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Product Monography

I. INTRODUCTION

Canine leishmaniasis is probably the most important dog disease in the Mediterranean countries. It is a parasitic disease with a high prevalence and potentially deadly, difficult to diagnose and not always with an effective treatment.

Over the years, the increasing use of immunological techniques, genetics and molecular, has greatly expanded the knowledge of canine leishmaniasis, which has led to a conceptual paradigm shift. Thus, it is now increasingly regarded more as an "immune system disease caused by a parasite" rather than as a simple "parasitic disease".

Advances in research on canine leishmaniasis over the past decades have shown that control of infection or disease progression is not in the pathogenicity of the parasite, but the characteristics of the immune response that is established in the dog after infection. However, this knowledge had not been used so far for the development of new therapies against this disease.

Leisguard® represents a new approach to the treatment of canine leishmaniasis, the first medicinal product intended to modulate the immune response of infected dog learned orienting towards an effective response rate in the control of the disease. Through its stimulating effect on the natural or innate immune response, **Leisguard®** also offers an alternative veterinary clinical therapeutic for the effective prevention of the disease.

II. Canine leishmaniasis and immune response.

II.1. Canine leishmaniasis.

Canine leishmaniasis is caused by different species of Leishmania protozoa, Leishmania infantum being the most representative.

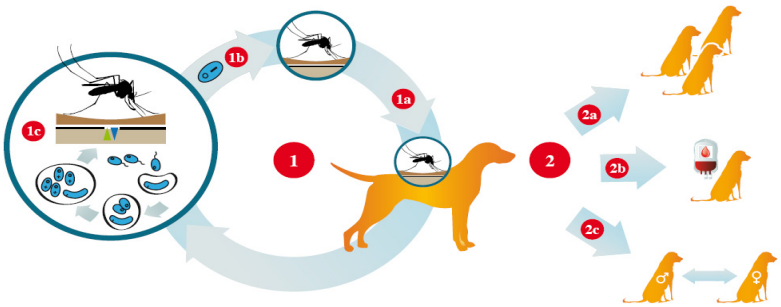
Leishmania is transmitted by the bite / biting flies of the genus Phlebotomus, Phlebotomus perniciosus primarily, although other routes have been described as transplacental, venereal or blood transfusions *(Figure 1).*

In most Mediterranean countries endemic for canine leishmaniasis, this vector usually has its period of activity between May and November. In Mediterranean countries the presence of vector is very high, both in peri-urban and rural areas, which has increased the prevalence of the disease to the high current levels.

Although prevalence data vary greatly from one area to another, different groups of canine leishmaniasis experts agree that in recent years the disease is spreading geographically and their prevalence in endemic areas is increasing gradually (Bourdeau et al., 2011; Paltrinieri et al., 2010, Solano-Gallego et al., 2011).

A key aspect in understanding the disease is the difference between infection and disease. Epidemiological studies carried out over recent years show that the percentage of infected dogs in areas where the disease is endemic is very high, but only part of them are seropositive and an even smaller part develop the disease (Baneth et al., 2008).

Figure 1.
Life cycle of Leishmania and the sandfly indicating also the routes of transmission alternatives proposed (Solano-Gallego et al., 2011).



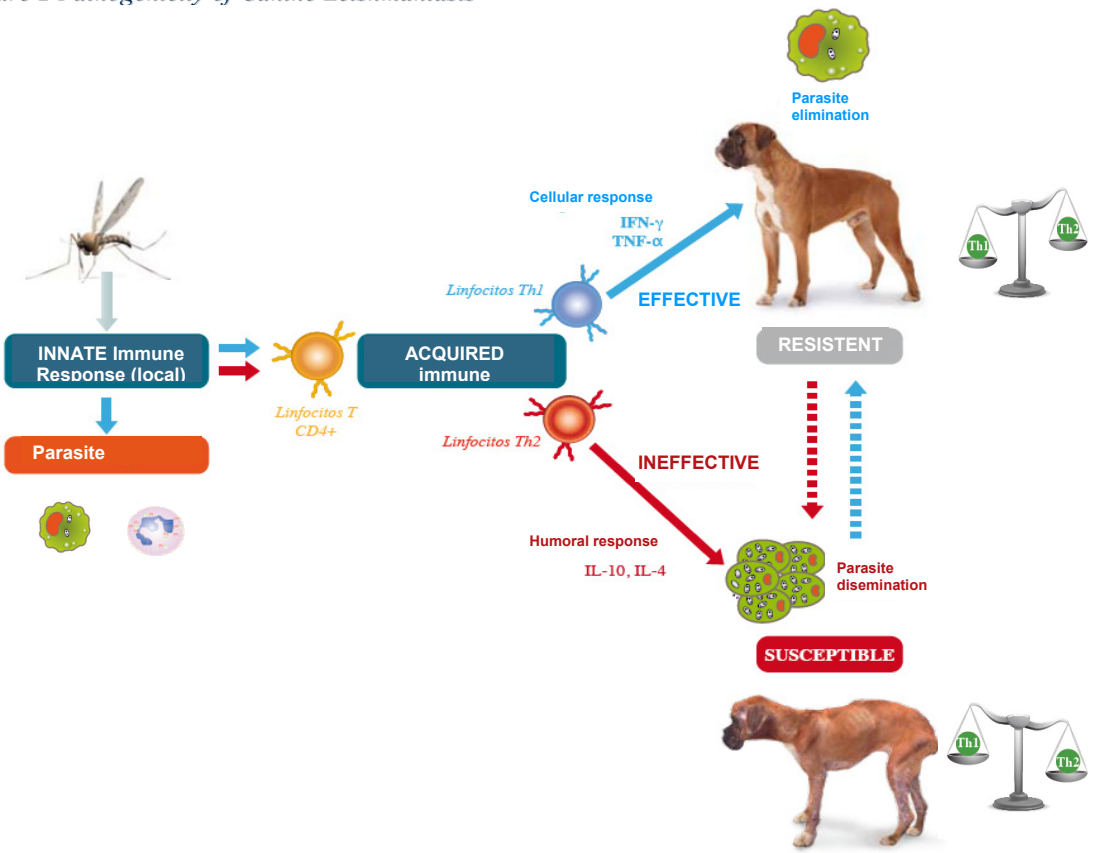
1. Classic stages of Leishmania infantum	*2. Other methods of unusual transmission*
1b Promastigote	*2aVertical*
1b Amastigote	*2b Blood transfusion*
1c Dissemination of parasites in the organs by infected macrophages.	*2c venereal transmission* *Other (not tested): Dog to dog (bites, wounds)*

II.2. The key role of the immune system in disease progression.

Today we know that once the parasite is inoculated by the sandfly in the dog's skin, the progression of infection may follow different paths (Baneth et al., 2008; Paltrinieri et al., 2010; Solano-Gallego et al., 2011).

On the one hand, in a small percentage of infected dogs is thought that the mechanisms of innate immunity may abort the infection locally, through the elimination of parasites by phagocytic cells that act as first line of defense *(Figure 2)*

Figure 2 Pathogenicity of Canine Leishmaniasis



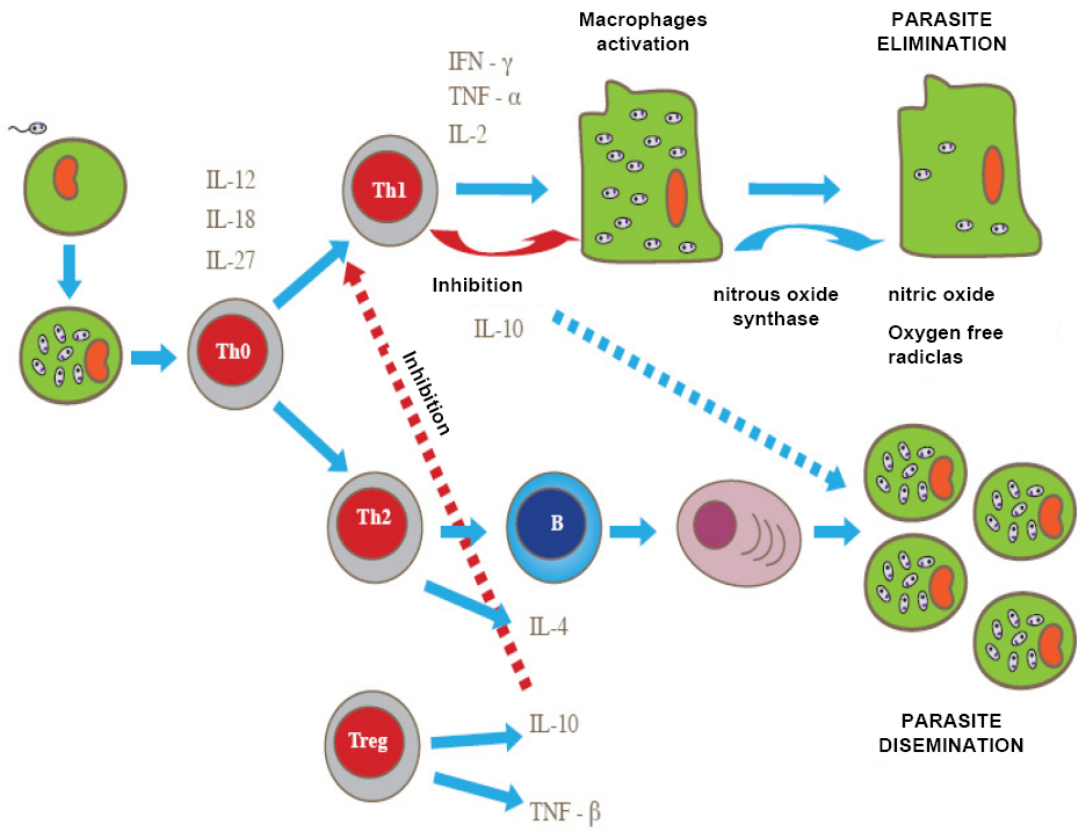
In most cases, however, the infection spreads locally and triggers the innate immune response and gives way to a specific acquired immune response. Depending on the type of acquired immune response is established, the infection progresses to clinical disease or remains controlled. In animals that developed an immune response predominantly cellular (probably most) occurs macrophage activation and consequent destruction of the parasite from the synthesis of oxygen free radicals, including nitric oxide. This type of response is known as **Th1-type immune response**.

By contrast, in animals in which the immune response is predominantly humoral profile, with overproduction of humoral (IgG1, IgG2) infection is not controlled and the

disease progresses. This other type of response is known as **Th2 type immune response.**

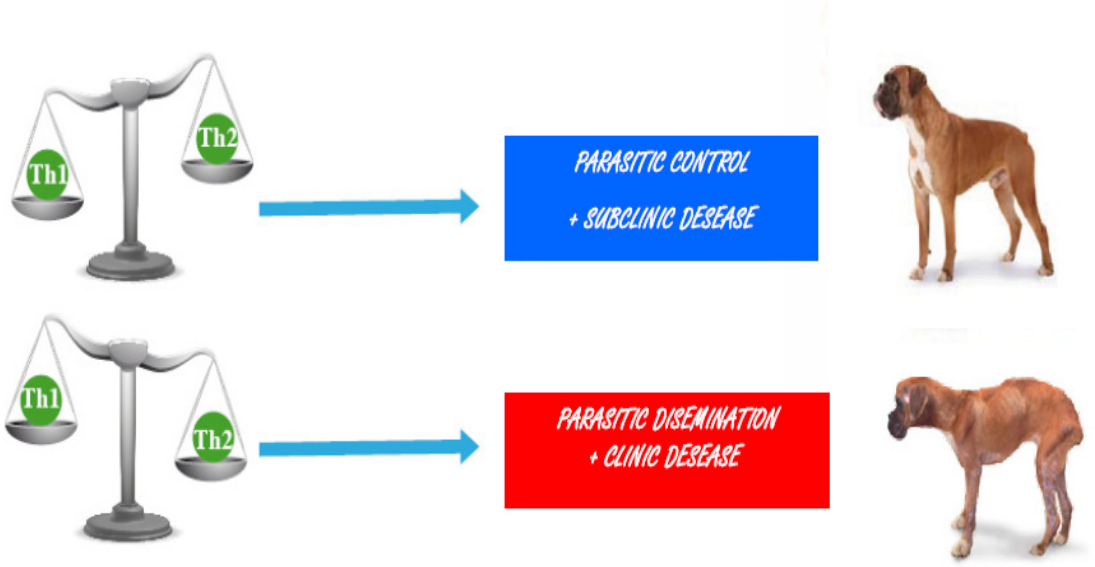
Recent advances in understanding the pathogenesis of canine leishmaniasis have been allowed to know that protective immunity (cellular) against this disease is mediated by T helper type responses 1 (Th1) involving the production of certain cytokines ("hormones" the immune system) that can stimulate and maintain that response over time. These cytokines such as IFN-γ, TNF-α or IL-2, among others, are directly or indirectly responsible for the proper activation of macrophages. By contrast, non-protective immunity (humoral) response is mediated by T helper type 2 (Th2) which involve the production of cytokines such as IL-10, IL-4 or TNF-β, which also inhibit the cell type immune response, stimulate overproduction of ab anti-Leishmania ineffective by plasma cells*(Figura 3).*

Figure 3.
Th1 and Th2 responses and their interactions in dogs infected with Leishmania (Baneth et al., 2008).



In contrast to other species where the response to Leishmania infection is highly polarized (Th1 or Th2), in the case of the dog is a mixed Th1/Th2 response, where control of the disease depends on the balance generated between both types(Figure 4)

Figure 4.
Clinical outcome according to the balance between the two types of immune response, cellular (Th1) and humoral (Th2) which is established after infection.



II.3. The importance of the innate immune response

Although the final control of canine leishmaniasis depends mainly on the acquired immune response mediated by T lymphocytes that is set after the first week of infection, currently gives equal importance to the cell populations involved in the innate response, when they are the first to come into contact with the parasite. It has been reported that these cells in addition to have initial control of infection, influence the routing of the learned response play a key role in the establishment of resistance or susceptibility to the disease (Bonilla-Escobar 2005).

Among these cell populations are monocyte-macrophages, neutrophils and the cells 'natural killer' (NK), among others. Neutrophils are the first to reach the skin after inoculation of parasites. A day or two later come NK cells and monocytes-macrophages became, the last ones, the predominant population during the early stage of infection.

It has been reported that some of the factors that determine susceptibility or resistance to leishmaniasis is due to functional differences in the monocyte-macrophages, being one of the cell populations with a more prominent role in Leishmania infection (Bonilla-Escobar,2005).

Monocyte-macrophages serve as 1) the parasite host cells, 2) antigen-presenting cells to T cells and 3) effector cells in the destruction of Leishmania. In the latter case, they may act as a primary barrier in the time of infection, or secondarily, after being activated by

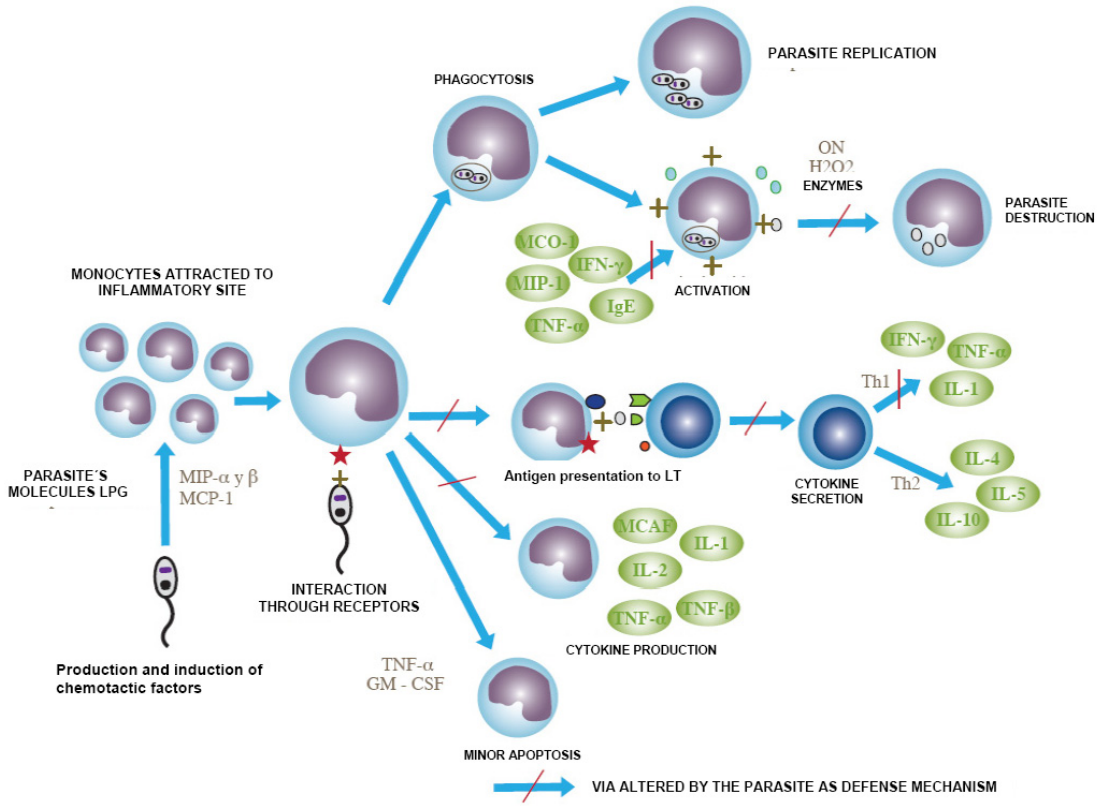
cytokines potentiating the Th1 response by T cells released during the acquired immune response. That is why changes in the activation of this cell population often result in the clinical development of the disease.

