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Review

# Leishmune<sup>®</sup> vaccine: The newest tool for prevention and control of canine visceral leishmaniosis and its potential as a transmission-blocking vaccine

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## Abstract

Canine visceral leishmaniosis is a life-threatening disease caused by *Leishmania infantum*. For quite some time, specialists in leishmaniosis have tried to develop more affordable and effective control measures against this disease. In this search, the first vaccine against canine visceral leishmaniosis was recently licensed in Brazil. In the light of recent research, the Leishmune<sup>®</sup> vaccine might be seen as the newest tool for prevention and control of canine visceral leishmaniosis. Moreover, the potential of the Leishmune<sup>®</sup> as a transmission-blocking vaccine has recently been demonstrated, indicating its usefulness in the control of zoonotic visceral leishmaniosis.

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**Keywords:** Zoonotic visceral leishmaniosis; Dogs; Vaccination; Prevention; Control

## Contents

1. Introduction . . . . .	2
2. Prevention of canine visceral leishmaniosis. . . . .	2
3. Leishmune <sup>®</sup> : the first vaccine against canine visceral leishmaniosis. . . . .	3
4. Experimental murine models. . . . .	3
5. Phase III trials: corroborating the results of murine models. . . . .	3
6. The FML-vaccine as an immunotherapeutic agent . . . . .	4
7. Leishmune <sup>®</sup> as a transmission-blocking vaccine . . . . .	4
8. Future research needs. . . . .	5
9. Final comment . . . . .	6
Acknowledgement . . . . .	6
References . . . . .	6

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## 1. Introduction

Canine visceral leishmaniosis is a life-threatening disease caused by infection with *Leishmania infantum* (Kinetoplastida: Trypanosomatidae), now recognized as synonymous with *Leishmania chagasi* (Mauricio et al., 2000; Dantas-Torres, 2006b), though some researchers have also considered these as subspecies (Lainson and Rangel, 2005). *L. infantum* is an obligate intracellular parasite and is primarily transmitted by the bite of an infected female phlebotomine sandfly (Diptera: Psychodidae). Other ways of transmission (e.g. by arthropods, such as ticks) have recently been considered (Dantas-Torres, 2006a). Domestic dogs have historically been pointed to as the major reservoir hosts of *L. infantum* for human infection (Ashford, 1996). For this reason, canine visceral leishmaniosis is considered a disease of both veterinary and public health importance.

When feeding on a parasitized dog, an uninfected female phlebotomine sandfly might ingest amastigotes of *L. infantum* that after a cascade of events in its gut will transform into metacyclic (infective) promastigotes (Sacks and Kamhawi, 2001) and eventually be transmitted to an uninfected vertebrate host, including human. Zoonotic visceral leishmaniosis is the most severe form of leishmaniosis and is often fatal if untreated (Desjeux, 2004). Early diagnosis and treatment of human cases, concomitantly with insecticide spraying of houses, are the most commonly used control measures against this zoonosis. Although culling of seropositive dogs is still being practiced in certain countries, particularly in Brazil (Lainson and Rangel, 2005), it is not universally acceptable, due to both ethical reasons (Gramiccia and Gradoni, 2005) and its low impact in situations of permanent transmission (Moreira et al., 2004). Moreover, culling of seropositive dogs is believed to be less effective than other strategies, such as vector control and canine vaccination (Dye, 1995; Tesh, 1995).

## 2. Prevention of canine visceral leishmaniosis

One of the most effective ways to prevent canine visceral leishmaniosis is to avoid the contact between dogs and phlebotomine sandflies. This is not an easy task. In Brazil, for example, *Lutzomyia longipalpis*

(the main vector of *L. infantum*) is highly adapted to domestic and peridomestic environments and feeds readily on a wide range of wild and domestic animals, particularly dogs (Deane and Deane, 1962; Grimaldi et al., 1989). So, how to protect dogs against phlebotomine sandfly bites?

