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CANINE VISCERAL LEISHMANIASIS AND AVAILABLE TREATMENTS - LITERATURE REVIEW

LEISHMANIOSE VISCERAL CANINA E OS TRATAMENTOS DISPONÍVEIS – REVISÃO DE LITERATURA

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RESUMO

A Leishamaniose Visceral Canina é umas das doenças zoonóticas causada por protozoários de maior ocorrência e preocupação mundial, o *Leishmania spp.*, que é transmitido através da picada do vetor flebótomo *Lutzomya longipalpis* fêmea. O cão é o mamífero mais acometido pela enfermidade no meio urbano, tornando-o o reservatório de maior importância para a manutenção da doença. Os métodos preventivos aplicados e existentes hoje no Brasil se baseiam no controle da proliferação do vetor e do seu contato com animais, utilizando coleiras e *pour-on* inseticidas e/ ou repelentes, evitando ou diminuindo o repasto em outros animais e humanos. O tratamento dos animais soropositivos com medicamentos de uso humano é proibido pela Portaria Interministerial Nº 1,426 de 2008. Entretanto em 2016 por meio da Nota Técnica Conjunta n° 001/2016 MAPA/Ministério da Saúde autoriza o registro e uso do produto MILTEFORAN® para tratamento de animais com Leishmaniose Visceral. Deste modo, o objetivo da confecção desse artigo é levantar os principais medicamentos e protocolos utilizados no tratamento da leishmaniose canina, levando em consideração que estes não proporcionarão a cura, mas para dar melhor qualidade e tempo de vida ao animal portador da doença, assim como, ser uma das medidas preventivas à saúde pública.

Palavras-Chave: cães, fármacos, leishmania, leishamanicida, leishmaniostáticos.

ABSTRACT

Canine Visceral Leishmaniasis is one of the zoonotic diseases caused by the most common and worrying protozoa in the world, *Leishmania spp.,* transmitted through the bite of the female phlebotomine sand fly vector *Lutzomya longipalpis*. In the urban environment, dogs are the mammals most affected by the disease, making them the most important reservoir for the maintenance of the disease. The preventive methods applied and currently existing in Brazil consist of controlling the proliferation of the vector and its contact with animals, using collars and *pour-on* insecticide and/or repellent, avoiding or reducing repast in other animals and humans. The treatment of seropositive animals with medicines for human use is prohibited by the Interministerial Ordinance Nº 1,426 of 2008. However, in 2016, by means of the Joint Technical Note Nº 001/2016 MAPA/Ministry of Health of Brazil authorizes the registration and use of

the MILTEFORAN® product for the treatment of animals with Visceral Leishmaniasis. Thus, the purpose of this article is to survey the main drugs and protocols used in the treatment of canine leishmaniasis, taking into consideration that these will not provide a cure, but can give a better quality and life span to the animal carrying the disease, as well as to serve as one of the preventive measures for public health.

Keywords: dogs, drugs, leishmania, leishamanicide, leishmaniostatics

INTRODUCTION

Leishmaniasis is an anthropozoonotic disease of worldwide importance, and this dates back to its discovery since the beginning of the 20th century by William Leishman and Charles Donovan, who found the protozoan in an Indian soldier. The evolution of the identification of this agent and its connection with the kala-azar disease happened in 1903 by Ross, who thus created the genus Leishmania and named it *Leishmania donovani* (MOURA, 2017; VARGAS & PINTO, 2017).

Rogers' cultivation in 1904 and Patton's observations in 1907 added important information for the next steps that would unravel the causes of the disease elsewhere in the world. Not to mention that, in 1885, in Bahia, Cerqueira had already raised the hypothesis that phlebotomine sand flies were the vectors of such disease. Other species of the genus continued to be reported, in 1908 *L. infantum* by Charles Nicole and later, in 1937, *L. chagasi*, by Chagas and Cunha, being the agent of visceral Leishmaniasis in America. The latter were afterwards identified as being the same species in molecular research and considered from this moment as synonymous (MAURÍCIO et al., 2000; MORAES, 2016).

The extent of the disease's involvement in the world is summarized as being endemic, in Europe, Asia, Africa and the Americas (VIOL, 2014). In Brazil, the leishmaniasis was concentrated in the North, Northeast, Center-West and Southeast regions. However, as of 2009, the South region was included with the involvement of indigenous human cases in the city of São Borja. After this, it was considered endemic throughout the national territory (PAYANO, 2018).