Numerous studies also suggest the important role of neutrophils during the early phase of Leishmania infection and relate their presence with less serious injuries and a lower parasite load (Zandbergen et al., 2002, Rousseau et al., 2001, Lima et al ., 1998; Smelt et al., 2000). Like monocitosmacrófagos, neutrophils also require to be activated more efficiently control the infection.

It has been reported that Leishmania, as a defense mechanism, interferes and alters the activation of both cell types by preventing the proper development of a protective response.*(Figure5)*

The ability of these cell populations to help win the battle down to the parasite and activated properly is a key point to consider for the development of new alternatives immunoprevention immunotherapy or, if you intervene early in the course of infection.

Figure 5.
General characteristics of the interaction between macrophages and Leishmania parasites. Leishmania induces monocyte arrival at the site of infection. To meet, interact, and the parasite is internalized. This entry into the cell can lead to antigen presentation, cytokine production, increased cell viability and the survival or destruction of the parasite, as the influence of different factors (Bonilla-Escobar, 2005).



II.4. Clinical disease and the importance of early detection

Although the intrinsic factors that make an individual animal progress towards immune control of the disease or to clinical disease is not yet fully known, genetics is probably the most important. There are breeds in which clinical disease is rare (Ibizan hound) and others which is very common (rottweiler, boxer, cocker, German shepherd). However, an important point to note is that the situation of "resistant" or "susceptible" is not definitive. An immunosuppressive disease, drug treatment or other factors may make an animal which for years has remained under control infection, develop clinical signs of disease.

In animals in which the infection progresses, the incubation period of the disease until the onset of clinical signs is very variable, from 3 months to 7 years, triggering during this period, several pathogenic mechanisms. On one hand, the infection spreads to many organs and systems (spleen, lymph nodes, skin and mucous membranes, liver, pancreas, testes, bowels ...), in which granulomatous inflammatory processes occur. In addition, there are circulating immune complexes deposited in renal glomeruli, uvea, blood vessels and joints. Deposition of immune complexes is a major cause of the clinical signs of disease. Furthermore, in the course of the disease occur other pathogenic mechanisms, such as the formation of auto-antibodies or chronic anemia. These pathogenic mechanisms are responsible for the pleomorphic clinical picture of the disease *(Figure 6).*

Figure 6.
Main clinical signs of canine leishmaniasis:

1. Skin lesions: exfoliative dermatitis, cutaneous ulcers and mucocutaneous junctions, cutaneous nodules.
2. Lymphadenopathy (reactive lymphoid hyperplasia).
3. Asthenia, anorexia, weight loss, muscle atrophy, hyperthermia mild.
4. Renal insufficiency (proteinuria, azotemia).
5. Serious eye (keratitis, uveitis, panophthalmitis, glaucoma).
6. Lameness (arthritis, myositis).
7. Epistaxis.
8. Chronic diarrhea large bowel (colitis).

Sometimes kidney failure is the only apparent sign (Baneth et al., 2008), so when it is detected clinically is likely to be an irreversible damage.

That is why, for a proper control of the disease, it is necessary to detect infected animals in the early stages of the disease, at which the probability of success of therapy is much higher.

As mentioned earlier, not all infected dogs develop the disease clinically. In many cases, dogs without clinical signs remain in this state for years and develop clinical disease only if other circumstances concur in any way compromise their cellular immune response. While we can say that an infected dog with clinical signs have developed a poor response (predominantly Th2) and one infected but with no signs has developed an effective response (predominantly Th1), it is now possible to predict in advance which the response will be for an specific animal, before being infected. Exceptions, as in the case of Ibizan hound, there are no scientifically proven evidence that breed, sex or age of the animal can orientate the immune response in one direction or another.

There is not available any diagnostic test, serological or other, which can be attributed prognostic value that can discern what animals are susceptible to disease and which will be defended successfully in contact with the parasite. Therefore, since we can not distinguish which will prospectively the behavior of each animal, the veterinary surgeon should concentrate their efforts on detecting the disease at the earliest possible stage. As in any other serious illness, early detection is the key to potential success of any therapy you want to set up and in this sense canine leishmaniasis is not an exception.

Despite this evidence, which has traditionally been considered a serological diagnosis with uncertain or lacking clinical confirmation should expect to see the evolution of the animal in subsequent reviews, before addressing any registered drug therapy for this disease, not without side effects and possible development of resistance, without the certainty that occur during this time active proliferation of the parasites. However, this practice involves some risk of disease progression and when you want to start therapy, the animal is in an advanced stage, thus compromising the success of treatment. As described in the following sections, **Leisguard®** offers to vets, an ideal tool for the therapeutic approach in the early stages of the disease.

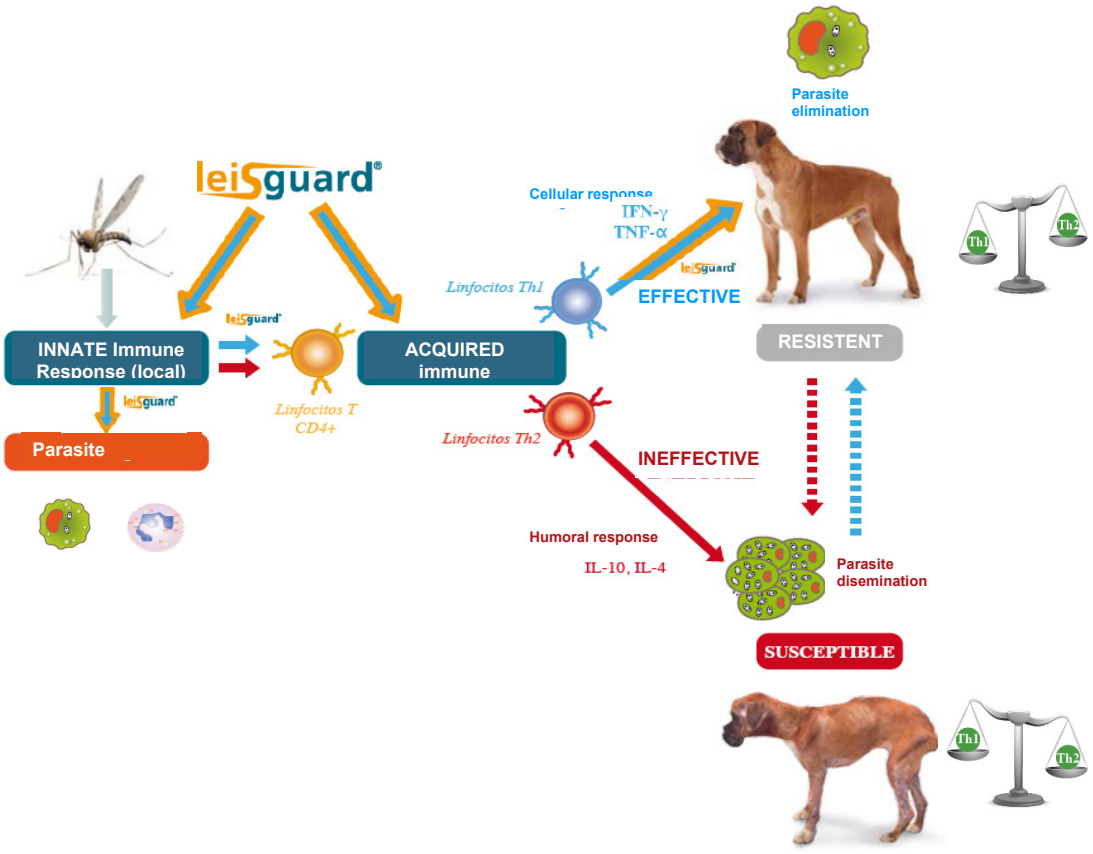
III. Leisguard ®, a new tool against canine leishmaniasis

III.1. What is Leisguard®?

Leisguard® is an oral suspension based on domperidone, indicated to decrease the risk of canine leishmaniasis in contact with the causative agent and control of clinical disease progression in mild or early stages of the disease.

Leisguard® acts on the dog's immune system, in the innate and the acquired response. In particular, increases the leishmanicidal potential of populations of phagocytic cells such as monocytes-macrophages and neutrophils, the first line of defense against Leishmania and a key element in the orientation of the acquired immune response. Through its effect on most cells of the immune system, **Leisguard®** contributes to the establishment of a predominantly cell-type response associated with the resistance to the advance of the clinical disease *(Figure 7)*.

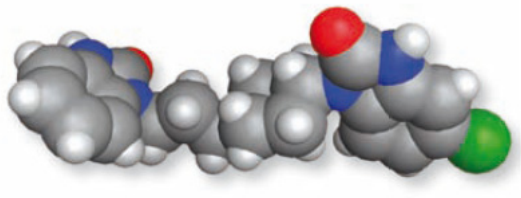
Figure 7.
Leisguard® action points on the immune response.



III.2. Its active ingredient, domperidone

Leisguard® active substance is domperidone (Figure 8), a benzimidazole derivative which acts through specific blockade of dopamine D2 receptors in the periphery.

Figure 8.
Chemical structure of domperidone (5-chloro-1-(1 - [3 - (2-oxo-2 ,3-dihydro-1H-benzo [d] imidazol-1-yl) propyl] piperidin-4-yl) - 1H-benzo [d] imidazol-2 (3H)-one).



Unlike other molecules that act likewise, domperidone practically does not cross the blood brain barrier, that is why not attributed any extra-pyramidal side effects (Reyntjens et al., 1978; Rooyen et al., 1981; Kohli et al ., 1983). This feature, together with the results of toxicological studies conducted during the development of **Leisguard®**, supported a wide margin of safety.

Domperidone has been used extensively in humans and in dogs like gastrokinetic and antiemetic agent, with both activities due to the blockade of D2 dopamine receptors at the level of vomiting center integrated in the medulla and upper digestive tract level, respectively (Brodgen et al., 1982; Reyntjens et al., 1982, Prakash et al., 1998, Takahashi et al., 1991; Johnson, 1992; Barone, 1999; Washabu Hall, 2000).

Less known is its endocrine hyperprolactinemia activity derived from the blockade of D2 dopamine receptors at the level of the pituitary gland. This blocking involves the acute release of prolactin accumulated in the pituitary gland which leads to a transient peak of a few hours in blood levels of this hormone (Kato et al., 1980, Fujino et al., 1980).

Numerous studies show that prolactin, in addition to participate in the hormonal regulation of reproductive function, also has a key role in the development and function

of the immune system, acting as a cytokine. Thus it has been described that prolactin has a great influence on the proliferation and differentiation of many immune system cells involved in both cell and humoral response, most of which have receptors for prolactin and also they synthesized it or have the potential to do it (Swarko-Sonta, 1992, Reber, 1993; Lastraa Vera et al., 2002; Chavez Rueda et al., 2005).

Specifically, it has been shown that, through the modulation of other cytokines, prolactin stimulates cellular type immune response by inducing NK cells and T lymphocytes to produce higher amount of IFN-γ which, in turn, stimulates phagocytic activity and the potential parasiticide NK cells, neutrophils and monocytes-macrophages to eliminate Leishmania charge (Matera et al., 1997 and 2000; Plocinski et al., 2007). Also is described, that prolactin promotes proper antigen presentation by macrophages and dendritic cells (Matera et al., 2001), an essential step for the establishment of an adequate acquired immune response, predominantly cellular, protective against leishmaniasis.

III.3. A dose and schedule carefully established

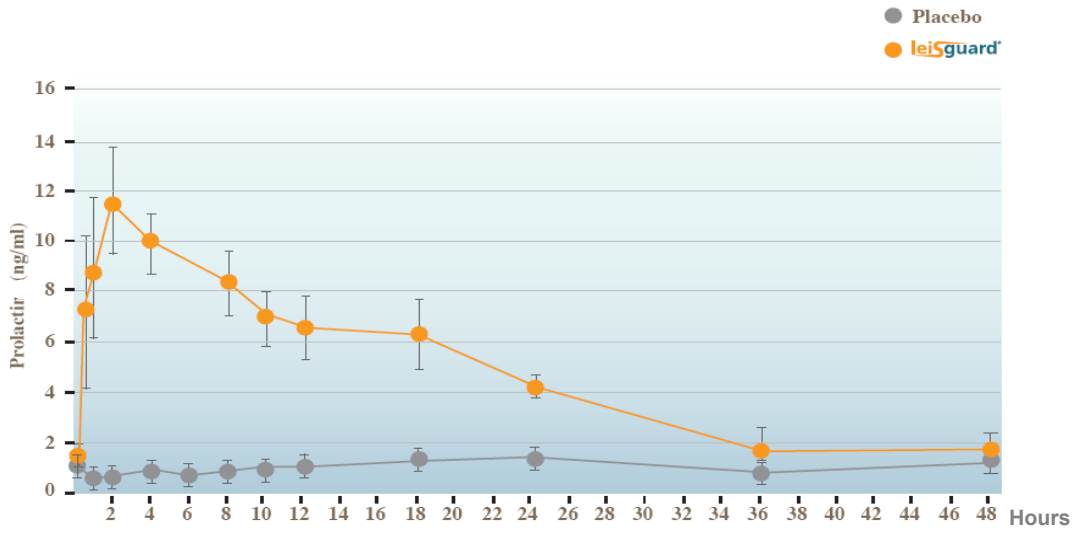
Both the dose and treatment regimen for **Leisguard®** in dogs, were set carefully in order to ensure maximum immunostimulant effectiveness of the cellular response. According to the literature, this effect is not obtained from a sustained increase in time of blood levels of prolactin but, what really stimulates the immune response is the periodic repetition of this hormone sharp peaks induced by the active substance of **Leisguard®** (Rovensky et al., 1995, 1996 and 1999).

The results of several studies in dogs have led to the conclusion that the dose of **Leisguard®** more likely to achieve a significant increase in blood prolactin corresponds to 1ml/10kg equivalent to 0.5mg/kg of domperidone. Thus, after oral administration of **Leisguard®** to such dose induces a peak of prolactin in blood whose maximum level is reached about two hours after administration of the product and then decrease gradually to recover their baseline values, between 24 and 36 hours *(Figure 9).*

This effect has been demonstrated in both males and females reaching a peak of prolactin very similar, although females departing from higher baseline values (Sabaté et al., 2005 and 2006a).

Figure9.

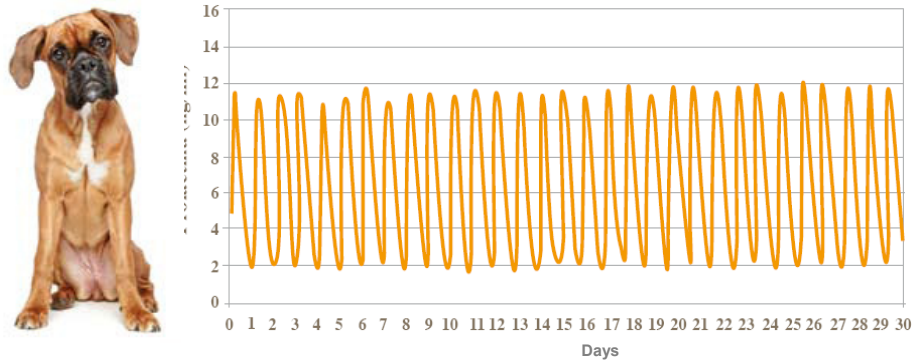
Pharmacokinetic profile of serum prolactin levels (mean ± SE) in dogs after administration of a dose of 1ml/10Kg of Leisguar®.



One of the peculiarities of this dose is that it allows repeat administration of **Leisguard®** every 24 hours without causing an accumulation of prolactin in blood. As a result keeps the magnitude of the daily peaks of the hormone across the treatment, ensuring the maximum immunostimulant efficiency on the cellular response.

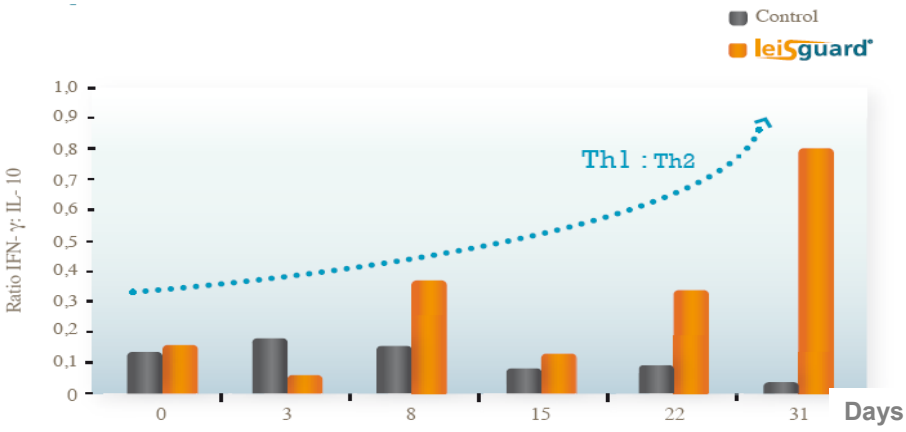
This has been confirmed in other studies whose results show that after repeated administration of 1ml/10kg/24h **Leisguard®** for 30 consecutive days: **i)** basal levels of prolactin remain stable throughout the treatment within physiological values, confirming the absence of accumulation, and **ii)** the magnitude of the daily peaks of prolactin is the same from the first until the last day of treatment, demonstrating the lack of accommodation of the response to repeated administration of the drug (Larraga et al., 2007; Sabaté et al., 2006b) *(Figure 10).*

Figure 10.
Simulation of the pharmacokinetic profile in dog serum prolactin after administration of a 30-day treatment with Leisguard ® (1ml/10kg/24h). Th1: Th2



On the other hand the effect of repeated administration of **Leisguard®** on the immune system and, specifically, on the cell type immune response has been confirmed by the results of another study (Larraga et al., 2007), in which the effect of treatment on acquired immune response by monitoring the ratio between the cytokine response enhancer of the cell type (Th1) and enhancer of the humoral response (Th2) synthesized by monocyte-macrophages from healthy dogs previously stimulated with nonspecific antigen of Leishmania infantum and treated with **Leisguard®** for a straight month (Figure 11).