Over the past 10 years or so, it has been demonstrated that the use of insecticide-impregnated dog collars is an effective way to prevent phlebotomine sandfly bites (Killick-Kendrick et al., 1997; Halbig et al., 2000; David et al., 2001; Maroli et al., 2001; Gavvani et al., 2002; Reithinger et al., 2004). These collars act both to repel and kill phlebotomine sandflies attempting to feed on dogs. Studies on laboratory-reared phlebotomine sandflies carried out in Brazil and France have revealed that deltamethrin-impregnated collars protect collared dogs from some 96% of the bites, for up to 34 weeks (Killick-Kendrick et al., 1997; David et al., 2001). Field studies have demonstrated that the mass application of insecticide-impregnated dog collars could reduce the incidence of canine leishmaniosis in intervention areas (Halbig et al., 2000; Gavvani et al., 2002; Reithinger et al., 2004). Theoretically, phlebotomine sandflies can seek alternative sources of blood (e.g., humans) when repelled from dogs. In contrast to what could be expected from theory, the community-wide application of deltamethrin-impregnated dog collars also reduced the risk of infection in Iranian children (Gavvani et al., 2002). This suggests that insecticide-impregnated dog collars might be useful to control leishmaniosis in dogs with a positive effect for the control of disease in humans. Although the impact of the use of insecticide-impregnated dog collars is greater than the culling of seropositive dogs (Gavvani et al., 2002; Reithinger et al., 2004), the effectiveness of this preventive measure is dependent on certain factors, such as collar coverage (i.e., % of dogs using insecticide-impregnated collars, in a given community) and loss rate (Reithinger et al., 2004).

The development of an effective vaccine against leishmaniosis has been the aim of many studies in recent years. The state of the art of vaccine candidates for use in humans has been reviewed elsewhere (Handman, 2001; Coler and Reed, 2005). Candidates for a canine vaccine include from live *Leishmania* parasites (first generation vaccines) to live recombinant bacteria expressing antigens of *Leishmania* and

antigen-encoding DNA plasmids (second-generation vaccines). Despite all the efforts (Dunan et al., 1989; Genaro et al., 1996; Mayrink et al., 1996; Mohebbali et al., 1998) and advances (Ramiro et al., 2003; Lemesre et al., 2005; Saldarriaga et al., 2006), there is no vaccine against leishmaniosis for use in humans and until recently there was no vaccine for use in dogs (Gradoni, 2001; Alvar et al., 2004).

### 3. Leishmune<sup>®</sup>: the first vaccine against canine visceral leishmaniosis

After some 25 years of research, a vaccine against canine visceral leishmaniosis has been licensed by the Brazilian Ministry of Agriculture, Livestock and Food Supply. The Leishmune<sup>®</sup> vaccine is produced by the Fort Dodge Animal Health and is the first vaccine against canine visceral leishmaniosis to become available worldwide.

The Leishmune<sup>®</sup> vaccine consists of a purified fraction isolated from *Leishmania donovani* plus a saponin adjuvant. The purified fraction, named fucose mannose ligand (FML), is a glycoproteic complex that strongly inhibits the *in vitro* infection of murine macrophages by promastigotes and amastigotes of *L. donovani* (Palatnik et al., 1989; Palatnik-de-Sousa et al., 1993). Present on the surface of *L. donovani* throughout its life cycle (Palatnik-de-Sousa et al., 1993), the FML is a potent immunogen in rabbits and mice (Palatnik-de-Sousa et al., 1993; Palatnik-de-Sousa et al., 1994; Santos et al., 1999; Santos et al., 2002). Additionally, it is a sensitive, predictive and specific antigen in serodiagnosis of human (Palatnik-de-Sousa et al., 1995) and canine visceral leishmaniosis (Cabrera et al., 1999). A more detailed characterization of the compounds of the FML fraction can be found elsewhere (Santos et al., 2003).

Experimental murine models (Palatnik-de-Sousa et al., 1994; Santos et al., 1999; Paraguai-de-Souza et al., 2001) and phase III trials (Silva et al., 2000; Borja-Cabrera et al., 2002) indicated that the Leishmune<sup>®</sup> vaccine is a promising option for prevention of canine visceral leishmaniosis in Brazil. Moreover, the potential of this vaccine as a transmission-blocking vaccine was recently shown (Nogueira et al., 2005; Saraiva et al., 2006), suggesting its usefulness in the control of zoonotic visceral leishmaniosis.

### 4. Experimental murine models

Vaccination with FML plus saponin induced a considerable reduction of parasite burden in liver of inbred female BALB/c mice (Palatnik-de-Sousa et al., 1994). Another similar study using Swiss Albino mice revealed that FML plus saponin enhanced the production of antibodies, mainly IgG2a subtype, and reduced the parasitic load with no side effects (Santos et al., 1999).