The predominance of cases reported both in Brazil and in other countries has a direct relation to the geographical, climatic, economic and cultural characteristics of the population. In addition, other influential fact is the urbanization of regions on the periphery of cities, which leads to the emergence of true epidemics in medium and large cities, even if well structured. To better illustrate this situation, in 2010, the Northeast had 47.1% of the cases, followed by the North (18.0%), Southeast (17.8%), Midwest (8.6%), and South (0.1 %) (MOURA, 2017).

Leishmaniasis is a disease caused by the mandatory intracellular protozoan. Its vector is the female *Lutzomya longipalpis* phlebotomine sand fly, an invertebrate host necessary for one part of its cycle, and the dog, a vertebrate host of great importance in the spread of the disease, as it has a greater susceptibility than other mammals, in addition to being the closest to the human population (MORAES, 2016).

The disease in dogs can often go unnoticed and/or asymptomatic and in humans, it can be confused with other diseases. Symptoms are nonspecific, as they depend on the host's immune response and the location of the deposition of immune complexes, which may be cutaneous or visceral.

The control of this disease is the greatest challenge of public health policies, both human and veterinary. Although there is a treatment for humans recently also authorized for dogs, the control of the phlebotomine sand flies is of outstanding importance, but of great difficulty, as it is a very resistant vector to repellent insecticides on the market, and adaptable to different environments, both rural and urban. To decrease the contact incidence of *Lutzomya sp.* with

vertebrate hosts, the use of repellents at homes and animal shelters is indicated, mainly during the hours of greatest activity of the vector, such as the twilight and the early evening. In addition, it is recommended the use of repellents in humans and animals exposed and with a positive diagnosis.

The existing treatment for humans has a satisfactory response. Although, according to the Interministerial Ordinance Nº. 1,426 of 2008, this treatment is not indicated nor authorized in dogs, in order to avoid possible resistance of the parasite to these drugs. Seropositive dogs that presented the disease until October 2016, when Technical Note Nº. 11/2016 authorized the marketing and use of the drug Milteforan® in these animals, had a euthanasia indication by DECREE Nº. 51,838 of 1963, resulting in a reduced number of animals susceptible to leishmaniasis transmission. However, this measure has always raised several discussions between veterinarians, tutors, and animal rights activists, with the justification that this is not the best and most effective measure to control the disease. Thus, the treatment was performed irregularly by veterinarians who were at risk of losing their records if they were reported, as they used products for human use and prohibited by the ordinance (VARGAS, 2017), and under the responsibility of the tutors by signing a term of commitment.

Given this information and the importance of this disease for the community, public and veterinary health, this project aims to detail the main drugs and protocols used for the treatment of dogs with leishmaniasis in a bibliographic review of the last years on this topic, whether authorized or not, according to the veterinarian's assessment, the animal's health status, and the tutor's financial availability.

LITERATURE REVIEW

AGENT

Leishmaniasis is an infectious-parasitic disease whose etiologic agent is the protozoan of the genus *Leishmania* spp., of the *Trypanosomatidae* family, order *Kinetoplastida* (MORAES, 2016). It includes single-celled digenetic species (heteroxenous), which gives it a wide antigenic variety. Within this group, the three most responsible for the Visceral Leishmaniasis infection are the *Leishmania donovani* in Asia and Africa, *Leishmania infantum* in Asia, Europe and Africa, and *Leishmania chagasi* in the Americas (MAIA, 2013). The order *Kinetoplastida* comprises organisms from a single mitochondria (kinetoplast) rich in mitochondrial DNA (KDNA) (SANTOS, 2007; MAIA, 2013; MORAES, 2016).

This parasite has two forms, a flagellate, the promastigote, which contains a round or oval nucleus, and the rod-shaped kinetoplast, with a long free flagellum emerging from its anterior part, found in the midgut of the female invertebrate vector. The other form, amastigote, does not have a flagellum, it is immobile and obligatory intracellular. It is spherical or spindle-shaped, with a large, rounded core, kinetoplast in the shape of a small rod and vacuoles (SANTOS, 2007; MAIA, 2013; MORAES, 2016).