Figure 11.
Evolution of the ratio of Th1: Th2 (IFN-γ: IL-10) in culture supernatant of monocytes-macrophages from healthy beagle dogs immunized with Leishmania antigen nonspecific infantum and treated with Leisguard ® (1ml/10kg/24h) for 4 consecutive weeks (n = 8) vs. untreated dogs a control group (n = 8). The study was conducted at the Center for Biological Research of the National Research Council (CIB-CSIC) (Spain)



As seen in Figure 11, the repeated administration of **Leisguard®** under the dose and schedule recommended (1ml/10kg/24h) involves a progressive orientation ratio of

cytokines Th1: Th2 to a predominantly Th1 profile, which achieves a significant increased after 1 month of treatment.

The appropriate dose and schedule of Leisguard ® that achieve a prolactin blood profile to stimulate a proper cell-type immune response (Th1) corresponds to 1ml/10kg/24h dog for 30 consecutive days.

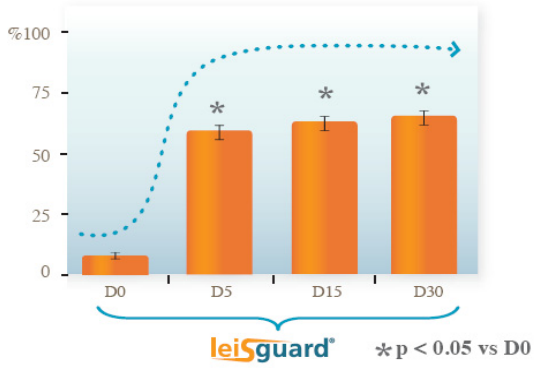
III.4. Stimulating effect of Leisguard ® on the innate immune response

It has been described that the establishment of an acquired immune response predominantly of cellular type (Th1), associated with resistance to disease is influenced largely by the natural or innate immune response (Bonilla-Escobar, 2005). This response is mediated by phagocytic cells such as monocytes-macrophages and neutrophils, which act as a protective barrier against infection also participating, some of them, in presenting antigens to T lymphocyte populations. To perfoms these functions, these cells must be properly activated.

The effect of **Leisguard®** on these cell populations was evaluated in two separate studies (Gomez-Ochoa et al., 2004 and 2008) using, for this, a technique previously validated: the test of NBT reduction or NBT, a test that can discriminate between activated and non activated phagocytic cells from a colorimetric reaction (Gómez-Ochoa et al., 2010a and 2012; Scarpona et al., 2010).

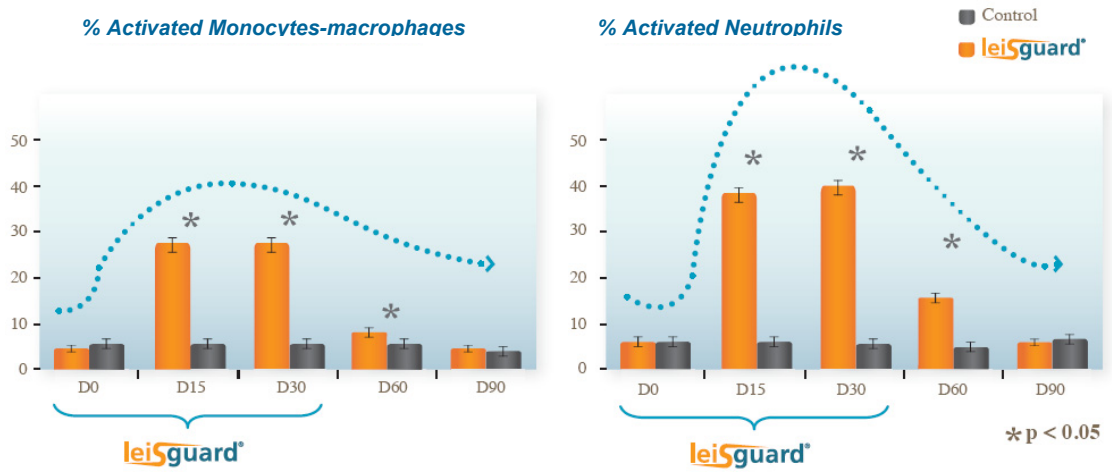
The results of the first study (Gómez-Ochoa et al., 2004) have highlighted the significant increase ($p < 0.05$)of the percentage of monocyte-macrophages and activated neutrophils from 5 th day of treatment, until the end of it (Figure 12).

Figure12.
Evolution of the percentage (Mean ± SE) of phagocytic cells (monocytes-macrophages and neutrophils) activated before and during a 30-day treatment with domperidone in healthy Beagle dogs (n = 20). The study was conducted in Animal Pathology Dept., University of Zaragoza (Spain).



The results of the second study (Gomez-Ochoa et al., 2008), in this case comparative study, confirmed the earlier study and showed that the significant activation of monocytes-macrophages and neutrophils induced by treatment with a 1ml/10kg **Leisguard®** / 24 for 30 consecutive days, exceed the treatment period, decreasing progressively upon completion (Figure 13).

Figure13.
Evolution of the percentage (Mean ± SE) of activated phagocytic cells before, during and after a 30-day treatment with Leisguard® (1ml/10kg/24h) in healthy dogs seronegative to Leishmania (n = 20). The study was conducted at the Department of Animal Pathology, University of Zaragoza (Spain).



As shown in Figure 13, the rates of activation in both cell populations gradually decrease after treatment until recovery the baseline values within two months from completion. The reason for this is that the study was conducted with healthy animals and, consequently, the phagocytic populations activated had not parasites to

phagocytise or antigens to process / present to lymphocytes T cell populations involved in the establishment of the acquired immune response . As a result it does not put in place feedback mechanisms of the cellular immune response (Th1) that in an infected animal, would have ensured their establishment and long-term survival, as demonstrated by the results of the study described in the following paragraph.

The administration of Leisguard® under the recommended dose and schedule involves stimulation of the innate immune response of the animal and the subsequent activation of phagocytic cell populations that act as a protective barrier against infection and are involved in antigen presentation to the acquired immune response populations.

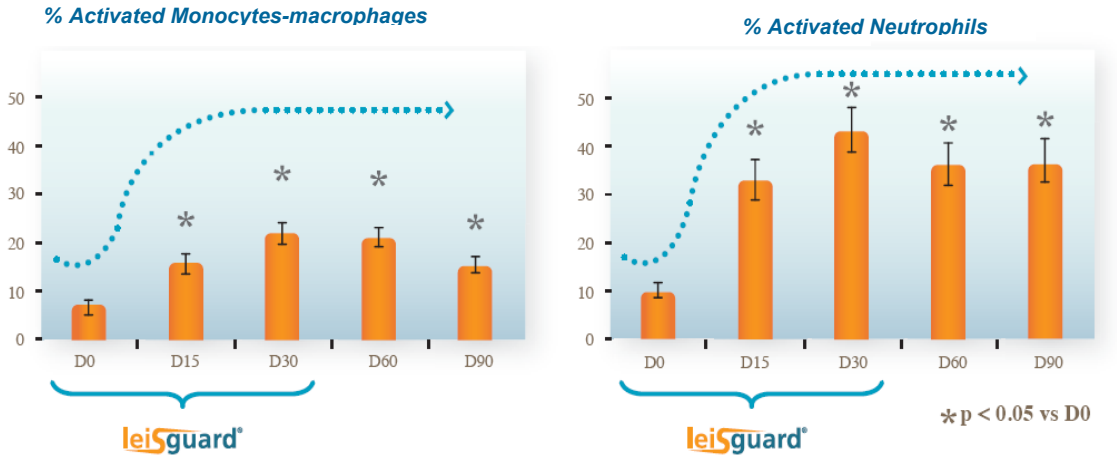
III.5. Stimulating effect of Leisguard® on the acquired immune response

As discussed above, administration of **Leisguard®** under the recommended dose and schedule leads to stimulation of the acquired immune response through an orientation ratio of Th1: Th2 profile to a predominantly Th1 (Larraga et al. , 2007). This effect is reflected ultimately in the activation of phagocytic cells responsible for parasite clearance such as macrophages and neutrophils.

This has been confirmed in a study conducted on dogs with mild leishmaniasis (Gomez-Ochoa et al., 2009a), the results showed that administration of **Leisguard®** to sick animals induces a significant increase in the percentage of monocyte-macrophages and neutrophils activated that, unlike what occurs in healthy animals exceeds the treatment period (Figure 14). This is because modifying the cytokine environment mentioned above, resulting from stimulation of the cellular immune response (Th1) launched, in turn, feedback mechanisms that facilitate their establishment and long-term survival.

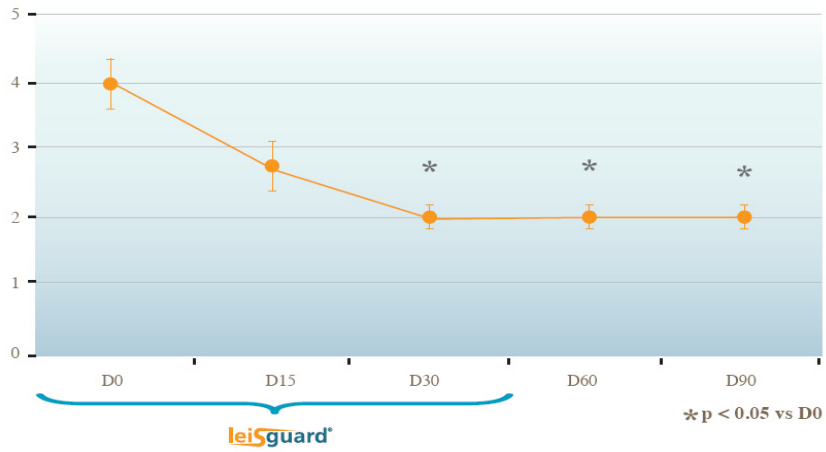
Figure14
Evolution of the percentage (Mean ± SE) phagocytic cells before, during and after a 30-day treatment with low dose Leisguard ® regimen on dogs naturally infected patients (n = 20), with a positive titer of antibodies -Leishmania (DAT-Direct Agglutination Test = 1/400 to 1/1600, equivalent to IFI - Indirect

immunofluorescence = 1/80 to 1/320) and mild clinical signs such as enlarged lymph nodes. The study was conducted at the Department of Animal Pathology, University of Zaragoza (Spain).



Throughout the study dogs experienced a gradual clinical improvement statistically significant ($p < 0.05$) respect to its initial state, which was correlated with the observed effect on the activity of phagocytic cells responsible for parasite clearance, confirming the effectiveness of the treatment (Figure 15).

Figure 15.
Clinical evolution of animals throughout the study, expressed as a Clinical Index (Mean ± SE) previously described in the literature (Penissi et al., 2005).



Leisguard® administration under the dose and schedule recommended involves stimulation of the acquired immune response of the animal and the subsequent activation time kept in the phagocytic cell populations responsible for eliminating the parasite.

III.6. Leisguard ® as a stimulant of the leishmanicidal activity of macrophages

The results of the studies described in the previous paragraph demonstrate clearly that **Leisguard®** influences the dog's immune system contributing to the establishment and permanence (in infected animals) of an immune response, predominantly of type cellular through the activation of phagocytes cell populations such as monocytes-macrophages and neutrophils.

Many studies have shown that activation trains cells to a better performance. Thus, in leishmaniasis, phagocytic cells responsible for eliminating the parasite such as monocytes, macrophages or NK cells, need to be activated to control the infection efficiently. In the case of monocyte-macrophage activation ensures an efficient respiratory burst or oxidative burst (one of the cytotoxic mechanisms used by these cells to parasite clearance) and an adequate expression of molecules for antigen presentation, which reflected in a successful response to the parasite (Bonilla-Escobar, 2005).

The beneficial effect of **Leisguard®** on the leishmanicidal ability of macrophages was confirmed from the results of a study conducted at the Department of Animal Pathology, University of Zaragoza with 10 dogs seronegative to Leishmania, from 2 to 8 years old, different breeds and sexes, to whom was given the specialty under the recommended dose and schedule for 30 consecutive days (Gomez-Ochoa et al., 2009b).

Before the treatment, half of it and after its completion (D0, D15 and D30) was extracted a blood sample from each animal, peripheral mononuclear cells were separated (monocytes) and plated in liquid for cultivation. After 10 days, Leishmania infantum promastigotes were added to the culture medium, and after 48 hours was evaluated the percentage of parasitized macrophages and the percentage of activated macrophages (positive NBT test).

The results put in evidence that the administration of **Leisguard®** induced a statistically significant decrease in the percentage of parasitized macrophages in the cultures mediums of samples obtained at D15 and D30 days of treatment compared to

baseline group (Figures 16 and 17). These results were correlated also with a significant increase in the percentage of activated macrophages.

Figure16.
Images of infected macrophages from samples obtained before initiation of treatment with Leisguard® (A) and at the end of it (B). In the photograph A shows the intact DNA of amastigotes in the cytoplasm of a macrophage while in macrophages B are three, two of them with fragmented DNA of amastigotes removed (diffuse image in the cytoplasm) and the other with amastigotes intact.

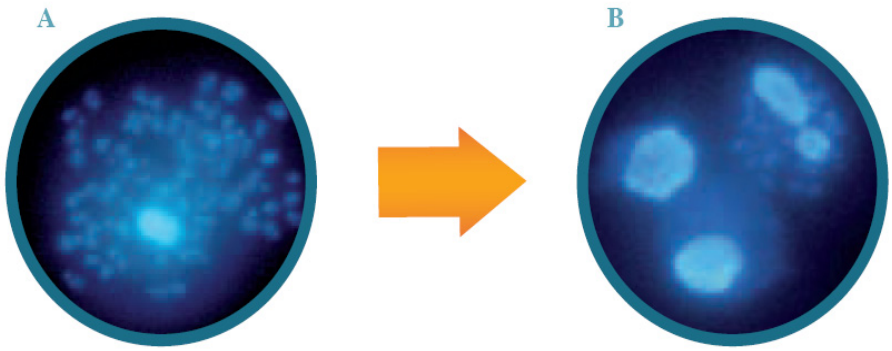
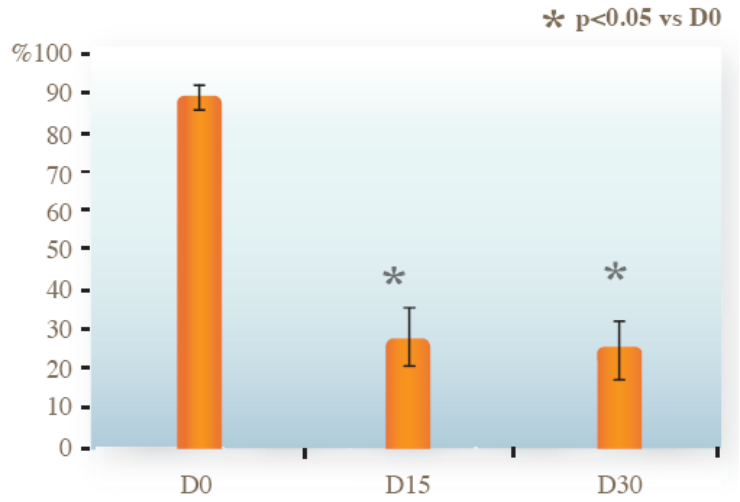


Figure17.
Evolution of the percentage (Mean ± SE) of infected macrophages after co-cultivation of Leishmania infantum with monocyte-macrophages from blood samples obtained during a 30-day treatment with low dose Leisguard® regimen in healthy animals.



IV. Clinical efficacy of Leisguard®

IV.1. Leisguard ® for control of the clinical progression of canine leishmaniasis

As discussed above, the progressive clinical progress in canine leishmaniasis is clearly related to the establishment of an acquired immune response predominantly humoral type (Th2) by the infected animal. As a consequence, T cells produce and release cytokines Th2-type, which induce overproduction of ineffective antibodies against the parasite and inhibit the activation of macrophages and NK cells, ultimately responsible for the elimination of Leishmania (Solano-Gallego et al., 2009).Thanks to that, the parasites can continue reproducing and the animal enters a negative feedback loop that can kill him unless he is put in place an effective therapeutic approach.

Drugs commonly used to treat or control of canine leishmaniasis act directly on the parasites through mechanisms of action leishmanicidal or leishmaniostatic, in an attempt to reduce the parasite load in the hope that the animal can reverse the situation and keep the disease subclinical state controlled. However, today it is known that the final achievement of this goal depends primarily on the ability of the dog to redirect ineffective response (Th2) to an effective response predominantly cell type (Th1), which is closely related with the resistance to disease progression.