The GP36, a glycoprotein that is present on the cell surface of both promastigote and amastigote forms of *L. donovani*, is the main antigen of the FML complex and is recognized by sera of rabbits (Palatnik-de-Sousa et al., 1993) and humans with visceral leishmaniosis (Palatnik-de-Sousa et al., 1996). The vaccination of Balb/c mice with purified GP36 plus saponin induced a significant protection, reducing the parasite burden in liver (Paraguai-de-Souza et al., 2001). When compared with controls, vaccinated mice showed enhanced lymphoproliferative response to GP36 and an increased level of specific IgG antibodies, particularly IgG2a. Moreover, the delayed-type hypersensitivity (DTH) reaction to promastigote lysate of *L. donovani* was greater among vaccinees than either saline or adjuvant controls. These preliminary observations in murine models suggested that the FML-vaccine could be an option to prevent canine visceral leishmaniosis.

### 5. Phase III trials: corroborating the results of murine models

Two phase III trials have been carried out in São Gonçalo do Amarante municipality, in the State of Rio Grande do Norte, where zoonotic visceral leishmaniosis is endemic. It is important to state that the first phase III trial (Silva et al., 2000) presented some methodological problems in the study design, as it was neither randomized nor blind. The second phase III trial (Borja-Cabrera et al., 2002), however, was a well-designed, randomized, blinded, controlled trial, from which a number of conclusions can be drawn. In both trials, dogs from the intervention group received three subcutaneous doses of the vaccine, at 21-day intervals, while those from the control group were treated with a single injection of sterile saline. In the first phase III

trial, after 2 years of experiment, 8% of the vaccinated dogs showed mild clinical signs of visceral leishmaniasis and no deaths occurred. In contrast, 33% of the controls developed clinical or fatal disease (Silva et al., 2000). In the second trial, after 3.5 years of study, 5% of the vaccinated dogs and 25% of the controls developed clinical and fatal diseases (Borja-Cabrera et al., 2002).

In both trials, all vaccinated dogs showed positive results in the DTH reaction to leishmanin (*L. donovani* lysate), within a period of 2 months (Silva et al., 2000) or 7 months (Borja-Cabrera et al., 2002) after vaccination. The DTH, also known as the Montenegro or leishmanin skin test (LST), is a useful tool for the evaluation of cell-mediated immunity in *Leishmania* infection in humans (Pearson and Sousa, 1996) as well as in dogs (Pinelli et al., 1994; Cardoso et al., 1998; Solano-Gallego et al., 2001). Since natural resistance to *Leishmania* infection is principally T-cell-mediated (Pinelli et al., 1995), the LST has been used as a marker of protective immunity. In contrast to what occurs in dogs with active leishmaniasis, most of the asymptomatic naturally infected dogs are LST positive (Cardoso et al., 1998; Solano-Gallego et al., 2000). Occasionally, one might ask if the protective immune response documented in the phase III trials could be in part induced by the LST rather than by the FML-vaccine. Although a single application of leishmanin can induce a *Leishmania*-specific cell-mediated immune response in humans (De Luca et al., 2003), it is unlikely to protect dogs from *Leishmania* infection (Cardoso et al., 1998).

As expected for a saponin vaccine (Santos et al., 2002), anti-FML antibodies were detected in 97% and 100% of the vaccinated dogs in the first (Silva et al., 2000) and second (Borja-Cabrera et al., 2002) trials, respectively. It is important to state, however, that anti-*Leishmania* antibodies do not reflect protection (Pinelli et al., 1994) and may even indicate susceptibility in the absence of positive T-cell proliferative response to *Leishmania* antigens (Gradoni, 2001).

The phase III trials indicated that the FML-vaccine induced a significant protective effect in vaccinated dogs, with a vaccine efficacy as high as 80% (Borja-Cabrera et al., 2002). These results corroborate that obtained from murine models (Palatnik-de-Sousa et al., 1994; Santos et al., 1999; Paraguai-de-Souza et al., 2001).

## 6. The FML-vaccine as an immunotherapeutic agent

A murine model of the immunotherapeutic treatment with FML-saponin vaccine against visceral leishmaniasis has generated promising results. Specific increases in IgG1, IgG2a and IgG2b antibodies, a DTH response to *L. donovani* lysate and *in vitro* ganglion cell proliferative response against FML-antigen, and decreased IL-10 levels were noted in vaccinated Balb/c mice (Santos et al., 2003). Furthermore, a notable decrease of parasitic burden in the liver was detected only in vaccinees.