INFECTIVE CYCLE

The spread of leishmaniasis and its development involve two essential hosts for the parasite's biological cycle to be complete, the invertebrate, female sandfly *Lutzomya longipalpis*, and the vertebrate, mainly mammals, such as canids and humans. The cycle can begin when the female phlebotomine sand fly, while ingesting blood by stinging a vertebrate host for the maturation of its eggs, ingests the amastigote form inside the cells of the monocytic phagocytic system found in the dermis of the infected host. These, in its intestine, will turn into pro-cyclical promastigote forms and later to the metacyclic form that will be present in the insect's proboscis (MORAES, 2016; MOURA, 2017; VIOL, et. al. 2014).

When the vector directly feeds on other animals, it inoculates the parasite in its metacyclic promastigote form in the vertebrate host, which is phagocytized by the cells of the peripheral mononuclear phagocytic system, mediated by receptors on the surface of the macrophage. Inside the parasitophorous vacuole of these cells, the promastigote returns to its amastigote form. Due to the resistance to the phagolysosome, it initiates the binary division within it until it breaks and releases several amastigote forms that will infect other cells, such as neutrophils and leukocytes, which will be phagocytosed again, then spreading through the lymphatic and hematological pathways to other organs such as the spleen, bone marrow, and liver. Thus, it occurs the chronic form of the disease, making the host liable to transmit the disease when bitten by the vector again, continuing the cycle (MAIA, 2013; PAYANO, 2018; SILVA, 2016; VIOL, 2014).

VECTOR, HOSTS, AND RESERVOIRS

The *leishmania* spp, as a heteroxenous parasite, needs more than one host to complete its biological cycle. It belongs to the order diptera, family *Psychodidae*, subfamily *Phlebotomine sand fliesinae*, of the species *Lutzomyia longipalpis*, and it is present in the five regions of Brazil. *L. cruzi* isspecific to the state of Mato Grosso do Sul. It is popularly known as sandflies and commonly as 'drain fly' and 'birigui', which is also the vector (MAIA, 2013; MOURA, 2017).

According to FORATTINI (1960), sandflies are dipterous, psychodidic insects, small, hairy, thin, and differ from other dipterans mainly for developing their entire larval stage in organic matter contained in the soil and not in the water. As adults, they have sexual dimorphism, feed on sap to maintain homeostasis, but females need a blood diet for ovarian maturation, and thus continue with oviposition and maintenance of the life cycle (MORAES, 2016).

These characteristics for its development, previously found in wild and rural environments, have advanced to urban regions, mainly peripheries of large centers, which lack basic conditions of hygiene and sanitation. In addition to the accumulation of garbage and organic matter present in chicken coops, pig pens, kennels, storerooms, and household items, this situation is aggravated in the rainy season and after, as the soil remains moist, which results in the pupae hatching. (MORAES, 2016).

Other important hosts for the evolution of *Leishmania spp*. are the vertebrates, in general wild mammals, such as foxes, marsupials, rodents, armadillos and sloths (MAIA). In a domestic environment, dogs (family kennels) are the main susceptible animals, as well as hosts and reservoirs, as they have high parasitism on the skin, which is also the place where the symptoms of *Leishmania spp.* appear the most. This situation facilitates the transmission of the protozoan to the phlebotomine sand fly when it feeds of other animals, including humans, whose role is of an accidental host (MOURA, 2017). According to PIRAJÁ (2013), the role of the cat is being questioned as a reservoir in urban centers with high rates of disease incidence in dogs and humans (MAIA, 2013).

PATHOGENY

The presence of the parasite in the vertebrate organism triggers an inadequate response of the immune system, which can be positive by eliminating it or contrary to the organism itself, promoting immune-mediated changes in all systems and organs. These can be of the cell type, with THelper (T) cell performance, or humoral, with the production of antibodies and B-lymphocytes (PAYANO, 2018; PINHÃO, 2009).

The presence of T cells is decisive in the evolution of the disease since they help macrophages to control the infection. They induce the production of interferon-γ (IFN-γ) and TNF-α by Th1 CD4 + and CD8 + cells, activating the macrophages that will phagocytize the

parasites, eliminating them. Another important factor is the production of interleukin (IL)-2, and the tumor necrotizing factor-α and IL-12 that act as protectors against the disease (PINHÃO, 2009).

