

EQUINE INFECTION WITH *LEISHMANIA* IN PORTUGAL

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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*.

Résumé :

INFECTION À *LEISHMANIA* CHEZ UN CHEVAL AU PORTUGAL
Les auteurs rapportent ici la première observation de leishmaniose équine au Portugal. L'infection a été diagnostiquée chez un animal présentant une lésion cutanée. Des anticorps antileishmaniens ont été mis en évidence par la technique de contre-immunoelectrophorèse, et l'ADN du parasite par PCR en temps réel. D'autres enquêtes devront être réalisées pour clarifier l'importance du cheval comme réservoir de *Leishmania* dans les foyers européens de leishmaniose.

MOTS CLÉS : cheval, leishmaniose, Europe, *Leishmania infantum*.

Leishmania infantum is the aetiological agent of leishmaniasis in Portugal, as in other southern european countries, where the dog is the domestic reservoir. However, other animals have been found parasitized in Europe, such as foxes in France (Rioux *et al.*, 1968), Italy (Gramiccia *et al.*, 1982), Portugal (Abranches *et al.*, 1984) and Spain (Fisa *et al.*, 1999), and black rats in Italy (Gradoni *et al.*, 1983) and Spain (Morillas-Márquez *et al.*, 1985), which have been considered wild reservoirs. Cats in Portugal (Durão *et al.*, 1994), Spain (Hervas *et al.*, 1999), France (Ozon *et al.*, 1998) and Italy (Poli *et al.*, 2002; Pennisi *et al.*, 2004), and a wolf in Portugal (Rebello, personal communication) have also been found parasitized and generally considered as accidental hosts.

Horses infected with *Leishmania* have been detected in South and Central America (Aguilar *et al.*, 1984; Oliveira-Neto *et al.*, 1988; Ramos-Vara *et al.* 1996), Germany (Koehler *et al.*, 2002) and Spain (Solano-Gállego *et al.*, 2003). In this report, we describe the first autochthonous case of equine infection due to *L. infantum* in Portugal.

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 A/N 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

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a final volume of 20 μ l. 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 \times 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.

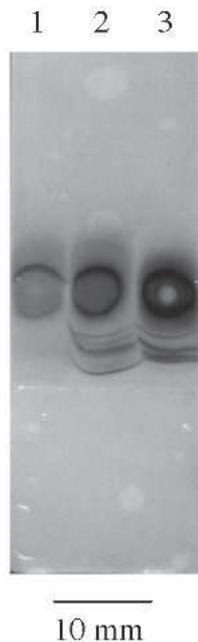


Fig. 1. – CIE result. Lane 1, negative control; lane 2, positive control; lane 3, horse sample.

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 viscerocutaneous infection in dogs and of visceral leishmaniasis in immunocompetent humans, while antibodies tend to be undetectable or not significant in the cutaneous human disease (Gradoni, 1999; Dedet & Pratlong, 2003). Likewise, the

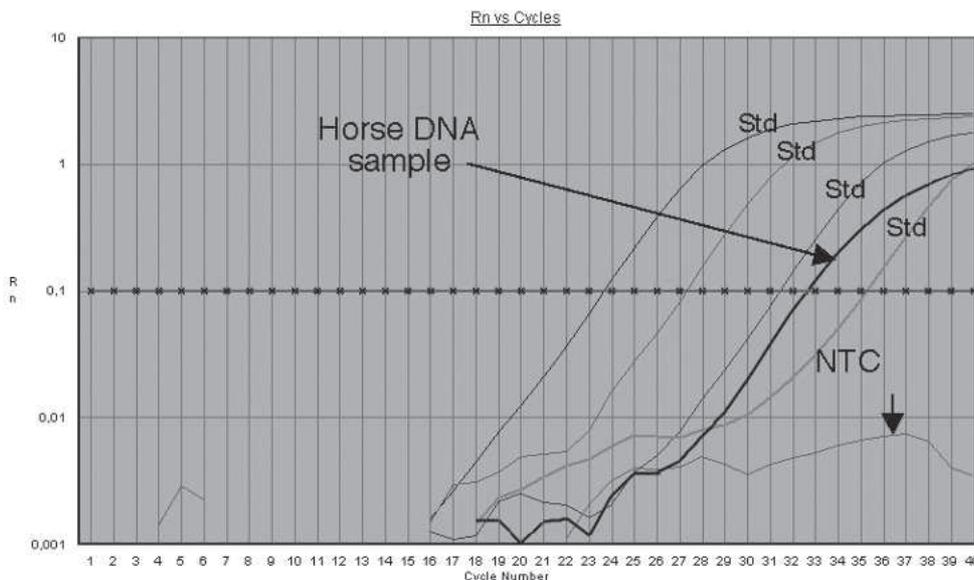


Fig. 2. – Real-time PCR result, showing the standard curves (Std), the horse sample and the negative control (NTC) curves obtained by plotting the normalised reporter (Rn) values against the cycle number.

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.*, 2003), 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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