The correlation between the stimulating effect of domperidone on the cellular immune response and clinical improvement of sick animals treated with this molecule under actual field conditions was demonstrated for the first time in a field trial conducted at the Veterinary Hospital the University of Zaragoza (Spain) (Gomez-Ochoa et al., 2009c). The study was conducted with 98 dogs naturally infected patients, which were monitored for 12 months after treatment for 30 days. The study results, showed a clear clinical improvement of dogs and a statistically significant decrease in the title of anti-Leishmania antibodies, especially in mild cases.

The clinical efficacy trials conducted with **Leisguard®** to support its therapeutic use are described below.

Clinical trials with Leisguard® for control of the clinical progression of canine leishmaniasis.

The therapeutic efficacy of **Leisguard®** in animals with natural infection has been confirmed in several field trials conducted in collaboration with various clinical veterinarians. One of them, carried out with 20 dogs has already been described in a previous section (Gómez-Ochoa et al., 2011) to highlight that the clinical improvement of dogs with natural infection during and after treatment is correlated with an increased percentage of activated monocytes-macrophages. The results of this study agree well with those of another study carried out by the same author with sick dogs (Gomez-Ochoa et al., 2009c).

Additional trials corresponds to a controlled field blind trial conducted with 41 seropositive dogs for Leishmania and mild clinical signs, including two different veterinary centers in Valencia and Zaragoza (Spain), (Gomez-Ochoa et al. , 2010b). The trial was carried out in accordance with the principles of Good Clinical Practice (VICH GL9-), with permission from the Spanish Agency of Medicines and Health Products.

The dogs, both sexes and different breeds, ages and weights were assigned randomly to two homogeneous groups: Treated with **Leisguard®** and Placebo (Figure 18). All animals had an antibody positive title anti-Leishmania mild to moderate (DAT = 1/400 to 1/1600, equivalent to IFI = 1/80 to 1/320) and mild clinical signs consistent with FMD (enlarged lymph nodes, skin lesions, etc ...). None of the animals showed clinical pathological or renal alterations.

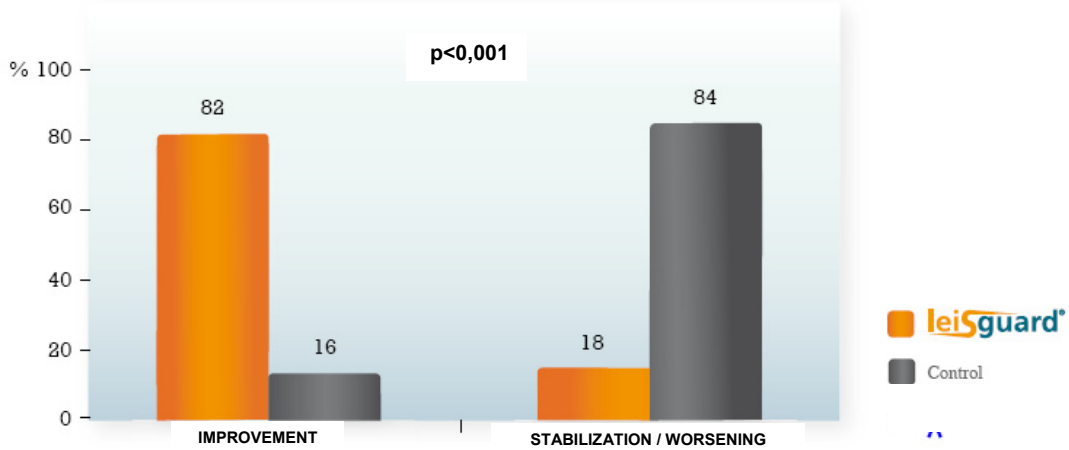
Figure 18.
Characteristics of dogs in each group and test of homogeneity between groups.

		leiSguard® (n=22)	Placebo (n=19)	P Value
SEX (n and %)	Males	9 (40.9%)	5 (26.3%)	0.514
	Females	13 (59.1%)	14 (73.7%)	
AGE (years)	Media ± DE	6 (2.7)	5 (2.1)	0.629
	Rango	3 - 13	1.5 - 10	
WEIGHT (kg)	Media ± DE	18.2 (9.52)	20.3 (8.96)	0.467
	Rango	4-36	4 - 41	
BREED (n and %)	Half-breed	17 (77.3%)	13 (68.4%)	0.534
	German Sheperd	1 (4.5%)	1 (5.3%)	
	Greyhound	3 (13.6%)	4 (21.1%)	
	Husky	1 (4.5%)	0 (0%)	
	Cocker	0 (0%)	1 (5.3%)	

The treated group animals received **Leisguard®** under a dose of 1ml/10kg/24h for 30 consecutive days. Placebo group animals received excipient of the specialty during the same period of time. In order to ensure the blind nature of the trial, both products were masked.

During a follow-up period of between 6 and 10 months (7 months on average) the animals were subjected to several clinical tests: before beginning of treatment (baseline), at 3 months (Intermediate) and 7 months (Final) after initiated. To evaluate the efficacy of **Leisguard®** in each of the clinical tests was evaluated and ranked 11 specific clinical and biochemical specific parameters for calculating a Clinical Index previously referenced in the bibliography (Penissi et al. 2005). During the study, animals in the placebo group experienced a significant decline ($p < 0.05$) of clinical status respect to its initial state, indicative of disease progression. By contrast, animals in the treated group experienced a significant clinical improvement ($p < 0.05$) evidential from 3 months of starting treatment. Specifically, while 84% of the dogs in the placebo group worsened or experienced no change, 82% of dogs treated group showed clinical improvement, the differences being observed between both groups were statistically significant ($p < 0.001$) (Figure 19).

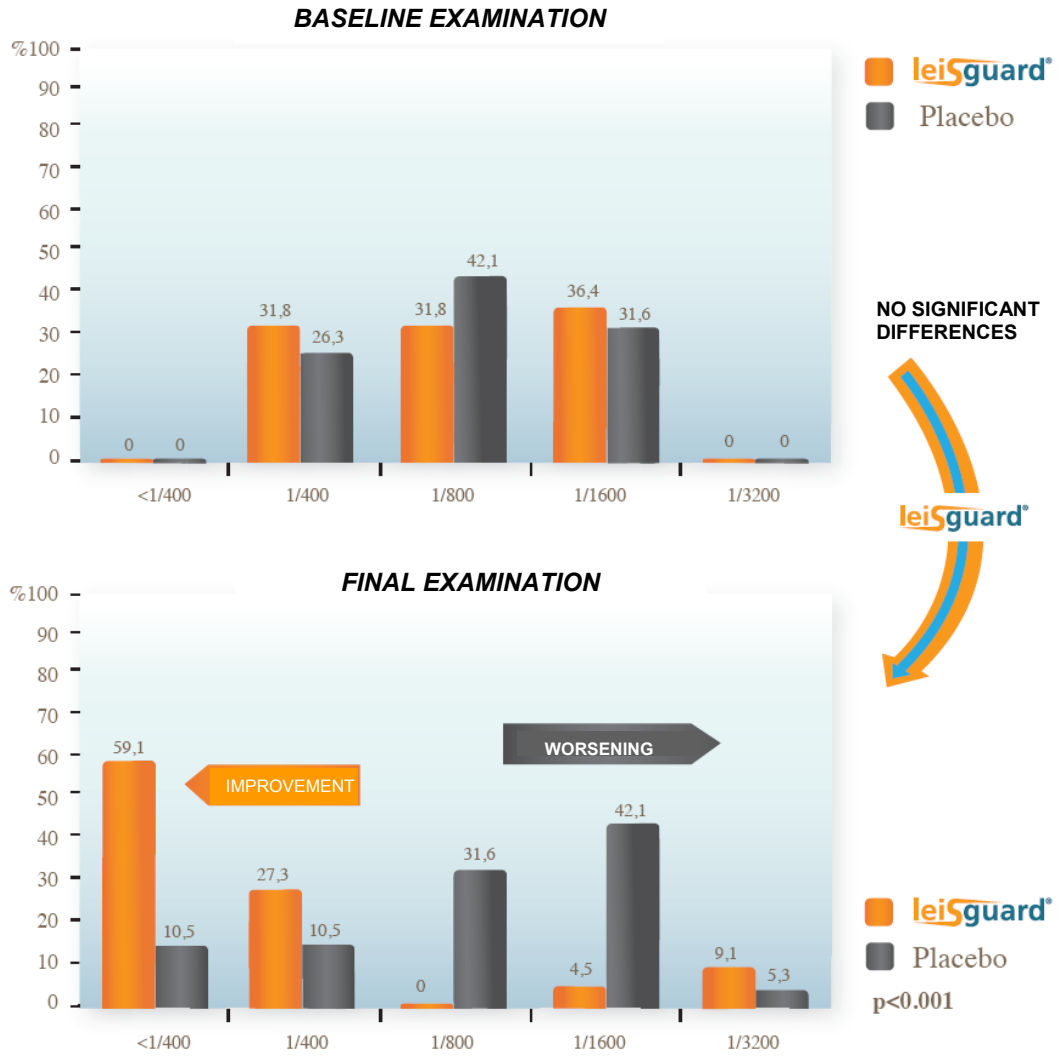
Figure 19.
Clinical status of the animals at the end of follow-up period compared to its initial state.



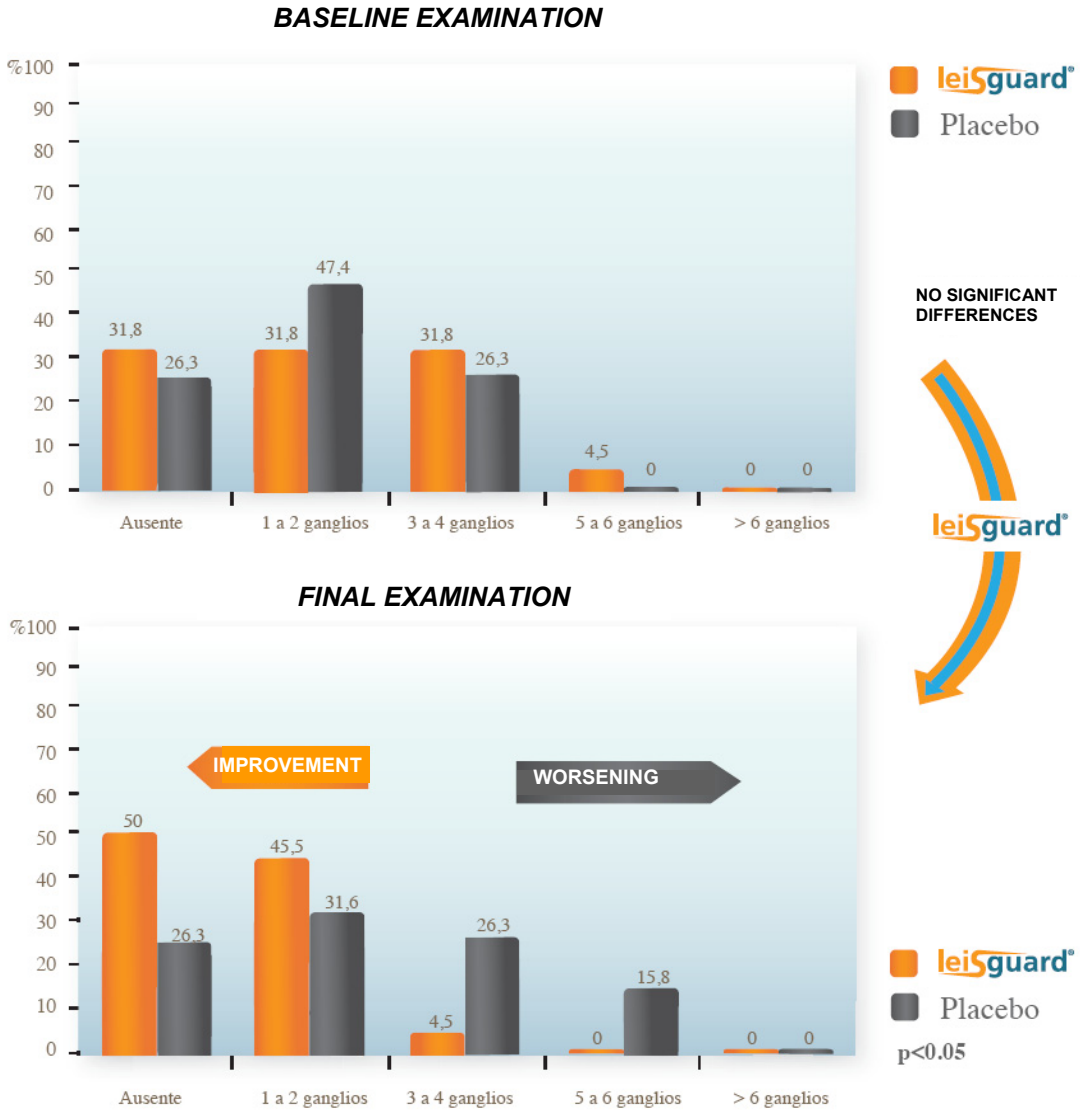
Among the most affected parameters highlighted, the degree of anti-Leishmania antibodies and the degree of enlarged lymph nodes. Thus at the end of follow-up period, treated animals with **Leisguard®** had experienced a significant improvement in both parameters, whereas in the placebo group animals they had worsened (Figure 20).

Figure20.
Changes in the title of Ab anti-Leishmania (A) and degree of lymphoadenomegaly (B) in both groups during the study.

A) ***Changes in the Title of Ab anti-Leishmania(%of animals)***

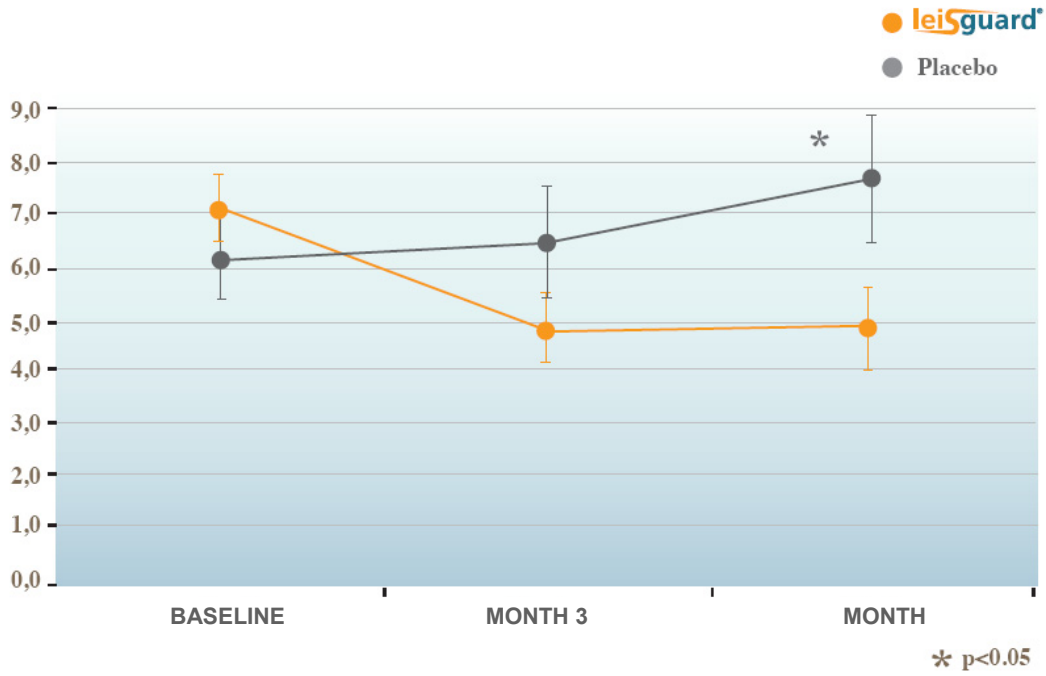


B) Changes in lymphadenomegaly degree (% of animals)



Finally, statistical analysis of the observed differences in clinical index between the two treated groups in each of the tests showed, first, the absence of statistically significant differences between baseline values (thus confirming the homogeneity between the two groups) and, secondly, the existence of statistically significant differences between groups at the end of the observation period (7 months), favouring the group treated with **Leisguard®** ($p < 0.05$) (Figure 21).

Figure 21.
Clinical Development Index (Mean ± SE) in both groups throughout the study.



Finally, none of the animals in the trial had clinical signs of intolerance to the treatment with **Leisguard®.**

Leisguard® is a safe and effective treatment for controlling the clinical progression of canine leishmaniasis in mild or early stages of the disease.

IV.2. Leisguard ® for the prevention of canine leishmaniasis

As discussed above, following inoculation of Leishmania parasites in the skin by the sandfly, it starts a local inflammatory process, with accumulation of resident cells and peripheral blood cells that migrate into the tissue through the vascular endothelium attracted by the presence of the parasite. These cell populations are non-specific defense of the animal to Leishmania, known as the innate immune response, in addition to be initial control of infection, influences in the immune specific system which develops the resistance or susceptibility to the disease (Bonilla-Escobar, 2005).

According to the studies described in the previous section, **Leisguard®** administration to healthy dogs involves the activation of these cell populations, in particular, its leishmanicidal potential, a key mechanism through which justifies its effectiveness in preventing canine leishmaniasis.

The clinical efficacy trials conducted with **Leisguard®** to support its preventive use are described below.