Th2 cells, on the other hand, induce the proliferation of B cells and the production of IL-4, IL-6 and IL-10, which are characteristic of the humoral response, resulting in plasmacytosis and increased production of immunoglobulins, mainly of the IgG class and in particular the subclasses IgG1, IgG2. These changes are responsible for the symptoms of the disease in the animal, for acting with the parasite antigens, promoting the production of immune complexes that accumulate in various organs, such as the spleen, liver and lymph nodes (MOURA, 2017; MAIA, 2013; SILVA, 2013). However, the pathological changes are not limited to the deposition of immune complexes, but also to the direct action of the parasite, which induces a non-suppurative inflammatory response in its location.

In this way, it is possible to assume that animals with a more exacerbated cellular response do not show clinical signs, but remain reservoirs. Animals with a more active humoral response, on the other hand, are animals that may present subclinical, self-limiting, or severe disease (MORAES, 2016; SANTANA, 2017). Dogs usually have a humoral response, and the disease may be unapparent for long periods, due to the incubation period from months to years, and it is not uncommon for animals that show signs of the disease to be diagnosed more than a year later, which is why the dog is the most important reservoir for public health (PINHÃO, 2009). According to CORRÊA et.al. (2007), in symptomatic dogs, there is a proliferation of the parasite in macrophages, due to a reduction in the number of CD4 + T lymphocytes, which suggests the absence of an effective immune response to eliminate the parasite (MOURA, 2017).

CLINICAL AND PATHOLOGICAL FINDINGS

The leishmaniasis has a systemic character, presenting a diversity of signs and changes depending on the animal's immune response, the location of the action, interaction, and deposition of the immune complexes and the injured tissues.

According to SHERDING (2006), MAIA (2013), and SILVA (2016), in the blood count, the changes found are thrombocytopenia, normochromic normocytic anemia, leukopenia associated with lymphopenia, leukocytosis with left shift and positive Coombs test. The biochemical examination may show increased levels of urea and alanine aminotransferase (ALT), normal or increased creatine, and low albumin concentration, with possibility of hyperproteinemia occurrence. In addition, it may show conformational and size changes in target organs due to deposition of immunocomplexes such as hepatosplenomegaly, splenomegaly, lymphadenomegaly, and hyperkeratosis, with the skin being one of the tissues most affected initially (NISHIDA and DELMASCHIO, 2017). Histologically, depositions of collagen fibers were observed, characterizing systemic fibrosis associated with tissue parasitism and degenerative and inflammatory processes (MORAES, 2016).

CLINICAL SIGNS

Due to the changes already mentioned, the clinical signs depend on the affected system, and these do not help in the diagnosis because they are nonspecific and can be confused with those of other diseases. Initially, the first signs are detected on the skin, as it is the location of the bite and deposition of the parasites, progressing to the viscera and returning to infect the skin (MORAES, 2016).

On the skin and appendages, about 56% to 90% of cases are noted, such as poor hair quality, furfuraceous desquamation, ulcers in the decubitus areas and at the tip of the ear, which are rarely pruriginous, hyperkeratosis of the nasal plane and plantar cushions, onychogryphosis (exaggerated nail growth), as well as nails fragility, making them brittle. Head alopecia, with

typical glasses around the eyes, ears, trunk, and limbs. Sterile pustular dermatitis and mucocutaneous nodular dermatitis are also noted. The signals appear according to the density of infected cells present, which is decisive in the transmissibility of these cells when the vector feeds directly on the infected animal (PAYANO, 2018; PINHÃO, 2009).

Ocular lesions such as mononuclear-plasmacytic inflammation of the uveal tract, diffuse or nodular conjunctivitis, nodular or suppurative blepharitis, diffuse or nodular scleritis, keratoconjunctivitis, glaucoma, panophthalmos, anterior or posterior uveitis, being granulomatous or diffuse, exophthalmos and discharge are the most common, affecting about 16% to 80% of dogs (PAYANO, 2018; SANTANA, 2017).

Concomitant to the signs described, the animal may show progressive weight loss, loss of appetite, anemia, anorexia, lethargy, and weakness, symptoms that may be due to parasitic competition for nutrients and kidney damage (PINHÃO, 2008). According to PINHÃO (2009), muscle atrophy and disturbances in the locomotor system may occur due to polio, and polyarthritis due to the deposition of immune complexes. There are more severe cases, such as osteolysis, mainly in the long bones, with the animals presenting pain on palpation and flexion/extension of the limbs.