The results from the murine model and other factors, such as the relative failure of chemotherapy against canine visceral leishmaniasis, have encouraged an investigation on the efficacy of the FML vaccine in immunotherapy of canine visceral leishmaniasis (Borja-Cabrera et al., 2004). Five mongrel dogs were experimentally infected, by the iv route, with  $10^8$  amastigotes of *L. donovani* and vaccinated after infection at days 127, 164 and 187 with FML plus QuilA saponin. Also, 21 FML-seropositive asymptomatic dogs from an area of canine leishmaniasis endemicity received three vaccine doses of FML plus saponin R. After 22 months of observation, no obits due to visceral leishmaniasis were recorded among the vaccinated dogs and 90% of these were still asymptomatic, healthy and parasite free. In contrast, 17 of 46 dogs from the control group died, within the same period (Borja-Cabrera et al., 2004).

Based on these results, it is possible to conclude that the FML-vaccine is able to prevent the development of canine visceral leishmaniasis, even in already infected dogs. Although the Leishmune<sup>®</sup> vaccine should be an option to the immunotherapy of canine visceral leishmaniasis, currently, there is no indication as to its use as an immunotherapeutic agent. According to the manufacturer, this vaccine is solely recommended for asymptomatic and seronegative dogs.

## 7. Leishmune<sup>®</sup> as a transmission-blocking vaccine

The first phase III trial of the FML-vaccine started in December 1996 and the dogs were followed-up for 24 months. Coincidentally or not, the incidence of

human cases of zoonotic visceral leishmaniasis in São Gonçalo do Amarante decreased from 15 cases in 1996 to 0 up to May 1998 (Silva et al., 2000). Thus, a key question appears: could the FML-vaccine have some impact on the incidence of human cases of zoonotic visceral leishmaniasis? Was it by reducing the infectivity of the canine population, blocking the transmission in the female phlebotomine sandfly, or both? Trying to answer these questions, the potential effect of Leishmune<sup>®</sup> vaccine to block the transmission of *Leishmania* parasites was investigated (Nogueira et al., 2005). Eleven months after vaccination, all vaccinated dogs were positive for anti-FML antibodies. Furthermore, vaccinated dogs showed PCR negative results and no clinical signs of leishmaniasis. In contrast, 25% of the controls presented clinical signs, 56.7% were PCR positive in lymph node, 15.7% were PCR positive in blood and 25% presented immunohistochemical positive reactions. The results from the aforementioned study, particularly the absence of parasites in the skin of vaccinated dogs, indicate that the Leishmune<sup>®</sup> vaccine might block the transmission of *L. infantum* among vaccinated dogs and therefore contribute for the control of canine visceral leishmaniasis.

Transmission-blocking vaccines are the ultimate goal for the control of certain digenetic parasites. For instance, malaria transmission-blocking vaccines aim to induce antibodies against the sexual stage antigens in order to block the development of the *Plasmodium* infective stage (sporozoites) in the female mosquito (Kaslow, 1997). The ability of the Leishmune<sup>®</sup> vaccine to inhibit *L. donovani* and '*L. chagasi*' (= *L. infantum*) procyclic promastigote-binding to dissected midguts of *L. longipalpis* was recently assessed (Saraiva et al., 2006). Both rate and intensity of infection were significantly higher among phlebotomine sandflies fed with  $1.5 \times 10^7$  amastigotes and either human blood or unvaccinated dog sera. The findings of this very interesting study clearly demonstrate that the Leishmune<sup>®</sup> vaccine is a potential transmission-blocking vaccine and therefore open a new perspective for the control of canine visceral leishmaniasis. Moreover, if antibodies of Leishmune<sup>®</sup> vaccinated dogs really prevent the development of metacyclic promastigotes in the female phlebotomine sandfly, this vaccine will certainly have some impact on the transmission of zoonotic visceral leishmaniasis.

## 8. Future research needs

The Leishmune<sup>®</sup> vaccine has offered the prospect of a new era for the prevention and control of canine visceral leishmaniasis and thereby of zoonotic visceral leishmaniasis. However, many questions remain to be addressed in future studies. Further field investigations with the Leishmune<sup>®</sup> vaccine are needed. These studies should be carried out in municipalities other than São Gonçalo do Amarante. Case-control studies might be useful to answer certain questions and estimate the effectiveness of this vaccine. For instance, it is important to know the possible risk factors for visceral leishmaniasis in vaccinated dogs.

A more detailed profile of the immune response developed after Leishmune<sup>®</sup> vaccination is also required. What are the consequences of the effects of this vaccine on Th1/Th2 balance? Is the amount of interferon-gamma secreted by peripheral blood mononuclear cells of vaccinated dogs greater than unvaccinated ones? This will improve the current understanding on the way this vaccine induces a protective immune response in certain dogs (and not in others) and also provide useful insights into the field of leishmaniasis vaccines.