Leisguard ® clinical trials for the prevention of Leishmaniasis

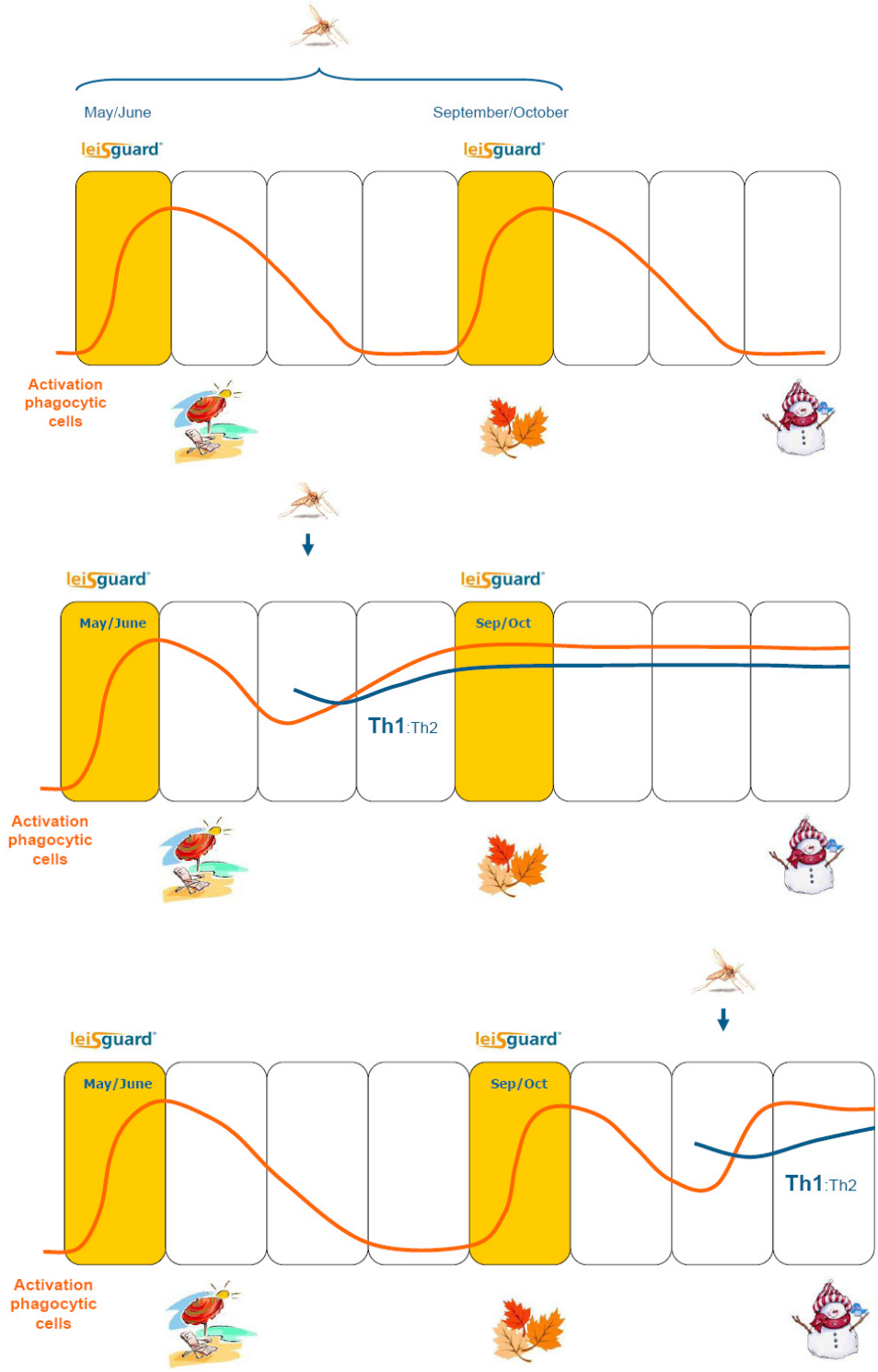
The effectiveness of **Leisguard®** to reduce the risk of Leishmania infection and subsequent development of clinical disease has been demonstrated in two field trials carried out over 400 dogs of multiple breeds, ages and weights, living in two endemic Mediterranean areas, with high and low prevalence.

One of the features of both trials is that instead of applying a single treatment with **Leisguard®** was applied strategic prevention program adapted to the specific risk of infection, consisting in the administration of two or three treatments spread over the year, looking the activity period of the vector. The theoretical basis of this program derived from the effect of **Leisguard®** on the activation of the innate immune response of infected dogs as described above.

As a reminder, **Leisguard®** induces the activation of phagocytic populations that constitute the first defense of the animal, increasing its leishmanicidal potential. If no contact with the parasite, the percentage of activated macrophages decreases gradually after treatment.

However, if the dog is infected during this period, activated macrophages are able to eliminate the parasite more effectively and introduce antigen more efficiently to lymphocyte populations contributing to the establishment of a learned response component with a predominantly cellular (Th1) , related to disease resistance. This response, in turn, ensures the continuous activation of phagocytic populations responsible for eliminating the parasite (Figure 22).

Figure 22.
Simulation of the state of activation of phagocytic cell populations (macrophages and neutrophils) along an established treatment program strategically watching the sandfly activity period.



Accordingly to it, periodic administration of various treatments for 30 days with **Leisguard®** throughout the year, set strategically in terms of risk of infection, matching two of them with the beginning and end of the period for which the vector would ensure

adequate stimulation of the immune system to deal with an infection during the period of risk.

In areas of low prevalence

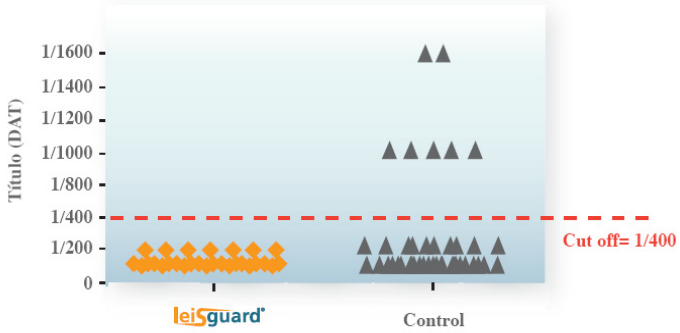
The preventive efficacy of **Leisguard®** in areas of low prevalence was demonstrated in a trial in Valladolid (Spain), with 240 healthy dogs and seronegative to Leishmania infantum (DAT <1/400), different breeds, sexes, weights and ages (Gomez-Ochoa et al., 2009d). The study was carried out in accordance with the principles of Good Clinical Practice (VICH GL9-), with permission from the Spanish Agency of Medicines and Health Products.

The study began the month of June, coinciding with the beginning of the period of vector activity and lasted 9 months. Half of the animals (n = 120) received two treatments with **Leisguard®**, one at the beginning and at the end of the period of vector activity in the area (June and September), under a 1ml/10kg/24h dose for 30 consecutive days. The remaining dogs were not treated. Throughout the study did not apply necklaces products or insect repellents to any of the animals.

All animals were clinically examined periodically to detect clinical signs consistent with FMD. At the end of the study was obtained a blood sample from each of the dogs to determine their degree of anti-Leishmania antibodies.

Throughout the study, most dogs had a normal clinical status except for some animals in both treatment groups who suffered superficial injuries resulting from fights. Seven animals in the untreated group showed the appearance of enlarged lymph nodes and alopecia over the last month. At the end of the study, these 7 animals were the only animals seropositive to Leishmania (DAT ≥ 1/400) (Figure 23). In these animals the infection was confirmed by direct observation of Leishmania amastigotes inside macrophages in some samples of lymph node and bone marrow obtained by puncture needle. All treated animals treated with **Leisguard®** remained seronegative without showing clinical signs.

Figure23.
Title of anti-Leishmania (DAT) dogs Leisguard® treated group (n = 120) and the control group (n = 120) at the end of treatment.



The differences observed between control and treated group in terms of disease incidence (5.83% vs 0%) were statistically significant (p <0.001), and demonstrate the great effectiveness of a prevention program such as that established in this study.

In high prevalence areas

The preventive efficacy of **Leisguard®** in areas of high prevalence was demonstrated in a trial in Valencia (Spain), with a total of 183 seronegative healthy dogs against Leishmania (IFI <1/80), over 24 breeds, both sexes, different ages and weights, residents in neighbourhoods around the city with a prevalence above 20%. The study was conducted with the permission of the Spanish Agency of Medicines and Health Products and was conducted in two phases:

Phase I

The first phase (Llinas et al., 2011th) lasted 21 months and was carried out with 90 dogs divided into two homogeneous groups: treated and control (Figure 24).

Figura 24.
Characteristics of dogs in both groups and homogeneity analysis.

		leiSguard®	Control	P Value
SEX (n and %)	Males Females	25 (56.8%) 19 (43.2%)	25 (54.3%) 21 (45.7%)	0.981
AGE (years)	Media±DE Rango	5 (2.2) 1 - 10	5 (2.3) 1 - 10	0.595
WEIGHT (kg)	Media±DE Rango	20.3 (10. 83)	20.4 (8.46) 7 - 43	0.683
BREED (n and %)	Half-breed Others*	13 (29.5%) 31 (70.5%)	23 (50.0%) 23 (50.0%)	0.606

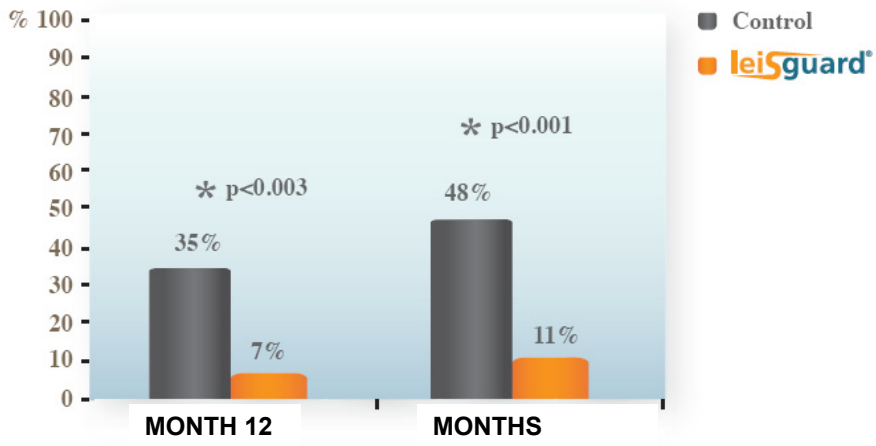
*24 different breeds

Animals from treated group (n = 44) were treated with **Leisguard®** under 1ml/10kg/24h dose for 30 consecutive days, every four months over 21 months. In all cases the first treatment is scheduled for early vector activity period (May-June). The control group animals received no treatment. The allocation of animals to each of the two groups were done randomly. All dog owners agreed not to apply necklaces products or insect repellents throughout the trial.

All animals were clinically examined periodically to detect clinical signs consistent with FMD. In each test was taken a blood sample from each dog to determine their degree of anti-Leishmania antibodies. When in one of the tests were observed clinical signs of disease (enlarged lymph nodes, dermatitis ...) and a positive title of antibodies (IFI ≥ 1/80), indicative of active infection and clinical progression of the disease, the animal was removed from study and treated according to clinical judgment of the veterinarian. The data obtained throughout the trial was carried out two statistical analysis: one at 12 months and another at 21 months.

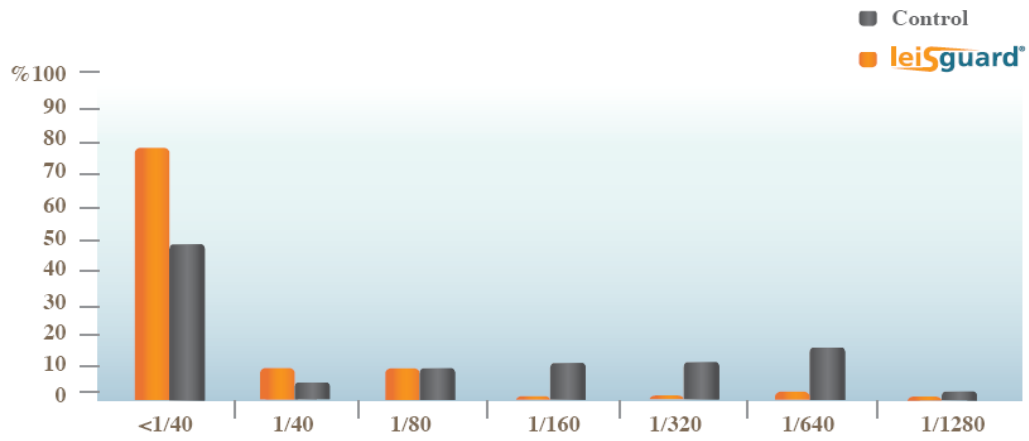
The percentage of infected animals (positive serology and clinical signs) was significantly lower in the group treated with **Leisguard®** than in the control group at both 12 months (7% vs. 35%, P = 0.003) and at 21 months (vs. 11%. 48%, P <0.001) (Figure 25).

Figure25.
Percentage of animals with active infection and disease progression in both groups at 12 and 21 months after the start of the prevention program with Leisguard® in the treatment group.



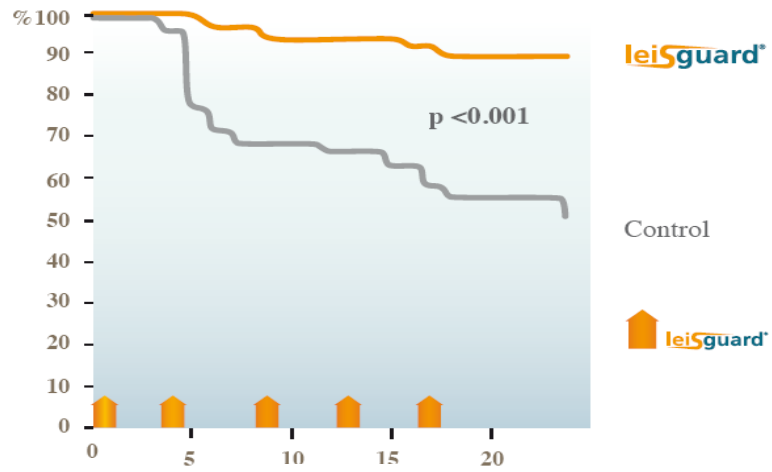
Furthermore, among seropositive dogs in both groups at the time of being removed from the study, evidence of anti-Leishmania antibodies were higher in the control group than in the group treated with **Leisguard®** (Figure 26).

Figure 26.
Distribution of the different titles of anti-Leishmania antibodies (IFI) among dogs in both groups dropped during the study.



There were also no statistically significant differences between groups ($p < 0.001$) in favor of **Leisguard®** treated group in relation to 'time to removal of animals' (Figure 27).

Figure 27.
Evolution curves of the percentage of clinically healthy seronegative animals in both groups throughout the study.



According to the cumulative percentages of healthy and sick dogs in both groups at the end of the study, the preventive efficacy attributable to the **Leisguard®** treatment

program under the conditions of this study was 80% (Figure 28). Also, according to these data, the probability of developing clinical disease (calculated in terms of odds ratio) is 7.2 times lower in the treated animals with **Leisguard®** than in untreated animals.

Figure 28.
Interpretation of results at 21 months.

	Diseased	Healthy	p-value
Control (n= 46)	22 (48%)	24 (52%)	p < 0.001
leiSguard® (n= 44)	5 (11%)	39 (89%)	

Preventive efficacy = 0.48 - 0.11 / 0.48 x 100= 80%

Odds ratio (OR) = (0.48 / 0.52) / (0.11 / 0.89) = 7.2 (I.C.95%=2.389-21.40)

Phase II

The aim of the second phase was to confirm the results of the first phase through its extension by including in the same vet clinic 93 new seronegative dogs (DAT <1/400) from the same geographical area as those of the first phase, over the next period of vector activity (Llinas et al., 2011b).

In this case, all dogs received a prevention program based on the administration of two treatments **Leisguard®**, one at the beginning and at the end of the period of vector activity (May / June and September / October) in a dose of 1ml / 10kg/24h for 30 consecutive days. The animals were subjected to regular clinical examinations over 9 months in order to detect clinical signs consistent with FMD. At the end of the trial was taken a new blood sample from each of the dogs to determine their title of anti-Leishmania antibodies. As in Phase I, throughout the monitoring period is not applied collars products or insect repellents to any of the dogs.

The results obtained were compared with those obtained in the control group of Phase I (historical control).

During the study, most dogs had a normal clinical status were not observed any signs compatible with canine leishmaniasis. However, serological analysis of blood samples obtained at the end of the study revealed the presence of 7 seropositive animals, 1 dog with a title DAT = 1/800 and 6 dogs with a degree DAT = 1/1600. The percentage of seropositive animals obtained in this second phase of the study was similar to that of seropositive animals obtained in the treated group (at 12 months) Phase I (7.5% and 7% respectively). Comparing this figure with that of seropositive animals in the untreated group of the first phase (historical control), the observed differences were statistically significant (7.5% vs 35%, P <0.001). According to these data, the preventive efficacy attributable to the prevention program proved to be 80%, thus confirming the results obtained in the first phase (Figure 29).

Finally, note that only 4 of the dogs included in **Leisguard®** treated group showed clinical signs of side effects attributable to treatment (2 galactorrhea, 1 loose stools and 1 diarrhea).

Figure 29.
Interpretation of results comparing the animals treated in Phase II with the untreated animals in Phase I, at 12 months.

	Diseased	Healthy	p-value
leiSguard® Fase II (n= 93)	7 (7.5%)	86 (92.5%)	p < 0.001
Control Fase I (n= 44)	16 (35%)	30 (65%)	

Same preventive efficacy = 80%

Leisguard® is a safe and effective in reducing the risk of developing active infection with Leishmania in case of contact with the parasite, when administered according to a strategic prevention program in endemic areas with low or high incidence of the disease.