Renal lesions are the main causes of death in animals with leishmaniasis and consequences of the deposition of immune complexes and LTCD4 + in the glomeruli resulting in membranoproliferative glomerulonephritis and interstitial nephritis. The insufficiency resulting from these changes leads to the appearance of symptoms such as vomiting, polydipsia, and, polyuria (PINHÃO, 2009). According to MARQUES (2008), the presence of proteins in the urine (proteinuria) can lead to the appearance of symptoms compatible with nephrotic syndrome, such as hypoalbuminemia, ascites, peripheral edema, and hypercholesterolemia (SANTANA, 2016).

Coagulation disorders due to vasculitis and thrombocytopenia, and platelet dysfunction due to uremia may occur, with the animal unilateral epistaxis, which may also be due to nasal ulcers (PINHÃ, 2009). Other typical findings, according to Ciaramella & Corona (2003), indicates that 89% of the affected dogs have lymphadenomegaly, which can be restricted to only one of the lymph nodes or be generalized. Splenomegaly occurs in 54% of the cases (PINHÃO, 2009).

It is important to note that the clinical manifestations depend on the density of parasites present on the skin, lymph nodes, spleen, and bone marrow, as well as on the animal's immune response.

DIAGNOSIS

The importance of the correct and rapid diagnosis of animals for leishmaniasis does not apply only to those with clinical signs and those suspect of having the disease, but also to clinically healthy dogs from endemic areas. The tests used to detect the agent in the animal's organism can be serological, parasitological, and molecular (PAYANO, 2018).

In Brazil, the Ministry of Health indicates the combination of two serological tests to confirm the disease in the animal, in addition to the clinical observations previously carried out. This is, the Enzyme Immunoadsorption Assay (ELISA), and Indirect Immunofluorescence Reaction (IFA). Since March 2012, a new rapid diagnosis method was introduced, offered by the state through Biomanguinhos/Fiocruz, the Dual Path Platform (DPP®) based on immunochromatography, also used in a seroprevalence study in canine sample and census surveys. In cases of suspected animals with positive serology in non-endemic regions, direct parasitological research using Fine-Needle Aspiration Cytology (FNAC) is recommended for confirmation (MAIA, 2013; MORAES, 2016).

Even with different means of diagnosis, there are factors that can mask the results, both for false positives and for false negatives, such as in the methods of indirect

immunofluorescence assay (IFA) and enzyme-linked immunosorbent assay (ELISA). Among these factors, are hemoparasitic diseases such as *Erlichia canis* and *Trypanosoma cruzi* (*T. cruzi*), low or no humoral response, and their respective seroconversion and opportunistic infections due to immunosuppression resulting from the disease (MORAES, 2016; PINHÃO, 2009). For confirmation, in addition to using parasitological research, it is possible to use molecular DNA research by the polymerase chain reaction (PCR) of the parasite in the animal's organism.

Other serological tests can be used, such as complement fixation test (CFT), direct agglutination test (DAT), and immunoelectrophoresis (PAYANO, 2018), in addition to plate culture with specific medium and immunodiffusion (PINHÃO, 2009).

TREATMENT

According to the Technical Norms to Combat Leishmaniasis established in DECREE Nº. 51.838 / 1963, seropositive animals that present the disease must be euthanized, without cruelty, decreasing the reservoirs of the disease, reducing transmission by the phlebotomine sand flies to other healthy animals and for humans. However, this decree aimed, mainly, the rural population, which at that time was the most affected by the disease. With the advance of cities towards rural proximity and their disorderly growth, without proper urbanization and sanitation, leishmaniasis has become an urban problem that affects not only the periphery but also the large urban centers of practically all Brazilian cities. Therefore, the control measures adopted for the rural environment started to be applied to urban domestic animals. However, the proximity of these animals to their tutors and the status of belonging to the family initiated several controversial surveys regarding the control measure adopted until then for seropositive animals, the euthanasia. With that, veterinarians also opposed to euthanasia and began to treat animals with the medications and protocols used in humans with leishmaniasis since until that moment there was no prohibition for this procedure.

With the increase of uncontrolled treatments in dogs, in 2008, with INTERMINISTERIAL ORDINANCE Nº. 1,426, the Ministry of Health of Brazil prohibited the treatment of seropositive animals with medicines for use in human treatment or products not registered by the Ministry of Agriculture, Livestock and Food Supply (MAPA), for this purpose in animals. This measure was adopted after the Pan American Health Organization (PAHO) World Health Organization (WHO) on Visceral Leishmaniasis in Las Américas reports, from November 23th to 25th, 2005, and of the Ministry of Health of Brazil, in 2007. The reports corroborate the ineffectiveness of canine treatment for curing the disease, decrease transmissibility and, mainly, with the risk of this use inducing the resistance of the agent to drugs in treatment in humans, keeping, in this way, infected animals as reservoirs in the urban environment, increasing the chances of contamination of other animals and humans (NISHIDA and DELMASCHIO, 2017).