The initial cost of the vaccination program (i.e., three vaccine doses, with 21-day intervals) with the Leishmune<sup>®</sup> vaccine is about \$100 per animal. Unfortunately, most of the dog owners living in poor rural and suburban areas of Brazil cannot afford it. Thus, it is important to evaluate the cost-effectiveness of a canine vaccination program not only to prevent individual cases, but also to control canine leishmaniasis in a community-based perspective. Certainly, this strategy should replace the controversial culling of seropositive dogs and it would contribute effectively for the control of zoonotic visceral leishmaniasis by reducing the proportion of infectious dogs, blocking the vectorial transmission, or both. To reduce the costs, the vaccination of dogs against visceral leishmaniasis could coincide with anti-rabies vaccination campaigns (Lainson and Rangel, 2005).

To date, there is no serological test to distinguish antibodies induced by the Leishmune<sup>®</sup> vaccine from those produced due to natural infection with *L. infantum*. The IgG1 subset appears to be associated to natural infection and IgG2 to a humoral response induced by the FML-vaccine (Oliveira-Mendes et al.,

2003). Undoubtedly, this subject must be addressed in future studies. A test capable of differentiating vaccinated dogs from naturally infected ones could help public health workers decide if a given seropositive dog should be culled or not.

The non-infectious condition of dogs vaccinated with the FML-vaccine has been suggested by the normal proportions of CD4 and CD21 lymphocytes detected in peripheral blood mononuclear cells of these animals (Borja-Cabrera et al., 2004). However, the non-infectious condition of the vaccinated dogs must be confirmed by xenodiagnosis (Gradoni, 2001) in order to reinforce the usefulness of this vaccine as a control measure for zoonotic visceral leishmaniasis.

## 9. Final comment

Unfortunately, it is not known whether the Leishmune<sup>®</sup> vaccine will present any positive impact on public health. As the vaccine was designed for dogs, it can be seen as an important solution to the animal problem. Obviously, it would be better if the Leishmune<sup>®</sup> vaccine also had an impact on the incidence of zoonotic visceral leishmaniasis in humans. If so, the control of this disease will turn into a reality and be more possible than ever. But this only time (and research) will tell for sure.

