Detection of *Leishmania infantum* in canine peripheral blood

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CANINE leishmaniosis is a severe systemic disease caused by *Leishmania* species parasites, which are transmitted by the bite of haematophagous phlebotomine sandflies. Dogs are a major reservoir host for human visceral leishmaniosis. During the course of the disease there is a generalised spreading of the parasite, and many organs can be colonised. *Leishmania* species amastigotes have been observed in the mononuclear phagocytic system, including the spleen, lymph nodes, bone marrow, liver, pancreas, testes, joints, bones, urinary bladder, intestinal lamina propria, lung, thyroid (Cortese and others 1999), choroid plexus (Nieto and others 1996), urine and semen (Riera and Valladares 1996), as well as in endothelial cells and fibroblasts (Hervas-Rodriguez and others 1996). The presence of *Leishmania* species amastigotes in peripheral blood has been rarely described (Schalm 1979, Ruiz de Gopegui and Espada 1998). This short communication describes the detection of numerous free and intraneutrophilic *Leishmania infantum* amastigotes in the peripheral blood of a leishmaniotic dog.

An 11-year-old male pinscher was referred during February 2004 with a one-month history of gastrointestinal disorders. The owner reported that the dog had dysorexia, weight loss, recurrent diarrhoea and abdominal distension after eating. Physical examination revealed emaciation, depression and dehydration; the mucous membranes were pale and the lymph nodes were moderately enlarged.

Haematological analysis revealed a normochromic-normocytic hyporegenerative anaemia (red blood cell count 2·56 x 10^{12}/litre, haematocrit 16·2 per cent, haemoglobin 62 g/litre, reticulocytes 3 per cent, reticulocyte production index 0·43) and leucocytosis (white blood cell count 20·9 x 10^{9}/litre) with neutrophilia (95 per cent) and lymphopenia (5 per cent). The platelet count was adequate (342 x 10^{9}/litre). Unexpectedly, a May-Grünwald-Giemsa-stained peripheral blood smear revealed numerous, 2 to 4 µm, ovoid or spherical red inclusions inside the neutrophils; these were identified as *L infantum* amastigotes (Fig 1). Many free parasites were also observed (Fig 2). Abnormal laboratory findings included an increased serum concentration of alkaline phosphatase (2152 iu/litre), gamma-glutamyl transferase (124 iu/litre) and urea (3·3 g/litre), and a polyclonal gammopathy.

Ultrasonography of the liver was performed and revealed acute hepatitis, chronic cholecystitis with small biliary calculi and cholangioectasis. A hepatic biopsy was not performed.

The immunofluorescent antibody test (IFAT) for *Leishmania*-specific antibodies yielded a positive titre of 1/320. However, serological tests also showed the presence of *Ehrlichia canis*-specific antibodies (IFAT titre 1/120). The diagnosis of leishmaniosis was confirmed by the observation of many amastigotes, within and outside macrophages, in specimens obtained from the bone marrow (Fig 3). Treatment with a combination of 50 mg/kg N-methyl-glucamine antimoniate (Glucantime; FarmitaliaCarlo Ebra) twice a day for 10 days, administered subcutaneously, and 10 mg/kg doxycycline monohydrate (Vibravet; Pfizer), administered orally, was attempted. After 10 days no clinical improvement was detected, and a complete blood count revealed a significant decrease in leucocytes (5·5 x 10^{9}/litre) and polymorphonuclear neutrophil granulocytes (PMNs) (63 per cent), and an increase in the regenerative response (reticulocytes 7 per cent, reticulocyte production index 1). Examination of a peripheral blood smear microscopically showed that the parasites had disappeared. The general condition of the dog progressively deteriorated, and it died 20 days after the treatment had begun.

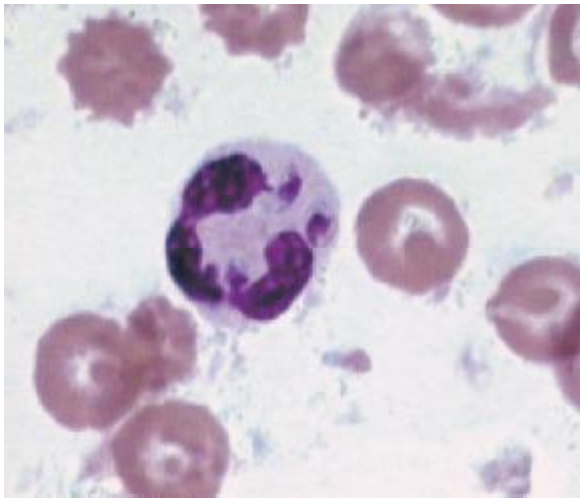

FIG 1: Intraneutrophilic *Leishmania infantum* amastigotes in a peripheral blood smear from a dog. May-Grünwald-Giemsa. x 100

The finding of *Leishmania* species amastigotes in peripheral blood is rare. In human beings, few cases have been reported (Sharma and others 2000, Fiorini and others 2002). In the peripheral blood of a leishmaniotic dog, Ruiz de Gopegui and Espada (1998) observed a single intracytoplasmic inclusion in a monocyte; previously, Schalm (1979) detected a single neutrophil with an inclusion later identified as *Leishmania donovani*. In contrast, in the present case a significant number of *Leishmania* species amastigotes were observed in peripheral blood, both within and outside neutrophils.