Even with this situation, the animals continued to be treated with various drugs and protocols to reduce the parasitic load and clinical signs. The treatments aimed improving the living conditions of these animals and increasing their life expectancy, since a cure is not possible due to the location of the parasite being intracellular and in some less vascularized tissues, such as skin, vitreous humor and keratinized tissues, which makes it difficult for drugs to pass through these structures and barriers. Thus, the exact dosages to achieve a favorable result are variable according to the state of the animal and the type of disease affecting the animal (PINHÃO, 2009). This attitude of tutors and veterinarians, if discovered, is liable to civil penalties and professional ethics in accordance with art. 268 of the Penal Code, Law Nº. 6.437 / 1977, Decree-Law Nº. 467/1969, and the Code of Medical Ethics, respectively (BRASIL, 2008).

The treatment for humans in Brazil has as first option the Pentavalent Antimonial drug,

Glucantime® (N-methylglucamine antimoniate), acting on the bioenergetic mechanism of the amastigote form of *Leishmania spp*. through glycolysis and beta-oxidation, which occur in organelles called glycosome. The second option for cases that are refractory to antimonial is Amphotericin B, or its liposomal version, which is less toxic, used in pregnant women and immunosuppressed individuals, as HIV carriers. It acts in both amastigotes and promastigotes. Other drugs used in combination are paromomycin or aminosidine, imidazoles, purines (allopurinol), imiquimod (immunomodulatory agent), sitamaquine, and anticancer medicine (MORAES, 2016).

In Europe, the use of Miltefosine (hexadecilfosfocoline, HepC), the Milteforan® in animals with leishmaniasis occurs since 2007. However, the commercialization of this drug in Brazil was not approved by MAPA, making it inaccessible. Despite that, through Joint Technical Note Nº. 001/2016, signed by MAPA and the Ministry of Health of Brazil, the registration of the product Milteforan® (Virbac Animal Health) was authorized for the treatment of visceral leishmaniasis in dogs in Brazil since it is not used in the treatment of humans (MOURA, 2017).

The use of this medication must follow the manufacturer's protocols and instructions for use, as well as be accompanied by periodic clinical reevaluation by the veterinarian responsible for the treatment, to monitor the parasitic infestation in the animal so that the appropriate measures are taken as soon as possible, as a new treatment cycle. In addition to the medication, the veterinarian must instruct the tutor on prophylactic measures to avoid contact between the phlebotomine sand fly vector in the infected animal and other animals and humans, avoiding the reinfection and proliferation of the disease. Another very important piece of information is that this authorization is not recognized as a measure of the sanitary control of the disease, but rather an individual option of the guardian and of the veterinarian in treating dogs to give them survival with health quality (BRASIL, 2016).

Still in 2016, the Ministry of Health of Brazil established a Working Group within its scope, by Ordinance Nº. 2,684, consisting of representatives of the Health Surveillance, Federal Veterinary Medical Council, Animal Rights Activists, Tropical Medicine Health, among others, to develop studies with the purpose of reviewing the guidelines for surveillance and management of visceral leishmaniasis reservoirs. Thus, in 2017, MAPA published the Normative Instruction Nº. 35, which establishes the rules and procedures that must be adopted by veterinarians regarding the use and prescription of special control substances for veterinary use, including in this document the Miltefosine.

Therefore, in order to use and prescribe the treatment protocol in animals with leishmaniasis, the veterinarian must acquire the drug through the notification of acquisition, containing the mandatory and necessary information, described in the ordinance. The responsible veterinarians must register themselves in the sector responsible for the inspection service of veterinary products in the Federal Superintendence of Agriculture in the State where they work. It is necessary to keep a report and record of stock control and use of the medication in a book for this purpose, present at the establishment, for inspection purposes. In case the veterinarian does not comply with the provisions of this ordinance, they may be assessed and penalized (BRASIL, 2017).