V. How to use Leisguard ® in practice?

V.1. the product

Leisguard® is supplied in vials containing 60 ml of suspension at a concentration of 5 mg of domperidone/ml, to be administered at 1 ml per 10kg body weight (equivalent to 0.5 mg / kg of active ingredient). The product comes with two dosing syringes that allow precise dosing regardless of the weight of the animal. A unit of 60 ml is enough for a 30-day treatment for a dog of 20kg. In case of some remaining product, it can be used in a second course of treatment as long as you give it within 8 months after first opening.

In all tests with **Leisguard®,** the product has been accepted without problems by dogs, either by administering it directly into the mouth, or by incorporating it into food. In fact, according to the pharmacokinetics and bioavailability trials conducted with **Leisguard®**, when administered with food it reaches higher plasma levels of active ingredient than when administered in the fasted state (Figure 30). It has also been found that regardless of mode of administration (forced or feed), achieved prolactin peaks are very similar (Figure 31). Thus the administration in the food is highly recommended when treating dogs kept individually. If there is no guarantee that the patient would eat teh complete meal that contains its full dose of **Leisguard®,** for example when several dogs live together in the same household, it is preferable to ensure correct dosage, administering it directly into the mouth.

However, repeated administration of doses that are too high or too low could affect prolactin peaks and its return to the baseline levels, which could modify its efficacy in both prevention and treatment of leishmaniasis. Since the adequate stimulus for the cellular response is obtained from a succession of transient prolactin peaks (Rovensky et al., 1995, 1996 and 1999), which occur when **Leisguard®** is administered to the exact dose and schedule recommended, it is necesary determine the animal's weight and administer accurately **Leisguard®** using the syringe.

Figure 30.
Domperidone plasma levels after administration of a dose of Leisguard® fasting or with food (Mean ± SD).

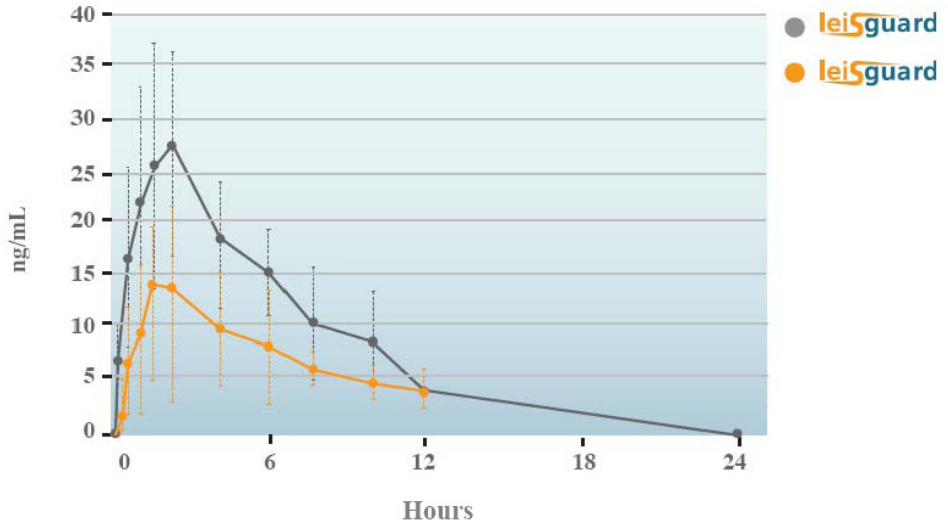
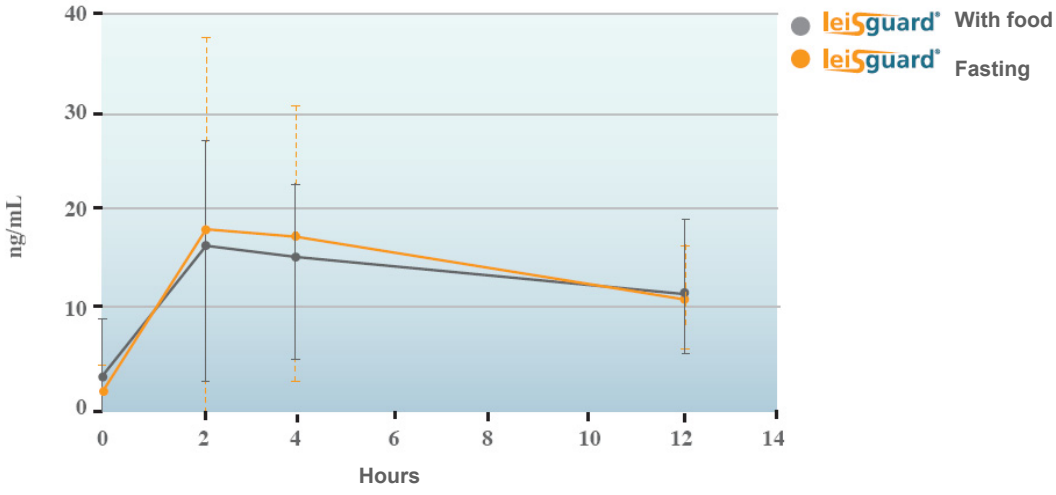


Figure 31.
Prolactin plasma levels after administration of a dose of Leisguard® fasting or with food (Mean ± SD).



V.2. An excellent safety profile

Domperidone (active ingredient of **Leisguard®**) practically does not cross the blood brain barrier, that´s why there are not attributed extrapyramidal side effects (Reyntjens et al., 1978; Rooyen et al., 1981, Kohli et al., 1983). **Leisguard®** has a wide safety margin, as demonstrated in the clinical trials conducted in which, after several courses of treatment administered to more than 300 dogs, only isolated cases of galactorrhea, loose stools or diarrhea have been described.

Moreover, in tolerance trials, it was administered at doses up to 5 times the therapeutic dose for a year without appreciable adverse effects observed. Therefore, it is not expected to produce any alteration in the patient in case of overdosage. Reproduction trials performed in experimental animals, showed no evidence of teratogenic or toxic effects, to the embryo nor to the mother, even at doses 20 times the recommended. However, since there are not enough well-controlled trials conducted in pregnant bitches a risk / benefit assessment should be performed before attempting to use Leisguard in this period. If administered to lactating dogs, as described in females of different species, is likely to induce an increase in milk production.

Given its mechanism of action, it should be used with caution in patients with previous episodes of pseudo pregnancy, as it might contribute to exacerbate the symptoms.

V.3. The patient: The importance of early diagnosis

As with any serious illness, early detection of Leishmania infection is essential to ensure the success of any therapy. However, as described above, healthy dogs may be developing a silent leishmaniasis. According Baneth et al. (2008), kidney disease may be the only apparent alteration in infected dogs. Therefore, to know exactly the status of the patient, it is necessary to supplement the clinical examination with specific diagnostic tests. Depending on the results, appropriate therapy should be established, either preventive or therapeutic.

Recent studies have shown that the diagnostic technique that presents the highest potential to detect early infection by Leishmania infantum is ELISA (Enzyme-Linked

Immunosorbent Assay), while other tests widely used in leishmaniasis like IFAT and PCR, have underperformed. (Rodriguez-Cortes et al., 2010). It is also very important that whatever the technique used, the test must be quantitative since it provides information about degrees of seropositivity that may have some prognostic value, and greater sensitivity. In fact, groups of experts in leishmaniasis recommend that when a rapid qualitative test is used, if a positive result is obtained, it would be equally necessary to re-evaluate the patient using a quantitative test (Cardoso et al., 2004a; Podaliri et al., 2011; Solano-Gallego et al., 2011).

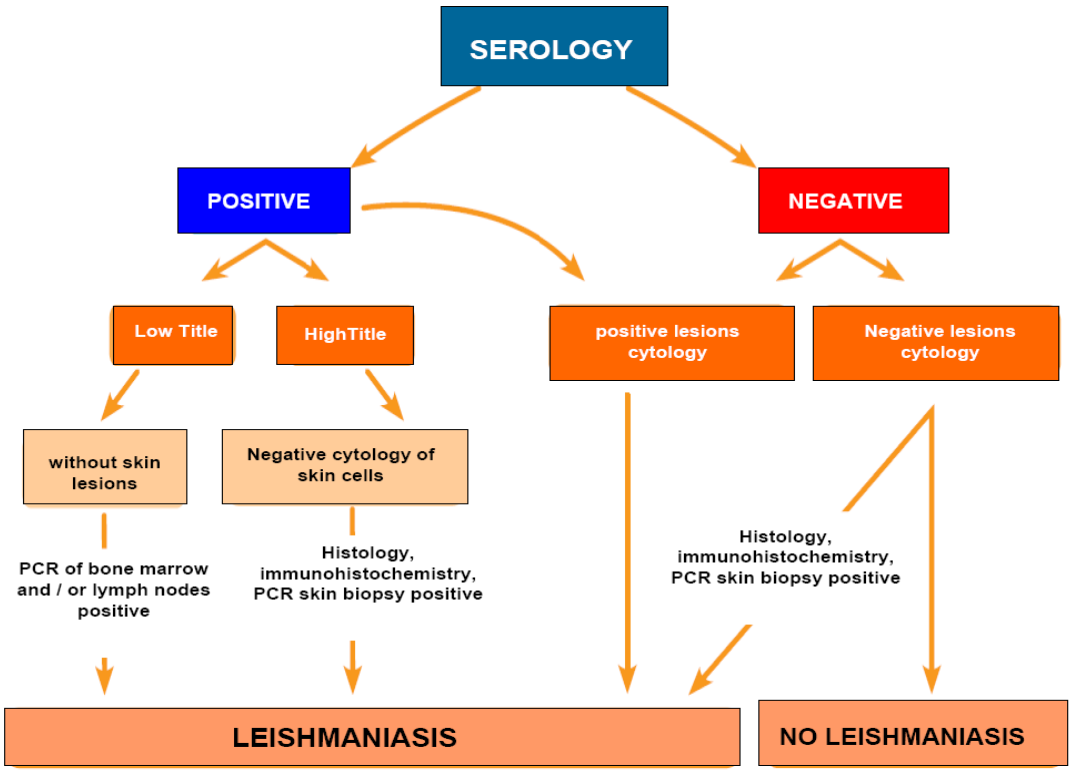
In a recent trial in the Autonomous University of Barcelona, different commercial serological tests for detection of infection were compared and showed that the performance of quantitative ELISA test is significantly higher to the "fast" tests or qualitative. Among them, the best commercial test to detect individuals infected with Leishmania infantum is **Leiscan® Leishmania Elisa Test**, resulting in the individual measures of performance of 98% in sensitivity and accuracy and negative predictive value 0.93 in. On aggregate, measures such as the area under the ROC curve (Receiver Operating Characteristic) was also significantly superior to all qualitative tests (Rodriguez Cortes et al., 2010).

Therefore, the systematic use of quantitative serology, also in asymptomatic dogs, has been shown essential for good early diagnosis of infection, which is the fundamental basis for successful therapy. Based on the serology results, we can establish the action plan for each patient, whether therapeutic or preventative.

V.4. What to do after a positive early diagnosis?

As mentioned above, even in the absence of other clinical signs or diagnostic tests, the serological result allows us to establish the initial protocol of action, especially if there has been a quantitative assessment, because we can act depending on the degree of seropositivity (Figure32).

Figure 32.
Serological diagnosis of leishmaniasis step by step (L. Ferrer and Roura X, 2012).

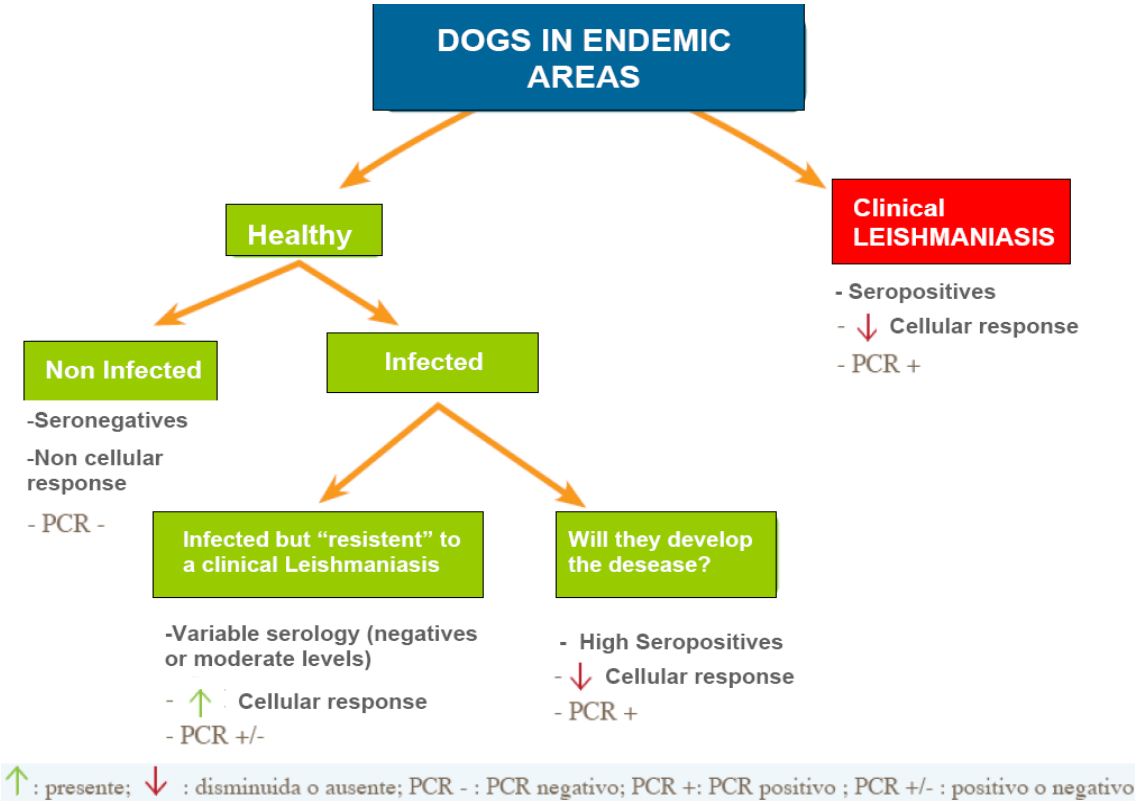


To a **high seropositive** dog **(Leiscan® Rz> 1.5; IFI> 1/160)**, we have to consider that the patient may be developing leishmaniasis (Oliva et al., 2006). Therefore, additional confirmatory tests should be performed to determine the clinical condition. Apart from a clinical examination, the presence of the parasite should be evidenced by isolation or PCR, and any possible clinic-pathological changes should be asessed, including the protein profile and renal function.

Depending on the results of all tests, we can determine the clinical status of the animal and depending on its severity, choose the most appropriate treatment. Figure 34 summarizes the recommended therapies according to the clinical status of each animal.

If after complete diagnosis it is determined that the patient is simply an exposed or infected dogs with mild symptoms, the use of **Leisguard®** as monotherapy has proved to be sufficient to reduce the clinical symptoms and antibody titer. However, if there is a high serology, it is likely that we are in advanced cases and with greater severity and a poor cellular immune response (Figure 33).

Figure33.
States clinical and immune response of dogs living in an endemic area of Leishmania infantum. (Solano-Gallego et al., 2009).



HIGH SEROPOSITIVE

In these cases it is advisable to reduce the parasite load with leishmaniostatic or leishmanicidal products, then use **Leisguard®** so that it can have maximum immunomodulatory effect and improve patient prognosis.

According to current recommendations (Solano-Gallego et al., 2011) the patient should be re-evaluated **after 30 days of treatment with a leishmanicidal or leishmaniostatic, at which point you can start a first course of treatment with Leisguard®** at the dose and schedule recommended(1ml/10kg/24h x 30d).

These patients should be re-evaluated every 3-4 months for one year and it is recommended to **repeat the treatment with Leisguard®** quarterly, coinciding with clinical follow-up examinations. Also, get serology tested every 6 months. If after 6-12 months after initiation of therapy the patient is stabilized (obvious clinical improvement, normalization of serum protein and stabilization or reduction of antibody levels) may keep the patient in a **relapse prevention program with Leisguard®** as described below (**Leispro® program**).

To a low seropositive (Leiscan ® Rz 1.1-1.5; 1/80-1/160 IFI) and in the absence of other signs related to the disease, we have a dog that has received the bite of infective

sandflies and has established an immune response against Leishmania. However, we can not distinguish whether this response will be effective Th1 and may eventually control the disease itself or is inclined towards an ineffective Th2 response.

Until the appearance of **Leisguard®**, in these cases, given the absence of a treatment acting therapeutically and preventively at a time, it has been historically recommended not to treat the patient and wait until a subsequent control will clear the doubts in terms of their serological and clinical evolution. This strategy carries the risk of the disease progressing silently, and when treatment is started the animal is in advanced stages and uncertain prognosis. However, the risk/benefit current recommendations so far considered the the risks of dealing with the therapies available to date in terms of possible adverse effects and generation of resistance that affect both canine and human disease, exceeded the benefits that could be obtained. However, the implementation of this recommendation in many clinical situations, in which other factors are involved, has been uneven.

With **Leisguard®**, this conflict can be solved since its administration to patients who have been exposed to the parasite but it is doubtful that they are developing the disease can only help them overcome their own situation without creating any significant side effects or resistance. Therefore, the risk/benefit of using **Leisguard®** to any low or questionable serology is clearly positive and may keep healthy a percentage of cases, which currently can not be known beforehand, preventing the disease progresses to advanced stages.