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## References

- Alvar, J., Canavate, C., Molina, R., Moreno, J., Nieto, J., 2004. Canine leishmaniasis. *Adv. Parasitol.* 57, 1–88.
- Ashford, R.W., 1996. Leishmaniasis reservoirs and their significance in control. *Clin. Dermatol.* 14, 523–532.
- Borja-Cabrera, G.P., Correia-Pontes, N.N., da Silva, V.O., Paraguai-de-Souza, E., Santos, W.R., Gomes, E.M., Luz, K.G., Palatnik, M., Palatnik-de-Sousa, C.B., 2002. Long lasting protection against canine kala-azar using the FML-QuilA saponin vaccine in an endemic area of Brazil (Sao Goncalo do Amarante, RN). *Vaccine* 20, 3277–3284.
- Borja-Cabrera, G.P., Cruz-Mendes, A., Paraguai-de-Souza, E., Hashimoto-Okada, L.Y., Trivellato, F.A.A., Kawasaki, J.K., Costa, A.C., Reis, A.B., Genaro, O., Batista, L.M., Palatnik, M., Palatnik-de-Sousa, C.B., 2004. Effective immunotherapy against canine visceral leishmaniasis with the FML-vaccine. *Vaccine* 22, 2234–2243.
- Cabrera, G.P., Da Silva, V.O., Da Costa, R.T., Reis, A.B., Mayrink, W., Genaro, O., Palatnik-de-Sousa, C.B., 1999. The FML-ELISA assay in diagnosis and prognosis of canine visceral leishmaniasis. *Am. J. Trop. Med. Hyg.* 61, 296–301.
- Cardoso, L., Neto, F., Sousa, J.C., Rodrigues, M., Cabral, M., 1998. Use of a leishmanin skin test in the detection of canine *Leishmania*-specific cellular immunity. *Vet. Parasitol.* 79, 213–220.
- Coler, R.N., Reed, S.G., 2005. Second-generation vaccines against leishmaniasis. *Trends Parasitol.* 21, 244–249.
- Dantas-Torres, F., 2006a. Do any insects other than phlebotomine sandflies (Diptera: Psychodidae) transmit *Leishmania infantum* (Kinetoplastida: Trypanosomatidae) from dog to dog? *Vet. Parasitol.* 136, 379–380.
- Dantas-Torres, F., 2006b. *Leishmania infantum* versus *Leishmania chagasi*: do not forget the law of priority. *Mem. Inst. Oswaldo Cruz* 101, 117–118.
- David, J.R., Stamm, L.M., Bezerra, H.S., Souza, R.N., Killick-Kendrick, R., Lima, J.W., 2001. Deltamethrin-impregnated dog collars have a potent anti-feeding and insecticidal effect on *Lutzomyia longipalpis* and *Lutzomyia migonei*. *Mem. Inst. Oswaldo Cruz* 96, 839–847.
- De Luca, P.M., Mayrink, W., Santiago, M.A., Nogueira, R., Conceicao-Silva, F., Melo, G., Mendonça, S.C., 2003. Randomized, double-blind, placebo-controlled study on the immunogenicity of the leishmanin skin test. *Trans. R. Soc. Trop. Med. Hyg.* 97, 709–712.
- Deane, L.M., Deane, M.P., 1962. Visceral leishmaniasis in Brazil: geographical distribution and transmission. *Rev. Inst. Med. Trop. Sao Paulo* 4, 198–212.
- Desjeux, P., 2004. Leishmaniasis. *Nat. Rev. Microbiol.* 2, 692–693.
- Dunan, S., Frommel, D., Monjour, L., Ogunkolade, B.W., Cruz, A., Quilici, M., 1989. Vaccination trial against canine visceral leishmaniasis. *Parasite Immunol.* 11, 397–402.
- Dye, C., 1995. The logic of visceral leishmaniasis control. *Am. J. Trop. Med. Hyg.* 55, 125–130.
- Gavagni, A.S., Hodjati, M.H., Mohite, H., Davies, C.R., 2002. Effect of insecticide-impregnated dog collars on incidence of zoonotic visceral leishmaniasis in Iranian children: a matched-cluster randomised trial. *Lancet* 360, 374–379.
- Genaro, O., Pinto, J.A., Costa, C.A., França-Silva, J.C., Costa, R.T., Silva, J.C., Sanguinetti, L.S.R., Vieira, E.P., Toledo, V.P.C.P., Mayrink, W., 1996. Phase III randomized double blind clinical trial on the efficacy of a vaccine against canine visceral leishmaniasis in urban area of Montes Claros, MG, Brazil. *Mem. Inst. Oswaldo Cruz* 91 (Suppl.) 166.