In Brazil, Milteforan® is an authorized medication for the treatment of dogs with leishmaniasis. Despite that, the main drugs used will be presented below, whether authorized or not, according to the staging carried out by the veterinarian. This list takes into account the state of the animal's health and commitment of the tutor to continue the protocol until the goals are reached, such as reducing parasitic load, avoiding contamination of the phlebotomine sand flies, and the clinical improvement of the animal. It is also known that in most cases, it is a long-term treatment, expensive, and with chances of recurrence after the end of the treatment without the complete elimination of the parasite in the organism of the animal and that the

parasitic reassessment must be carried out periodically according to the guidance of the responsible veterinarian.

DRUGS

Allopurinol

This drug has the principle of inhibiting xanthine oxidase to reduce the concentration of urate in the blood serum. In the parasite, it is metabolized and converted to inosine, an inactive form capable of being incorporated into its DNA. Thus, hypoxanthine-guanine phosphoribosyltransferase (HGPRT) is inhibited, which interrupts the synthesis of RNA and prevents the synthesis of parasite protein, inhibiting its multiplication and leading to its death, being classified as leishmaniostatic (SCHIMMING, 2012; PAYANO, 2018).

The recommended dosage is 10 to 20mg/kg, twice a day, orally. Due to its low toxicity, it can be maintained for the entire life of the animal, to maintain a low parasitemia and avoid infection of the phlebotomine sand flies and, consequently, the spread of the parasite (SCHIMMIG, 2012; PAYANO, 2018; PINHÃO, 2009). This drug is also an ally to improve clinical conditions, normalizing hematological and biochemical parameters (resolution of anemia, increase in the albumin/globulin ratio, and restoration of CD4 + T cell levels) (SILVA, 2016).

The adverse effects may be fever, leukopenia, skin disorders, and elevations of low-intensity enzymes. The liver and kidney functions should also be regularly evaluated, since xanthinuria and xanthine urolith formation may occur, especially if there is concomitant liver disease (PINHÃO, 2009; SCHIMMIG, 2012).

Despite good results, allopurinol used alone cannot reduce the parasitic load to levels of remission, even with high rates of animals with low parasitemia on the skin. It should be used in conjunction with leishmaniostatic protocols and to maintain the treatment (MORAES, 2016; PNHÃO, 2009).

Amphotericin B

Amphotericin B has a fungicidal action due to the mechanism of joining with ergosterol in the fungal cell membrane, which results in osmotic imbalance, leading to cell death. In parasites, it also has a recognized action, such as in *Leishmania spp*, which has its membrane affected with the appearance of aqueous pores. These characteristics make it a leishmanicidal drug, significantly reducing clinical manifestations, parasitic load, and antibody levels (PAYANO, 2018; PINHÃO, 2009; SILVA, 2016).

Its adverse effects on animals occur mainly because of its nephrotoxicity, as it acts on the cells of the renal tubules, inducing renal vasoconstriction. For this reason, creatinine levels must be monitored throughout the treatment.

The indicated dosage is 0,5mg/kg, every three days, for three months. It should be administered slowly via intravenous, and phlebitis, glomerulonephritis, hyperthermia, anaphylaxis, and anorexia may occur during the treatment. (PINHÃO, 2009).

Liposomal Amphotericin B

A version created to reduce adverse effects and direct the drug to the site of infection. As it is incorporated into liposomes, it reacts less with cell wall cholesterol, decreasing its nephrotoxicity. It has an even higher cost than its precursor, but it is a good option for nephropathic dogs (PINHÃO, 2009).

The indicated dosage is 1 to 2.5 mg/kg/day, for 7 days.

Pentavalent antimonial

According to FAYET (2000), the two compounds - sodium stibogluconate and meglumine antimoniate - are leishmanicidal drugs for altering the glycolysis, β-oxidation of fatty acids, and fixing the carbon dioxide of the parasite, as well as inhibiting glycosomal enzymes phosphofructokinase and phosphoenolpyruvate carboxykinase, which leads to the parasite death (PINHÃO, 2009).

The protocol used is 100 mg intravenously or subcutaneously, every 24 hours, for 3 to 4 weeks. It has several side effects, such as vomiting, diarrhea, muscle pain and fibrosis, abscess formation, thrombophlebitis, and renal failure caused by the high rate of circulating immune complexes due to the death of the parasites. Thus, it is necessary to monitor renal functions during and after treatment (MAIA, 2013; PINHÃO, 2009).