LOW SEROPOSITIVE

In these cases, subject to the discretion of the veterinarian who carried out other diagnostic tests making it possible to better characterize the patient, **treatment with Leisguard®, at the recommended dose and schedule (1ml/10kg/24h x 30d) should be started immediately after positive diagnosis**. Depending on the risk of the area and time of year when the animal is, it is advisable to **repeat a course of treatment at 4 months of the first** and, following the current recommendations, make a new **serological test at 6 months**. Where the antibody titles are stable or decreased and the absence of other signs, we can **include the patient in a prevention program with Leisguard®** as described below (**Leispro® program**). Otherwise, make a complete assessment of the patient as described above.

V.5. What to do after a doubtful early diagnosis?

If we get a **doubtful serology (Leiscan® Rz 0.9-1.1 or IFI 1/80)** is not possible to know if the dog is positive or not and if there is no evidence the presence of the parasite by other techniques it is advisable to repeat the analytical test after 6 months to confirm or refute the diagnosis. Even if these animals are seropositive they would be in a very low titer, probably indicating that this is a simple infection, well controlled by the animal's own immune system or are still in a very early stage.

DOUBTFUL

In either case, in case of a **questionable serological result it is advisable to start preventive treatment** with **Leisguard®** to the recommended dose and schedule, immediately. In this way we increase the chances that by repeating the serology after 6 months obtain an effective immune response through the reduction or negativity of the antibody titer. As in the previous case, if the patient's course has been favourable, it can begin **a prevention program with Leisguard® adapted to the circumstances of the dog (Leispro® program).**

CLINICAL STATUS	SEROLOGY RESULTS	PARASITOLOGY (PCR/ direct Obs /cultivo)	CLINICAL SIGNS	CLINICAL-PATHOLOGICAL ALTERATIONS	THERAPY	MONITORING	WHY *Leisguard®*?
Exposed, infected or with mild disease	- +/- +	+ + ? -	Asymptomatic or lymphadenopathy, papular dermatitis	Non alterations Normal renal profile (Creatinine< 1,4 mg/dl; UPC < 0.5)	**LEISGUARD** (1ml/10 kg/d x 30d)	***Repeat LEISGUARD alter 4 months.*** ***LEISCAN and clinical re-evaluation after 6 months.*** ***If controlled:*** ***Preventive Program LEISPRO depending on the risk*** ***If LEISCAN alter 6 m > initial. Complete Re-evaluación***	*There is no certainty that these dogs are sick, but we can not know if they are at the start of a clinical process with risk of worseness.* ***LEISGUARD** will help to establish an effective Th1 response, if necessary.* *If LEISCAN alter 6 m < initial, the preventive program **LEISPRO**, will protect the dogs in case of future exposure to the parasite.*
MILD Leishmaniosis	+ a +++	+	+Dermatitis exfoliative, onychogryphosis, ulcers, anorexia, weight loss, epistaxis, fever	Non-regenerative anemia, hypergammaglobulinemia, hypoalbuminemia. serum hyperviscosity Normal renal profile (Creatinine <1.4 mg / dl; UPC <1)	Leihsmanicide and/or Leishmaniostatic	**Clinical evaluation after 30d.** **Continuation:** **LEISGUARD (1ml/10kg/d x 30d).** **Clinical Re-evaluation each 3-4 meses + LEISGUARD quartely x1 year.** **LEISCAN each 6 m** ***If controlled:*** ***Preventive Program LEISPRO depending on the risk***	*The immune response of these dogs is predominantly Th2 balance and tend to worsen.* Treatment with leishmanicidal initially decrease the parasitic burden, but **LEISGUARD** *rebalance the immune response towards Th1 immune response for the animal to stabilize in the longer term.* If **LEISCAN** *and semiannual clinical controls show a stabilization of the patient, the preventive program **LEISPRO**, will protect these dogs of any recurrence or in case of new exposures to the parasite.*
SEVERE Leishmaniosis	++ a +++	+	+ lesions for inmunocompl.: uveitis, arthritis, glomerulonephritis	+ chronic kidney disease IRIS I UPC >1 ó IRIS II (Creatinine 1,4-2 mg/dl)	Leihsmanicide and/or Leishmaniostatic + IRIS for renal functionality		
Leishmaniosis clínica grave	++ a +++	+	+ pulmonary embolism, nephrotic syndrome	+ Chronic kidney disease IRIS III (Creatinine >5 mg/dl. UPC>5)	Leishmaniostatic + IRIS for renal functionality		**LEISGUARD** *will help to rebalance the immune response towards Th1, without damaging the kidney.*

V.6. What to do after a negative early diagnosis?

In the case of a negative **serological result (Leiscan® Rz <0.9; IFI <1/80)** and in the absence of other signs or clinicopathologic abnormalities, we have a healthy patient but which we can not know whether if contacting infective sandflies will control infection itself or if it will eventually develop the disease.

In these cases, we can consider a preventive program, combining strategic use **Leisguard®** with serological monitoring with **Leiscan®,** adjusted to the characteristics of the area and the lifestyle of the patient **(Leispro Program ®).**

Valorate the risk

If the dog lives in an endemic area it is at risk of getting the disease. In these cases, the main factor that should concern us is the prevalence of living area and seasonal period that may be infecting sandflies.

The higher prevalence and longer seasonal period the greater the risk of contact between the dog and the vector and therefore the greater the risk of infection. Epidemiological studies have determined the prevalence of different endemic areas that are helpful for making risk management decisions (in paragraph VIII shows the data so far available). Roughly, Franco et al. (2011) propose **three levels of seroprevalence: Low (<5%), Medium (5-20%) or high (> 20%)** used as a reference for decision-making.

However, we must take into account that there can be large differences in prevalence between similar dog populations living a few miles away because of the environmental variables that determine the abundance of sandflies in a given area (Cardoso et al., 2004a) so it is important to know the epidemiological characteristics of the area where the patient resides.

The **density of sandflies** plays a key role in the emergence and spread of the disease (Martin Sanchez et al., 2009). The sandflies breed in areas where organic matter accumulates and retain a high relative humidity (animal burrows, foot of trees and shrubs) and humanized environments that meet these conditions, such as fuelwood, farms, gardens, sewers, garbage, etc.. Humanized environments but with plenty of green areas (eg residential areas on the outskirts of cities) is where a higher density of

sandflies and therefore the risk of transmission in these areas increases up to 70% (Nieto 2004) .

Keep in mind that although the populations of sandflies are high in early summer (June-July), they are less infectious, since there has been little time to extract the parasite and infected dogs. Instead, sandflies that live at the end of the season (late September and throughout October) are more likely to be infective, so the risk of infection is greater. Depending on the climate, the **period of activity** is highly dependent and varies depending on the year (Lucientes 2004, Oliva et al 2006). In the more southern areas, the sandfly can become active in late February and end in early December, while in northern areas it begins in May and ends in early November (Lucientes 2004). With the altitude rainfall increases and temperature decreases wich are worse conditions for the presence of sandflies (Galvez et al., 2011).

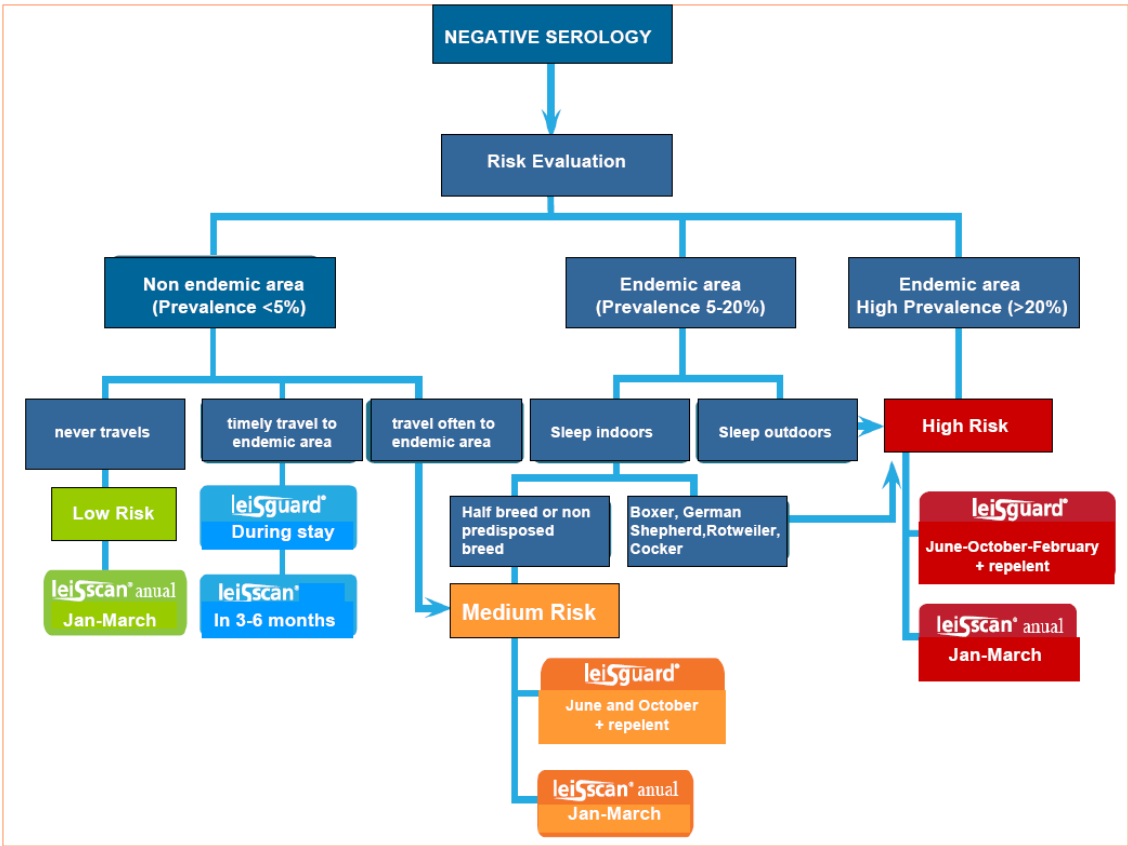
However, the presence of sandflies is not the only risk factor we must take into account for assessing the prevalence of an area. Other factors such as breed and **way of life** of the dog must be considered. It has been reported that pure **breeds** and particularly the Boxer, Rottweiler, Cocker Spaniel and German Shepherd are particularly sensitive, while the mongrel dogs from endemic areas and individual breeds like Ibizan Hound are more resistant (Nieto 2004, Solano-Gallego et al. , 2011). In the way of life is of fundamental importance if the dog lives primarily outside or inside the house and especially over during the night hours (Sousa et al., 2011). From sunset to midnight are the peak hours of the sandfly activity (Lucientes 2004). So if the dog sleeps outdoors makes the risk of infection (Odds Ratio) is 3.3 times greater than if it was sleeping inside the house. Therefore, guard dogs show a risk of infection 3-4 fold. Residence in an urban environment is also a risk factor compared to rural areas, probably due to the existence of gardens and a higher density of dogs (Cortés et al., 2007, Martin Sanchez et al., 2009, Sousa et al., 2011).

Finally, **nutritional status** is a determining factor for the establishment of the disease due to its direct effect on the immune status of host (Nieto 2004). It has been reported in animals with clinical leishmaniosis who were exposed to situations of malnutrition (eg by competition from other dogs in communities), are cured by simply eating well. This

is also one of the reasons why human leishmaniasis mainly affects poor countries with high rates of bad nutrition (WHO 2010).

In any situation of risk described **Leisguard®** can be used as an effective disease prevention. However, the pattern should be established taking these factors into account and in accordance with the decision tree described in Figure 35.

Figure 35.
Recommendations of the preventive use of Leisguard ® as the risk of infection (seronegative animals).



NEGATIVE

In case of negative serology, we must take into account the degree of prevalence of each area. (Low <5%, 5-20% Medium or High> 20%). Based on this classification, we propose different actions that are intended to serve of orientation for the veterinary surgeon(preventive program **Leispro®**). At this point it is convenient to use the latest information on the degree of prevalence available in the area where the dog resides or generate it from the consolidation of the results of serological screening tests in the same area or veterinary clinic.

While it may vary according to the criteria used, several epidemiological studies show that approximately 50% of seropositive animals show any symptoms or clinical pathologic alterations associated with disease (Galvez et al., 2010, Marty et al., 2007; Solano -Gallego et al., 2001; Brandonisio et al., 1992). Therefore, based on the percentage of dogs with clinical leishmaniasis it could also be extrapolated, for guidance, which may be the prevalence of the disease in a specific group.

Non-endemic area (low prevalence <5%)

In areas of weather conditions that do not allow high densities of sandflies or the density of dogs is very low, we find very small population of seropositive dogs. This makes the risk of contracting the disease through contact with the vector in dogs residing in these areas low. However, in these areas leishmaniasis cases usually appear when the dog was infected during a relatively long stay in an endemic area. Additionally, in recent times different routes of infection such as maternal-fetal (Nieto 2004) or by blood transfusions (Solano-Gallego et al., 2011) or tick bites (Podaliri et al., 2011) have been described and could also take place in these regions although its incidence is much lower than transmission through the sandfly.

Therefore, if the dog lives in this area without ever travelling to risk areas is very unlikely that would get in contact with the parasite. In these cases, however, it is also advisable to make a serological test once a year. In this way, we can act quickly in case of infection by any route without having to administer any type of product to the dog.

In general, we recommend performing a serological test in winter, preferably between January and March, as will have already sufficient time elapsed from the sandfly season to seroconvert in case of a possible infection.

The case of the travelling dog

If the dog, normally resident in this area makes frequent excursions into endemic areas, such as weekends, it will require a preventive program in line with the prevalence of

these areas and not the habitual residence. If visiting an endemic area is timely, for example on summer vacation, a preventive program should be applied accordingly. In this case, we recommend treating with **Leisguard®** during the stay of the dog in the danger zone. According to available cell activation, the immunomodulatory effect of domperidone is already evident at 5 days after initiation of therapy (Gomez Ochoa et al., 2004) and may occur even earlier. Therefore, in practice, the preventive treatment with **Leisguard®** may be simultaneous to the arrival at the area of risk. However, even if the stay in the endemic area is short, it is necessary to maintain treatment to complete the recommended regimen of 30 consecutive days.

If the stay is prolonged for over a month, you can set a second period of treatment, leaving a maximum of 3 months of rest between them. Additionally, we recommend a serological test within 3-6 months after returning from the holiday period, in order to detect possible signs of infection.

Endemic area (Average prevalence 5-20%)

In the case of dogs living in endemic areas with a prevalence of between 5 and 20% it will be necessary to take measures to avoid infection. These dogs are at obvious risk of infection, even if they make little life outdoors.

In these areas, the activity of the sandfly season often extends from May to October, although there may be variations from year to year due to weather conditions. In these cases it is advisable to establish a preventive program with **Leisguard®** consisting of two annual treatments at the beginning of the epidemiological season and at the end, typically in June and October. This program has proven effective in reducing dramatically the risk of contracting the disease in the clinical trials described above. Additionally, we recommend the use of insecticide repellents (either as a collar or as spot-on) that, acting independently, have a complementary effect that makes the risk of contracting the disease even lower.

Since there is no 100% effective prevention, it must not be overlooked early diagnosis, as it offers the possibility to intervene with greater success guarantees, if necessary. As described above, the moment that would be suitable for making a serological screening in these areas would be in winter, preferably between January and March. In this regard

we must remember that **Leisguard®** does not have a preventive effect on the bite of the sandfly, but prevents the development of clinical disease. Thus the finding of low seropositivity in a dog during the prevention program indicates only that it had contact with the parasite, but not that the dog is developing the disease.

Endemic area (High prevalence> 20%)

In areas where prevalence has been reported very high (> 20%) usually converge habitat and favourable climate to the multiplication of sandflies and a high density of dogs. These circumstances make that infective sandflies can be found well into Fall season and since the beginning of Spring, without a well-defined epidemiological season. Therefore, in these areas is necessary to establish a more intensive prevention program comprising administering **Leisguard®** with a quarterly frequency. In most cases it corresponds to treatments in June in October and in February. This regimen has proven effective in clinical trials discussed earlier in which the probability of infection was reduced 7.2 times.

This pattern would also be advisable for those dogs that, evein if living in areas of lower prevalence, are exposed to additional risk factors, mainly the way of life outdoors in the night hours (for example, guard dogs) or breeds described as particularly sensitive.

In these high risk areas there is a large exposure to sandflies during most periods of the year, so it is advisable not to neglect other preventive measures such as use insecticide repellents and try to avoid the dog to be or sleep in areas with presence of sandflies at night.

In this way, there are several trials that attributed to the use of collars or spot-on products based on permethrin a significant preventive efficacy due to its repellent effect on sandflies (Ferroglio et al., 2008, Miró et al., 2007a; Foglia Manzillo et al., 2006).

Given that the effect of these products to reduce the exposure of dogs to sandflies bites is independent of the immunomodulatory effect of **Leisguard®**, you can calculate what wold be the effect of their combined use. In this respect, taking into account the results of trials Manzillo Foglia et al., (2006) and Llinás et al. (2011th), both carried out in areas of high prevalence, the preventive efficacy attributable to the use of collars with **Leisguard®** on a quarterly schedule would be 98%. It is therefore highly advisable to combine any repellent products registered with **Leisguard®** to achieve a degree of protection virtually complete.

Finally, even with these measures implemented, it is recommended that a serological test should be performed once a year in order to respond quickly to any sign of infection.