- Gradoni, L., 2001. An update on antileishmanial vaccine candidates and prospects for a canine *Leishmania* vaccine. *Vet. Parasitol.* 100, 87–103.
- Gramiccia, M., Gradoni, L., 2005. The current status of zoonotic leishmaniasis and approaches to disease control. *Int. J. Parasitol.* 35, 1169–1180.
- Grimaldi Jr., G., Tesh, R.B., McMahon-Pratt, D., 1989. A review of the geographic distribution and epidemiology of leishmaniasis in the New World. *Am. J. Trop. Med. Hyg.* 41, 687–725.
- Halbig, P., Hodjati, M.H., Mazloumi-Gavagni, A.S., Mohite, H., Davies, C.R., 2000. Further evidence that deltamethrin-impregnated collars protect domestic dogs from sandfly bites. *Med. Vet. Entomol.* 14, 223–226.
- Handman, E., 2001. Leishmaniasis: current status of vaccine development. *Clin. Microbiol. Rev.* 14, 229–243.
- Kaslow, D.C., 1997. Transmission-blocking vaccines: uses and current status of development. *Int. J. Parasitol.* 27, 183–189.
- Killick-Kendrick, R., Killick-Kendrick, M., Focheux, M.C., Dereure, J., Puech, M.P., Cadiergues, M.C., 1997. Protection of dogs from bites of phlebotomine sandflies by deltamethrin collars for control of canine leishmaniasis. *Med. Vet. Entomol.* 11, 105–111.
- Lainson, R., Rangel, E.F., 2005. *Lutzomyia longipalpis* and the eco-epidemiology of American visceral leishmaniasis, with particular reference to Brazil: a review. *Mem. Inst. Oswaldo Cruz.* 100, 811–827.
- Lemesre, J.L., Holzmüller, P., Cavaleyra, M., Goncalves, R.B., Hottin, G., Papierok, G., 2005. Protection against experimental visceral leishmaniasis infection in dogs immunized with purified excreted secreted antigens of *Leishmania infantum* promastigotes. *Vaccine* 23, 2825–2840.
- Maroli, M., Mizzoni, V., Siragusa, C., D'Orazi, A., Gradoni, L., 2001. Evidence for an impact on the incidence of canine leishmaniasis by the mass use of deltamethrin-impregnated dog collars in southern Italy. *Med. Vet. Entomol.* 15, 358–363.
- Mauricio, I.L., Stothard, J.R., Miles, M.A., 2000. The strange case of *Leishmania chagasi*. *Parasitol. Today* 16, 188–189.
- Mayrink, W., Genaro, O., Silva, J.C.F., da Costa, R.T., Tafuri, W.L., Peixoto Toledo, V.P.C., da Silva, A.R., Barbosa Reis, A., Williams, P., da Costa, C.A., 1996. Phases I and II open clinical trials of a vaccine against *Leishmania chagasi* infections in dogs. *Mem. Inst. Oswaldo Cruz* 91, 695–697.
- Mohebbi, M., Fallah, E., Jamshidi, S., Hajjaran, H., 1998. Vaccine trial against canine visceral leishmaniasis in the Islamic Republic of Iran. *Rev. Santé Méditerranée Orientale* 4, 234–238.
- Moreira Jr., E.D., Mendes-de-Souza, V.M., Sreenivasan, M., Nascimento, E.G., Pontes-de-Carvalho, L., 2004. Assessment of an optimized dog-culling program in the dynamics of canine *Leishmania* transmission. *Vet. Parasitol.* 122, 245–252.
- Nogueira, F.S., Moreira, M.A., Borja-Cabrera, G.P., Santos, F.N., Menz, I., Parra, L.E., Xu, Z., Chu, H.J., Palatnik-de-Souza, C.B., Luvizotto, M.C., 2005. Leishmune vaccine blocks the transmission of canine visceral leishmaniasis: absence of *Leishmania* parasites in blood, skin and lymph nodes of vaccinated exposed dogs. *Vaccine* 23, 4805–4810.
- Oliveira-Mendes, C., Paraguai-de-Souza, E., Borja-Cabrera, G.P., Batista, L.M.M., dos Santos, M.A., Parra, L.E., Menz, I., Palatnik, M., Palatnik-de-Souza, C.B., 2003. IgG1/IgG2 antibody dichotomy in sera of vaccinated or naturally infected dogs with visceral leishmaniasis. *Vaccine* 21, 2589–2597.
- Palatnik, C.B., Borojevic, R., Previato, J.O., Mendonça-Previato, L., 1989. Inhibition of *Leishmania donovani* promastigote internalization into murine macrophages by chemically defined parasite glycoconjugate. *Infect. Immun.* 57, 754–763.
- Palatnik-de-Souza, C.B., Dutra, H.S., Borojevic, R., 1993. *Leishmania donovani* surface glycoconjugate GP36 is the major immunogen component of the fucose-mannose ligand (FML). *Acta Trop.* 53, 59–72.
- Palatnik-de-Souza, C.B., Paraguai-de-Souza, E., Gomes, E.M., Borojevic, R., 1994. Experimental murine *Leishmania donovani* infection: immunoprotection by the fucose-mannose ligand (FML). *Braz. J. Med. Biol. Res.* 27, 547–551.
- Palatnik-de-Souza, C.B., Gomes, E.M., Paraguai-de-Souza, E., Palatnik, M., Luz, K., Borojevic, R., 1995. *Leishmania donovani*: titration of antibodies to the fucose-mannose ligand as an aid in diagnosis and prognosis of visceral leishmaniasis. *Trans. R. Soc. Trop. Med. Hyg.* 89, 390–393.
- Palatnik-de-Souza, C.B., Gomes, E.M., de Souza, E.P., dos Santos, W.R., de Macedo, S.R., de Medeiros, L.V., Luz, K., 1996. The FML (fucose mannose ligand) of *Leishmania donovani*: a new tool in diagnosis, prognosis, transfusional control and vaccination against human kala-azar. *Rev. Soc. Bras. Med. Trop.* 29, 153–163.
- Paraguai-de-Souza, E., Bernardo, R.R., Palatnik, M., Palatnik-de-Souza, C.B., 2001. Vaccination of Balb/c mice against experimental visceral leishmaniasis with the GP36 glycoprotein antigen of *Leishmania donovani*. *Vaccine* 19, 3104–3115.
- Pearson, R.D., Sousa, A.Q., 1996. Clinical spectrum of leishmaniasis. *Clin. Infect. Dis.* 22, 1–13.
- Pinelli, E., Killick-Kendrick, R., Wagenaar, J., Bernadina, W., del Real, G., Ruitenber, J., 1994. Cellular and humoral immune responses in dogs experimentally and naturally infected with *Leishmania infantum*. *Infect. Immun.* 62, 229–235.
- Pinelli, E., Gonzalo, R.M., Boog, C.J., Rutten, V.P., Gebhard, D., del Real, G., Ruitenber, E.J., 1995. *Leishmania infantum*-specific T cell lines derived from asymptomatic dogs that lyse infected macrophages in a major histocompatibility complex-restricted manner. *Eur. J. Immunol.* 25, 1594–1600.
- Ramiro, M.J., Zarate, J.J., Hanke, T., Rodríguez, D., Rodríguez, J.R., Esteban, M., Lucientes, J., Castillo, J.A., Larraga, V., 2003. Protection in dogs against visceral leishmaniasis caused by *Leishmania infantum* is achieved by immunization with a heterologous prime-boost regime using DNA and vaccinia recombinant vectors expressing LACK. *Vaccine* 21, 2474–2484.
- Reithinger, R., Coleman, P.G., Alexander, B., Vieira, E.P., Assis, G., Davies, C.R., 2004. Are insecticide-impregnated dog collars a feasible alternative to dog culling as a strategy for controlling canine visceral leishmaniasis in Brazil? *Int. J. Parasitol.* 34, 55–62.
- Sacks, D., Kamhawi, S., 2001. Molecular aspects of parasite-vector and vector-host interactions in leishmaniasis. *Annu. Rev. Microbiol.* 55, 453–483.