Macrophages are well known as the principal effector cells in both the innate and acquired immune responses to leishmaniosis. However, the role played by PMNs in controlling leishmanial infection is still unclear. In vitro studies of the interaction between human phagocytes and *Leishmania*

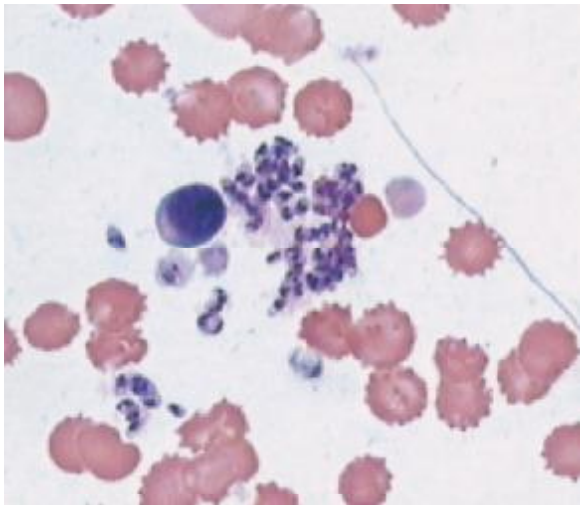

FIG 2: Numerous free *Leishmania infantum* amastigotes in a peripheral blood smear from a dog. May-Grünwald-Giemsa. x 100

Veterinary Record (2005) **156,** 151-152

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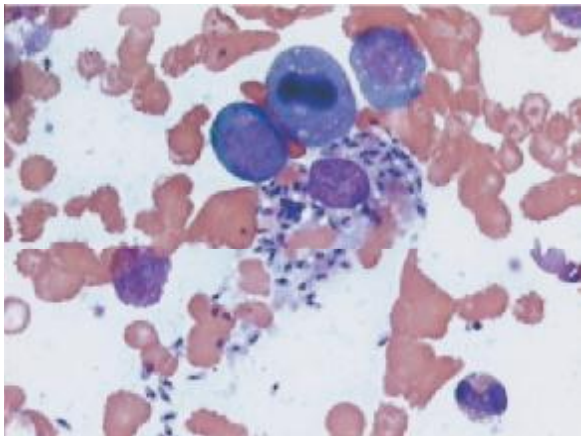

FIG 3: Numerous *Leishmania infantum* amastigotes within and outside macrophages, in a bone marrow smear from a dog. May-Grünwald-Giemsa. x 100

species (Chang 1981, Pearson and others 1987) showed that both neutrophils and eosinophils were able to ingest and kill the parasites, and that the phagocytic efficiency and the killing ability of neutrophils was higher than that of monocytes and eosinophils. In addition, by studying the development of disseminated *Leishmania major* in susceptible BALB/c mice, Hill (1986) found that both mononuclear phagocytes and neutrophils may be vehicles for the transport of the parasite in blood. Pompeu and others (1991) showed that PMNs predominated during the early phase of infection in BALB/c mice infected with *Leishmania amazonensis*, and suggested an important parasiticidal role for these cells at least in the early phase of leishmanial infection. Finally, Laufs and others (2002) suggested a dual role for PMNs in the early response to *L major* infection. On one hand, PMNs can rapidly eliminate the intracellular parasite; however, *Leishmania* species can survive intracellularly within PMNs for the first hours or days after infection. Brandonisio and others (1996) demonstrated a phagocytic and killing ability by the PMNs of dogs, suggesting a possible role for these cells in the defence against canine leishmaniosis.

In the present case, the massive detection of *Leishmania* species amastigotes in peripheral blood, during a period outside the transmission season (April to October), seems in contrast with previous data about the role played by PMNs in the early phase of *Leishmania* infection. Moreover, the authors' clinical experience in a leishmaniosis-endemic area confirms that *Leishmania* species amastigotes are only rarely detected in peripheral blood. In fact, routine microscopic examination of numerous blood smears of leishmaniotic dogs had never revealed the presence of *L infantum* amastigotes. For these reasons, the role of PMNs in canine leishmaniosis, the transmission of the disease and the current control measures need further study. The authors also recommend that a careful microscopic examination of blood smears from all dogs with leishmaniosis should be performed, especially in cases of *Leishmania* species and *E canis* co-infection, where the effects are still not well known.

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Veterinary Record 2005 156: 151-152
doi: 10.1136/vr.156.5.151

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