, lameness and a tongue lesion). The most common presentation of *L. infantum* includes dermatological lesions, generalised lymphadenomegaly and weight loss. Gastrointestinal signs are considered uncommon with frequencies of between 3 and 30 per cent reported in the literature. Clinical signs may include small and/or large intestinal diarrhoea, haematochezia, melena and vomiting. Often, they are attributed to hepatic or renal insufficiency during the course of the disease; however, individual cases presenting with gastrointestinal signs as the primary problem are described. In dogs infected with *L. infantum*, diffuse enterocolitis has been demonstrated on histopathology. The pathogenesis by which *L. infantum* causes gastrointestinal signs in these cases is poorly understood. It is thought that the organisms activate the lamina propria macrophages and dendritic cells, leading to lymphocyte proliferation; however, the immunological response to the parasites appears to be different in different segments of the intestinal tract.
Given that *L. infantum* is not frequently encountered in the UK and that such cases do not usually present primarily with gastrointestinal signs, before endoscopy it might have been assumed that the present case had an idiopathic IBD and the case inappropriately treated with immunosuppressive drugs, which could have resulted in very serious or even fatal complications for the dog. This case highlights the importance of obtaining histopathological samples in patients with chronic enteropathy before embarking on treatment.

In *Leishmania* seropositive patients, immunohistochemistry can enable identification of amastigotes in cases when they are not seen with routine haematoxylin and eosin staining and Giemsa staining.45 49 Interestingly cases without gastrointestinal signs have been shown to have gross endoscopic gastrointestinal tract inflammation, granulomatous changes on histopathology and even *Leishmania* amastigotes present.50

Oral lesions including single32 or multiple25–27 tongue nodules, multiple papules,28 and ulcers of the lingual mucosa24 29 have been described in a few cases of leishmaniosis, but these are rare. In most of the cases with lingual lesions, patients also had more classical clinical signs of systemic disease; however, they have been reported as a sole presenting clinical sign.25–27 Mucosal lesions have been attributed to migration of infected macrophages to sites of microtrauma or by direct infection of the parasite by accidental chewing of infected sandflies.43

The chronic right hindlimb lameness and elbow discomfort reported in this patient resolved with treatment for leishmaniosis. This suggests leishmaniosis may have been the cause; however, further investigations including arthrocentesis for cytology to detect amastigotes or PCR to detect *Leishmania* DNA in the joint fluid were not performed. Lameness affecting one or more limbs, joint pain, reluctance to move, stiffness and crepitation with or without other systemic signs have been infrequently reported with leishmaniosis.14–16 24 Lameness results from the direct presence of the parasite within the joint causing a granulomatous
inflammation or a type 3 hypersensitivity reaction with deposition of immune complexes within the joint.\textsuperscript{15,24}

The detection of high serum antibody concentrations is associated with high tissue parasite loads and disease. Therefore, a high result strongly supports the diagnosis and is conclusive in dogs with a travel history, compatible clinical signs and/or clinicopathological abnormalities (eg, non-regenerative anaemia, hyperglobulinaemia, proteinuria).\textsuperscript{1,36} The presence of a low/moderate antibody level does not necessarily indicate the disease, with low titres detected in subclinical carriers. In patients with low/moderate titres and compatible clinical signs, additional investigations (eg, cytology, histopathology, PCR) are necessary to confirm or exclude clinical leishmaniosis as a cause of the clinical signs.\textsuperscript{36}

As phlebotomine sandflies cannot survive in the UK, there is no endemic transmission of \textit{L. infantum}; however, infection is increasingly identified in imported dogs.\textsuperscript{5,7} The number of dogs either returning to the UK after the holidays in endemic countries or being imported for rehoming in the UK from rescue centres in endemic countries is increasing.\textsuperscript{7} Because of the potentially prolonged incubation period and variable presentation, it is very important to obtain a full travel history in all patients undergoing investigation for chronic disease to identify these dogs even if they have been in the UK for many years.

In addition to acquiring infection from sandflies overseas, vertical and venereal transmission,\textsuperscript{51-53} transmission through infected blood transfusions\textsuperscript{54,55} and direct dog-to-dog transmission through bite wounds\textsuperscript{56} have been reported. Although these situations are rare, as the number of infected dogs in the UK increases the risk of these types of transmission will increase.

General awareness of \textit{L. infantum} is growing among practitioners; however, an awareness of the more unusual presentations of this disease is also important. The authors hope that this
non-classical presentation of *L infantum* with predominantly gastrointestinal signs and a markedly prolonged incubation period will further increase the frequency with which leishmaniosis is included on the lists of differential diagnoses.
Figure 1: Ileum. The lamina propria is expanded by granulomatous inflammation admixed with fewer neutrophils. Numerous *Leishmania* species organisms are present within macrophages (arrows). Haematoxylin and eosin-stained x 400.

Figure 2: Colon. The lamina propria is expanded by granulomatous inflammation admixed with fewer neutrophils. Numerous *Leishmania* species organisms which stain positively with Giemsa are present within macrophages (arrows). Giemsa-stained x 400.
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