- Saldarriaga, O.A., Travi, B.L., Park, W., Perez, L.E., Melby, P.C., 2006. Immunogenicity of a multicomponent DNA vaccine against visceral leishmaniasis in dogs. *Vaccine* 24, 1928–1940.
- Santos, W.R., Paraguai-de-Souza, E., Palatnik, M., Palatnik-de-Sousa, C.B., 1999. Vaccination of Swiss Albino mice against experimental visceral leishmaniasis with the FML antigen of *Leishmania donovani*. *Vaccine* 17, 2554–2561.
- Santos, W.R., de Lima, V.M.F., Paraguai de Souza, E., Bernardo, R.R., Palatnik, M., Palatnik de Sousa, C.B., 2002. Saponins IL12 and BCG adjuvant in the FML-vaccine formulation against murine visceral leishmaniasis. *Vaccine* 21, 30–43.
- Santos, W.R., Aguiar, I.A., Paraguai-de-Souza, E., de Lima, V.M., Palatnik, M., Palatnik-de-Sousa, C.B., 2003. Immunotherapy against murine experimental visceral leishmaniasis with the FML-vaccine. *Vaccine* 21, 4668–4676.
- Saraiva, E.M., Barbosa, A.D., Santos, F.N., Borja-Cabrera, G.P., Nico, D., Souza, L.O., Mendes-Aguiar, C.D., de Souza, E.P., Fampa, P., Parra, L.E., Menz Jr., I.J.G., de Oliveira, S.M., Palatnik-de-Sousa, C.B., 2006. The FML-vaccine (Leishmune<sup>®</sup>) against canine visceral leishmaniasis: a transmission blocking vaccine. *Vaccine* 24, 2423–2431.
- Silva, V.O., Borja-Cabrera, G.P., Correia-Pontes, N.N., de Souza, E.P., Luz, K.G., Palatnik, M., Palatnik-de-Sousa, C.B., 2000. A phase III trial of efficacy of the FML-vaccine against canine kala-azar in an endemic area of Brazil (Sao Goncalo do Amaranto, RN). *Vaccine* 19, 1082–1092.
- Solano-Gallego, L., Llull, J., Ramos, G., Riera, C., Arboix, M., Alberola, J., Ferrer, L., 2000. The Ibizaian hound presents a predominantly cellular immune response against natural *Leishmania* infection. *Vet. Parasitol.* 90, 37–45.
- Solano-Gallego, L., Llull, J., Arboix, M., Ferrer, L., Alberola, J., 2001. Evaluation of the efficacy of two leishmanins in asymptomatic dogs. *Vet. Parasitol.* 102, 163–166.
- Tesh, R.B., 1995. Control of zoonotic visceral leishmaniasis: is it time to change strategies? *Am. J. Trop. Med. Hyg.* 52, 287–292.