VI. FAQ´s

- If a bitch being treated with Leisguard® in the preventive regimen becomes pregnant, should I continue administering the product or is it better to stop treating?

As specified in the leaflet, studies performed with experimental animals did not evidenced adverse effects during pregnancy. However, given that there are not enough well-controlled studies in pregnant bitches, a risk/benefit assessment should be done by the practitioner. This assessment should take into account the dog's risk level, especially considering the prevalence in the geographical area where it lives, season, breed and whether the animal sleeps outdoors or indoors.

- If a bitch is treated with Leisguard® in the prevention regimen, and one of the treatment courses coincides with the lactacting period, should I continue administering the product or is it better to stop treating?

Specific studies in lactating bitches have not been performed. In other species, including humans, an increase in milk production has been described when treated with the active principle of Leisguard® during lactation, so it is likely that treatment with **Leisguard®** induces the same effect in lactating bitches. Moreover, during lactation there is a physiological increase in prolactin that could grant itself an adequate degree of protection against leishmaniasis. Therefore, if milk production of the dog is normal, you can skip the treatment course coinciding with this period or, depending on the risk level and season, awaiting lactation termination to resume the treatment with **Leisguard®.**

- In a diseased animal, can I administer Leisguard® together with other medications?

Association of Leisguard® with antacids such as omeprazole or cimetidine is not recommended, neither it is with dopaminergic molecules as dopamine or dobutamine. On the other hand, the active principle of Leisguard is antagonistic to cabergoline. No other interaction with other medications has been described in clinical trials conducted up to date.

From the standpoint of efficiency, the mechanism of action of **Leisguard®** is completely independent of leishmaniostatics or leishmanicidal products commonly used in dogs.

In fact, treatment concomitantly with allopurinol has already described with a good response and without any adverse effect.

- In the preventive use, what is recommended, the use of Leisguard® or vaccine against Leishmania?

The basis of both treatments is similar, since the two are focused on improving the effective cellular immune response against leishmaniasis and thus prevent the development of the clinical disease.

The main difference is that vaccines carry an antigen that will cause a certain amount of specific antibody production even if the animal is not infected, while **Leisguard®** boosts the immune response without causing by itself a serological response. The production of antibodies after vaccination has the potential to interfere with the serological diagnosis of dogs (EMEA 2011). Thus, vaccination may decrease the effectiveness of early detection campaigns. **Leisguard®** does not interfere with serology, thereby allowing the detection of seropositive dogs earlier, simpler and safer than if they were vaccinated. Moreover, the use of vaccines in seropositive animals is not indicated, being very advisable to use **Leisguard®** in these cases.

Regarding clinical efficacy in high prevalence areas **Leisguard®** has demonstrated preventive efficacy* 80%. On the other hand, the 'probability' or 'risk' of developing clinical leishmaniasis (calculated in terms of chance or odds **) is 7.2 times lower in the treated animals with **Leisguard®** than in untreated patients.

Finally the protective cell activation occurs within 5 days after starting treatment with **Leisguard®.**

* Real efficacy attributable to the treatment program once discounted all cases without being treated / vaccinated nor had contracted the disease.

** 'Probability' that a dog has of getting sick respect to the probabilituy of remaining healthy, depending on if treated / vaccinated or not.

- Can I combine Leisguard ® treatment with Leishmania vaccines?

Both, **Leisguard®** and vaccines against Leishmania pursue the activation of cellular immune responses. Their mechanisms of action are compatible and it is likely, although there have been no trials that conclusively demonstrate this, that their combined use increases the effectiveness of both.

Moreover, protection by **Leisguard®** cell activation occurs within 5 days after starting treatment, the earliest available. Therefore, the use of **Leisguard®** for the primary vaccination program could also provide protection much earlier, which can be very necessary primovaccination is administered during the season of abundance of sandflies.

- Are there differences in efficacy based on the hour of the day that Leisguard® is administered?

In dogs, unlike humans, there is not a clear circadian rhythm of plasmatic prolactin, so there is not a better hour than other for treating . Moreover, prolactin peaks achieved with treatment with **Leisguard®** are significantly higher than baseline (in the absence of lactation), so its effect is independent of small variations in the basal prolactin.

- From what age can administer Leisguard ®?

Leisguard® is a very safe drug in adults as in puppies and it is not necessary to wait to reach a minimum age for starting treatment. From the point of view of efficiency we can get adequate protection from the first weeks of life due to the effect of **Leisguard®** on innate immunity.

In fact, it has been reported that prolactin plays a key role in the development and maturation of the immune system of mammals (Swarko-Sonta, 1992).

- Are there any problems associated with reduction of the Th2 response?

In infected dogs, **Leisguard®** enhances the Th1 response, but does not eliminate the Th2 response. As in dogs resistant to disease, a rebalancing occurs between the two responses, so there remains some degree of humoral response coexisting with cell effective response. Specific trials have shown that **Leisguard®** does not affect the speed or intensity of seroconversion after the use of common viral vaccines in puppies (Salichs et al., 2006a, Salichs et al., 2006b).

- Can I cause hyperprolactinemia by administering Leisguard ®?

Leisguard® used at the recommended doses causes completely reversible increases in serum prolactin (peaks), so there is not accumulation or sustained hyperprolactinemia over time. These peaks of prolactin are responsible for the activation of protective cellular immunity against infection by Leishmania and are of much lower magnitude

than those found in a lactating bitch, returning each day to baseline. It is therefore very rare to induce undesirable effects in the treated animals.

However, when using higher doses or different patterns, differences in the magnitude of the prolactin daily peaks may occur, which could affect the efficacy of the treatment. Similarly, lower doses may be insufficient to produce a complete release of pituitary prolactin.

Therefore, dose adjustment for the weight of the animal is necessary to respect as much as possible the recommended dosage schedule.

- Can we alter the levels of other hormones in addition to prolactin?

Due to its anti-dopaminergic effect, in humans, it has been observed a certain increase of the TSH which has not shown any clinical relevance. Moreover, it has been shown that this does not affect the levels of 18-hidroxicorticosterona, cortisol, renin, angiotensin, aldosterone, or growth hormone.

- Is it true that domperidone may induce cardiac abnormalities?

Some human studies have shown that when administered intravenously at higher doses than recommended for **Leisguard®** it may increase the risk of QT prolongation and ventricular arrhythmias. However, specific studies conducted in dogs demonstated no cardiac effect administering doses much higher than the recommended, even when administered intravenously.

- Can I give Leisguard® to a bitch that has suffered episodes of pseudopregnancy?

Yes, but must be used with caution since it is likely that these patients will achieve higher levels of prolactin, so that could potentially induce some degree of galactorrhea. If this sign is presented in mild form it is a benign effect because it is indicative that there are protective levels of prolactin. If these effects are too intense and do not disappear upon discontinuation of treatment cabergoline may be given to normalize the patient.

However, cabergoline should never be administered concomitantly with **Leisguard®** as they have antagonistic effects.

- Can we manage Leisguard ® for periods of less than 30 days without affecting its clinical efficacy?

Leisguard® effect on transient activation of phagocytic cells is observed since shortly after initiation of treatment. However, the orientation of the immune system response to a durable, predominantly cell type (Th1), takes place gradually and is only significant towards the end of the 30 consecutive days of treatment. For this reason, in all trials conducted during the clinical development of **Leisguard®** it was administered for 30 consecutive days. There have been no studies demonstrating the efficacy of shorter treatments.

Consequently, shortening the treatmen period is not recommended as this may compromise its effectiveness.

- What if treatment is interrupted for a day?

The stimulating effect of **Leisguard®** on the immune response occurs as a result of repetition of the prolactin peaks that occur daily, after administration of each dose of product. Timely interruption of treatment (two or three doses maximum) should not significantly affect the efficacy. In this case, please return as soon as possible to treat and manage missing doses to complete 30 days of treatment. However, if the interruption exceeds three consecutive doses, the end of treatment efficacy can not be guaranteed, and it is recommended to initiate a new full treatment of 30 consecutive days with **Leisguard®.**

- Can Leisguard® induce the occurrence of autoimmune diseases?

The increase in blood prolactin levels above physiological values and sustained for a long time (hyperprolactinemia) has been effectively linked to the onset of autoimmune diseases. However, to attain these levels, it is necessary to increase not only the release, but also and significantly, the synthesis of prolactin.

The administration of **Leisguard®** produces a reversible prolactin peak within a physiological range (well below levels reached during lactation), which returns to baseline daily, without any accumulation taking place of the hormone. This is so because Leisguard® does not work by stimulating the synthesis of prolactin but only causes the release of prolactin accumulated each day in the pituitary in a physiological way.

Consequently, there is no basis for relating the administration of **Leisguard®** with the onset of autoimmune diseases, not having reported any instance or in humans or in dogs despite the widespread use of domperidone in both species for decades.

VII. TECHNICAL DATASHEET

NAME OF THE VETERINARY MEDICINAL PRODUCT

Leisguard 5 mg/ml Oral Suspension for Dogs

QUALITATIVE AND QUANTITATIVE COMPOSITION

Each ml contains:

Active substance:

Domperidone	5 mg

Excipients:

Methyl parahydroxybenzoate (E218)	1.80 mg
Propyl parahydroxybenzoate (E216)	0.20 mg
Quinoline yellow (E104)	0.20 mg

PHARMACEUTICAL FORM

Oral suspension

Yellow suspension

CLINICAL PARTICULARS

Target species

Dogs

Indications for use, specifying the target species

To reduce the risk of developing an active infection and clinical disease in case of contact with *Leishmania infantum*, through the enhancement of the cell-mediated immune response.

The efficacy of the product has been demonstrated in dogs under multiple natural parasite exposure in zones with high infection pressure.

Control of clinical progression of canine leishmaniosis at early stages of the disease (dogs with low to moderate positive antibody levels and mild clinical signs such as peripheral lymphadenopathy or papular dermatitis).

Contraindications

Do not use whenever stimulation of gastric motility might be dangerous eg. In the presence of gastrointestinal haemorrhage, mechanical obstruction or perforation.
Do not use in animals with a known hypersensitivity to domperidone or any of the excipients.
Do not use in animals with prolactin-secreting pituitary tumor.
Domperidone is metabolized by the liver, therefore it should not be administered to animals with liver failure.

Special warnings

In case of severe infections, adequate aetiological treatment should be established in order to lower the parasitic load prior to consider a treatment with this veterinary medicinal product. In all cases, and taking into account the highly variable evolution of the disease, close patient follow up is recommended in order to adapt the treatment to the clinical stage of the animal, as required.

Special precautions for use

Special precautions for use in animals

Administration of this veterinary medicinal product produces a transitory increase in plasma prolactin and could induce endocrine disturbances such as galactorrhoea. Therefore it should be used with caution in animals with previous episodes of pseudopregnancy.

Special precautions to be taken by the person administering the veterinary medicinal product to animals

People with known hypersensitivity to domperidone or any of the excipients should avoid contact with the veterinary medicinal product.
In case of accidental ingestion, seek medical advice and show the package leaflet or the label to the physician.
If you develop symptoms following exposure such as skin rash, you should seek medical advice and show this warning to the physician. Swelling of the face, lips or eyes, or difficulty with breathing are more serious symptoms and require urgent medical attention.

Do not smoke, eat or drink while handling the product.

Adverse reactions (frequency and seriousness)

At the dosages and duration recommended, this veterinary medicinal product is very well tolerated.

In clinical trials rare cases of galactorrhoea during treatment with Leisguard were reported. This is considered a consequence of the prolactine peaks induced by domperidone, which disappear after treatment discontinuation.

Use during pregnancy, lactation or lay

Pregnancy - Reproduction studies were performed in laboratory animals with no evidence of drug related teratogenic or embryotoxic effects. Signs of maternal toxicity were not seen in laboratory animals at doses 20 times higher than the recommended dose. However, there are no adequate and well controlled studies in pregnant bitches; therefore this drug should be used during pregnancy only in accordance with the benefit/risk assessment by the responsible veterinarian.

Lactation - Administration of domperidone to lactating females of several species has been shown to induce an increase of milk production. Administration of Leisguard to lactating bitches is likely to induce the same effect.

Interaction with other medicinal products and other forms of interaction

Cabergoline is a dopamine agonist that inhibits prolactin release from the pituitary gland. Therefore, its effects are antagonistic to those of domperidone.

Do not administer with stomach antacids such as omeprazole, cimetidine, or antacids

Domperidone should not be used with dopaminergic drugs such as dopamine or dobutamine

Amounts to be administered and administration route

0.5 mg/kg/d, equivalent to 1 ml/10 kg of Leisguard, once daily, during 4 consecutive weeks.

Leisguard may be administered directly into the mouth or mixed with food. To ensure a correct dosage, body weight should be determined as accurately as possible

Shake well before use

There are several schedules of dosing:

A) for reducing the risk of developing an active infection and clinical disease in case of contact with Leishmania infantum,

In seronegative animals that have never showed any sign of *Leishmania spp.* infection, but live or travel to an endemic area, domperidone treatments should be programmed, taking into account the temporary prevalence of leishmaniosis vectors *(Phlebotomus spp.)* in the geographic area of the patient location or destination.

In high prevalence areas or in climates with a long infective season, one treatment every four months should be administered. In the Mediterranean area, it would be advised to treat in June, October and February.

In low prevalence areas, one treatment period at the beginning of the infective season and another treatment shortly after the end may suffice.

In all cases, the treatment strategy must be established by the attending veterinarian in accordance with the local incidence of the disease and temporary presence of the infective vectors.

B) For the Control of clinical progression of canine leishmaniosis at early stages of the disease

The treatment should be started immediately after diagnosis in order to help animals to self-limit the disease.

Treatment with Leisguard may be repeated as needed, in accordance with the clinical and serological follow up performed by the attending veterinarian.

Overdose (symptoms, emergency procedures, antidotes), if necessary

In tolerance trials performed in dogs, this veterinary medicinal product has been administered at five times the recommended doses during periods up to one year with no noticeable adverse events.

Withdrawal period(s)

Not applicable.

PHARMACOLOGICAL PROPERTIES

Pharmacotherapeutic group: Propulsives

ATCvet Code: A03FA03

Pharmacodynamic properties

Domperidone is a dopamine antagonist that promotes the release of prolactin from the pituitary gland. Its repeated daily administration results in daily regular acute and reversible peaks in prolactin blood levels with stimulatory effects on the cellular immune system, leading to activation of phagocytic leukocytes and as a result, to an intracellular microorganism (*Leishmania spp.*) reduction, at "in vitro" conditions. Domperidone also has anti-emetic and gastrokinetic properties due to its antagonism of dopamine receptors.

Pharmacokinetic particulars

Absorption

In fasting dogs, domperidone is rapidly absorbed reaching peak plasma concentrations (Cmax) of 16.6 ng/mL at 2 hours after oral administration. Oral absolute bioavailability of domperidone is low (24%) due to an extensive first-pass metabolism in the gut wall and liver. Domperidone's bioavailability is not affected when taken with food.

In studies performed in dogs at oral dosages between 2.5 and 40 mg/kg domperidone does not accumulate or induce its own metabolism. Domperidone is 91-93% bound to plasma proteins.

Distribution

Distribution studies with radiolabelled drug in animals have shown wide tissue distribution, although it does not readily cross the blood-brain barrier. Small amounts of drug cross the placenta in rats.

Metabolism

Domperidone undergoes rapid and extensive hepatic metabolism by hydroxylation and N-dealkylation. Aromatic hydroxylation of domperidone yields (hydroxy-domperidone) which is the main metabolite found in faeces. N-dealkylated metabolites and their

conjugates can be detected in urine. None of the identified metabolites has any pharmacological activity.

Excretion

Elimination half-life (T1/2) is of 3.2 h. The distribution volume (Vd) of 3.3 L/kg, and plasma clearance (Cl) of 0.73 L/h/kg. The proportion of the drug excreted unchanged is small (15% of faecal excretion and approximately 2% of urinary excretion). The amount excreted in faeces or urine corresponds to 60% and 28% of the oral dose respectively. Very small amounts may be found in milk.

PHARMACEUTICAL PARTICULARS

List of excipients

Sorbitol liquid (non crystallising)
microcrystalline cellulose and carmellose sodium,
methyl parahydroxybenzoate
propyl parahydroxybenzoate
saccharin sodium
polysorbate 20
quinoline yellow
fruitmix flavouring
sodium hidroxide
purified water

Incompatibilities

In the absence of compatibility studies, this veterinary medicinal product must not be mixed with other veterinary medicinal products.

Shelf life

Shelf-life of the veterinary medicinal product as packaged for sale: 30 months
Shelf-life after first opening the immediate packaging: 8 months

Special precautions for storage

Store in the original package.

Protect from light.

Nature and composition of immediate packaging

A 60 ml high-density polyethylene (HDPE) bottle closed with a low density polyethylene (LDPE) adapter and a HDPE child-proof screw-cap.

Carton box with one bottle and two syringes (LDPE barrel, polystyrene (PS) plunger and LDPE piston), one graduated up to 3 ml and the other graduated up to 5 ml.

Special precautions for the disposal of unused veterinary medicinal products or waste materials derived from the use of such products.

Any unused veterinary medicinal product or waste materials derived from such veterinary medicinal products should be disposed of in accordance with local requirements.

MARKETING AUTHORISATION HOLDER

Laboratorios Dr. ESTEVE, S.A.
Avda. Mare de Déu de Montserrat, 221
08041 – Barcelona (Spain)

VIII. REFERENCIAS BIBLIOGRÁFICAS

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