Summary: The present report describes the first case of equine leishmaniasis in Portugal. Leishmania infection was detected in one animal, which presented an ulcerated skin lesion. Diagnosis was based on serology by CIE, and parasite DNA detection by real-time PCR using a probe specific for L. infantum. This finding requests further leishmaniasis equine surveys in order to clarify the role of the horse as reservoir host in European endemic areas.

KEY WORDS: horse, leishmaniasis, Europe, Leishmania infantum.

MATERIAL AND RESULTS

The present study was conducted within the framework of a canine epidemiological survey in the endemic Metropolitan Region of Lisbon. A serological screening was carried out in 13 horses living in a farm from the referred endemic area, where three dogs were previously diagnosed with leishmaniasis. The horses were born in Portugal and had never travelled abroad. Serological samples were analysed by counterimmunoelectrophoresis (CIE). The CIE was carried out according to the procedure of Campino et al. (1995), using cultured promastigotes of L. infantum MON-1 as antigen. Sera samples were used undiluted and all the reactions with at least one precipitation arc were considered positive.

DNA was extracted from skin biopsy samples (PCR template preparation kit, Roche, Germany) and analysed by real-time TaqMan PCR for Leishmania DNA detection (Rolão et al., 2004). The PCR primers (forward, 5'-GGTTAGCCGATGGTGGTCTT-3', reverse, 5'-GCTATATCATATGTCCAAGCACTTACCT-3') and the TaqMan® internal probe (5'-ACCACCTAAGGTCAACCC-3') (Applied Biosystems, Foster City, CA, USA) were designed from a kDNA minicircle sequence of a Mediterranean region isolate of L. infantum (Genebank AF169140). Briefly, 2 µl of each DNA sample were added to a reaction mix consisting of TaqMan® Universal PCR Master Mix and 1 µl of unlabeled primers and TaqMan® MGB probe (FAM™ dye-labeled) mix, in
a final volume of 20 µl. Optimal conditions for PCR amplification were: 95°C for 10 min and 40 cycles consisting of 95°C for 15 sec and 60°C for 60 sec. The PCR reactions were performed in the ABI PRISM 5700 System (Perkin-Elmer, Applied Biosystems).

One of the horses presented a single irregular ulcerative skin lesion of 2.5 × 1 cm in size in the right metatarsus, which evolved from a small erosion within two months. Analysis by CIE (Fig. 1) revealed the presence of anti-Leishmania antibodies in the horse serum. This 17-year-old male mixed-breed (Anglo-lusitano) horse was born in the south of Portugal and is living in the Metropolitan Region of Lisbon for more than six years.

A physical examination did not reveal other clinical signs. Leishmania DNA was detected in that skin lesion by real-time PCR (Fig. 2), confirming the infection. The lesion healed spontaneously and relapsed after three months. The other 12 horses did not present any clinical signs and the CIE results were negative.

**DISCUSSION**

Equine leishmaniasis is quite common in South and Central America (Shaw, 2002). The parasite incriminated has been identified as *L. braziliensis* and it has been pointed out that the horse acts as reservoir of *L. braziliensis* in peri-urban areas of Brazil. In Europe, equine leishmaniasis caused by *L. infantum* has been reported in Germany (Koehler et al., 2002) and in an endemic region from Spain (Solano-Gállego et al., 2003). However, the low level or the lack of *Leishmania* antibodies found supported the hypothesis that cutaneous leishmaniasis is the only clinical form in horses (Koehler et al., 2002). In the present case the detection of anti-*Leishmania* antibodies may be indicative of a concomitant visceral involvement. In dogs, the systemic disease caused by *L. infantum* is accompanied by cutaneous lesions, which are frequently the first clinical sign and the most common manifestation.

In fact, the presence of anti-*Leishmania* antibodies is a reliable marker of viscero-cutaneous infection in dogs and of visceral leishmaniasis in immunocompetent humans, while antibodies tend to be undetectable or not significant in the cutaneous human disease (Grandoni, 1999; Dedet & Pratlong, 2003). Likewise, the...
The occurrence of human visceral leishmaniasis with concomitant cutaneous lesions is frequent in immunocompromised individuals, whereas in immunocompetents the disease presents distinct clinical forms, cutaneous or visceral. In previous studies, the specificity of the CIE test was higher than 96% in canine and human visceral leishmaniasis in immunocompetents (unpublished observation). The irregular ulcerative skin lesion described in the present report is different from the multiple papulonodular lesions described in other equine leishmaniasis cases caused by *L. infantum* (Koehler et al., 2002; Solano-Gállego et al., 2005), suggesting that the pathogen is capable of producing a variety of cutaneous lesions in horse such as in canine and human hosts. To our knowledge, this has been the first leishmaniasis equine survey performed in Portugal. The study led to the finding of the first horse infected with *Leishmania* in that country, where endemic canine leishmaniasis has a prevalence of up to 20% and an increase in incidence of human disease has been observed in the last decade. Our results strongly suggest that equine infections with *L. infantum* need to be explored further in order to clarify the clinical form of the equine infections and the role of the horse as reservoir of the parasite in endemic areas.

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**REFERENCES**


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