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A single-centre, open-label, controlled, randomized clinical trial to assess the preventive efficacy of a domperidone-based treatment programme against clinical canine leishmaniasis in a high prevalence area

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ABSTRACT

The innate immune response acting immediately after initial infection with *Leishmania* parasites is known to play a relevant role in prevention against clinical progression of the disease. Domperidone is a dopamine D2 receptor antagonist that has shown to enhance the innate cell-mediated immune response.

The aim of this study was to assess the preventive efficacy of a domperidone-based treatment programme against clinical canine leishmaniasis (CanL) in a high prevalence area. The study was performed with 90 healthy, seronegative dogs of different sex, age, weight and breed from a single veterinary clinic located in Valencia (Spain). Dogs were randomly allocated into two groups. Dogs in one group (domperidone-treated group; $n = 44$) were administered an oral suspension of domperidone at 0.5 mg/kg bw/day during 30 consecutive days, every 4 months. Dogs in the other group (negative control group; $n = 46$) were left untreated. A 21-month follow-up period was implemented covering two seasonal phases of the sand fly vector. During this period all animals underwent periodic clinical examinations and blood samplings for anti-*Leishmania* serological testing. Dogs seropositive for *Leishmania* (IFAT antibody titre $\geq$ 1:80) plus at least one clinical sign consistent with CanL (indicative of active infection and incipient disease progression) were categorized as a 'prevention failure'. These dogs were withdrawn from the study after confirming the infection by direct observation of the parasite in smears of lymph nodes and/or bone marrow aspirates.

The cumulative percentage of 'prevention failure' after 12 months was significantly lower in the domperidone-treated group than in the negative control group (7% *versus* 35%, $p = 0.003$). Differences between groups persisted after 21 months (11% *versus* 48%, $p < 0.001$). The prevention rate provided by domperidone was 80% during the first 12 months and 77% throughout the complete 21-month follow-up period, with odds ratios of 7.3 ($p = 0.001$) and 7.15 ($p < 0.001$), respectively, this indicating that the risk for domperidone-treated dogs to develop the clinical disease is quite 7 times lower than for dogs left untreated.

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The results of this study demonstrate that the implementation of a strategic domperidone-based treatment programme consisting in quarterly repeated 30-day treatments with domperidone effectively reduces the risk to develop clinical CanL in areas with high prevalence of the disease.

1. Introduction

Canine leishmaniasis (CanL) is a zoonotic disease caused by a protozoan parasite (*Leishmania infantum* or its New World synonym *Leishmania chagasi*) and transmitted by the bite of a phlebotomine sand fly vector. Infected domestic dogs constitute the major reservoir of the parasite and play a key role in transmission to humans, in which the infection causes visceral leishmaniasis (Alvar et al., 2004; Gramiccia and Gradoni, 2005; Maroli et al., 2012). CanL is present in some areas of southern Europe, Africa, Asia, South and Central America (Baneth et al., 2008) and it has been recently reported in previously non-endemic areas of northern Europe, southern Canada and from northern Argentina to northern United States (Maroli et al., 2008; Otranto et al., 2009; Chamaille et al., 2010; Petersen and Barr, 2009; Mencke, 2011; Dantas-Torres et al., 2012). Expansion of human and canine leishmaniasis from endemic to non-endemic areas has been attributed to the geographical spread of sand fly vectors due to global warming, among other causes (Ferroglio et al., 2005; Shaw et al., 2009; Dantas-Torres et al., 2012). A unified medical-veterinary approach has recently been proposed to avoid the expansion of visceral leishmaniasis to new areas of the planet as a consequence of global warning. Prevention of the disease in dogs is one of the main action lines of this approach (Reis et al., 2010; Palatnik-de-Sousa and Day, 2011).

During several decades, prevention strategies against CanL have been mainly focused on the use of veterinary registered products containing synthetic pyrethroids, permethrin, or deltamethrin with a repellent effect against phlebotomine sand flies (Maroli et al., 2010; Solano-Gallego et al., 2011). A 'fructose mannose ligand' (FML)-based vaccine is commercialized in Brazil since the early 2000s and, more recently, one vaccine based on the recombinant A2 protein and another vaccine based on cultured *L. infantum* purified excreted/secreted antigens have been approved for use in dogs in Brazil and Europe, respectively (Solano-Gallego et al., 2011). A domperidone-based product has also been approved in several European countries for reduction of the risk of developing an active infection and clinical disease in case of contact with *L. infantum*, as well as for the control of clinical progression of CanL at early stages of the disease (HMA, 2013).

Domperidone is a dopamine D2 receptor antagonist developed during the 1970s as a prokinetic and antiemetic agent (Prakash and Wagstaff, 1998; Barone, 1999). In addition to these effects, domperidone has an immunomodulatory effect, mainly thorough a reversible increase of prolactin blood levels (Rovensky et al., 1995, 1996, 1999). Prolactin is a neuroendocrine hormone mainly produced in the pituitary gland that is largely known to play a stimulatory role of the immune system as a pro-inflammatory lymphocyte-derived cytokine (Reber, 1993; Hinterberger-Fischer, 2000; Vera-Lastra et al., 2002; Chavez-Rueda et al., 2005; Kelley et al., 2007). Specifically, an elevation of prolactin blood levels has been shown to induce an increase in the CD4 + Th1 subsets and the release of interleukin (IL)-2, IL-12, interferon (IFN)-γ and tumour necrosis factor (TNF)-α, leading to a natural killer (NK) cell and macrophage activation, followed by a decrease in CD4 + Th2 subsets and TNF-β (Di Carlo et al., 1993; Richards et al., 1998; Majumder et al., 2002).

In CanL, it is well known that resistance to disease progression is associated with a protective CD4 + Th1 cell-mediated immune response, while susceptibility to clinical disease is associated with a high humoral CD4 + Th2 mediated immune response and a reduced or depressed CD4 + Th1 cell-mediated immunity (Paltrinieri et al., 2010; Solano-Gallego et al., 2011). Through its stimulatory effect on the Th1 cell-mediated immune response, domperidone helps dogs with CanL to control and reduce clinical signs and anti-*Leishmania* antibody titres, particularly at early stages of the disease (Gómez-Ochoa et al., 2009).

In clinically healthy, seronegative dogs, repeated administration of domperidone has been shown to induce a significant increase in the percentage of activated phagocytic polymorphonuclear cells involved in the innate immune response such as neutrophils and macrophages (Gómez-Ochoa et al., 2012). These cells constitute the first line of defence encountered by *Leishmania* parasites when entering the susceptible host. Indeed, after initial infection, neutrophils and macrophages, among others, are recruited to the infection site and the interaction between them as well as with the parasites significantly influence the outcome of infection (Novais et al., 2009; Ribeiro-Gomes and Sacks, 2012). An appropriate activation of these cell populations is crucial for rapid elimination of the phagocytosed parasites and further antigen presentation by dendritic cells (Bonilla-Escobar, 2005; Liu and Uzonna, 2012).

According to the above mentioned, by activating the phagocytic polymorphonuclear cells involved in the innate immune response domperidone might hypothetically control the early infection by *Leishmania* parasites as well as prevent the progression of the disease. The present clinical trial was performed in order to contrast this hypothesis under real field conditions in a high prevalence area. The study was carried out under the approval of the Spanish Medicines Agency (AEMPS, *Agencia Española de Medicamentos y Productos Sanitarios*).