In Brazil, this drug is for human use, being distributed exclusively by the Ministry of Health, which makes it unavailable for treatment in dogs, unlike what happens in Europe, where, in addition to being the drug of choice, it is distributed for veterinary use as Glucantime® Merial without interfering in the treatment in humans (SCHIMMING, 2012).

Domperidone

According to REGUERA et. al. (2016), this benzimidazole-based drug is used as an antiemetic in humans, in addition to being a galactagogue, promoting the production of prolactin, as a D2 receptor antagonist, from the pituitary gland that improves the innate TH1 response and subsequent release of INTy, IL 2, IL 12, and TNF (PAYANO, 2018).

It is widely used in the early stages of the disease, helping to reduce the risk of developing the disease, but without being completely eliminated. The dosage is 0.5mg/kg/day, for up to four weeks (PAYANO, 2018).

Marbofloxacin

Second generation fluoroquinolone antibiotic, also acts as an immunomodulator, as it favors the production of NO, IL 6 and TNF.

Its dosage is 2mg/kg/day, for 28 days. However, it has high rates of recidivism (PAYANO, 2018).

Metronidazole and Ketoconazole

Metronidazole and ketoconazole are antifungals of the imidazole family, acting on bacteria, protozoa, and anaerobic agents. These drugs act on protein metabolism, inhibiting the synthesis of ergosterol and nucleic acids by blocking the sterol 14–demethylase. In addition, their use intensify glycogenolysis, which decreases the parasite's glycogen reserves, resulting in its death (NERY et. al., 2017).

The indicated dosage is 10mg/kg/day, for 40 days for ketoconazole, and 20mg/kg/day, for 30 days for metronidazole (NERY et. al., 2017).

Miltefosine

Recently approved by Technical Note Nº. 11/2016 MAPA, the drug based on hexadecyl phosphocholine, belonging to the group of alkylphosphocholines, registered commercially as Milteforan® (Virbac Animal Health), is an antineoplastic medicine that, according to REGUERA et. al. (2016), has as principle observed *in vitro* and *in vivo* the alteration of the metabolism of fatty acids and sterol. It activates the mechanism similar to cell apoptosis in the kinetoplast and mitochondria dysfunction. It also promotes an increase in the Th1 response, which increases IFNy levels, resulting from the stimulation of NO production and reactive oxygen radicals within the macrophage vacuole, eliminating parasites (MOURA, 2017; PAYANO, 2018; PINHÃO, 2009).

The recommended dosage is 2mg/kg orally, which can be mixed in the ration for 28 days, continuing in plasma concentrations for up to 28 days after the end of treatment. As it is a chemotherapy, the owners, who must wear gloves, must properly store and handle it. For this same reason, it should not be administered to pregnant or lactating female dogs, due to the high degree of teratogenicity (PINHÃO, 2009). According to PINHÃO (2009), the adverse effects reported are gastrointestinal, such as vomiting, diarrhea, and inappetence.

Similarly to other medications used for the treatment of leishmaniasis, it does not completely eliminate the parasite from the animal's organism. It is indicated the concomitant use with other drugs to maintain the treatment (PAYANO, 2018).

Pentamidine

This drug is an antifungal that inhibits the transcription and replication of the parasite's DNA. Despite its effectiveness, it has adverse effects such as abscess formation at the injection site, pain, local edema, hypoglycemia, irreversible liver destruction, nephrotoxicity and hypotension during administration due to its high toxicity (PAYANO, 2018; PINHÃO, 2009).

According to PINHÃO (2009), the recommended dosage is 4 mg/kg, once or three times a week, for at least 6 weeks, or intraperitoneally, diluted 1/10.

Prednisone

According to NOGUEIRA (2007), this glucocorticoid drug acts as an immunosuppressant, reducing humoral immunity by decreasing the production and action of antibodies, reducing the deposition of immune complexes in the body, and consequently, its effects.

The dosage indicated for either prednisone or prednisolone is 0.5 to 2 mg/kg/day over a period of one week to one month, depending on the reduction in the dosage of this drug, which must be progressive to avoid the effects arising from acute adrenocortical insufficiency (NOGUEIRA, 2007).

Aminosidine sulfate

This drug is an aminoglycoside that acts by blocking ribosomal protein synthesis and altering the permeability of the parasite's cytoplasmic wall (PAYANO, 2018; PINHÃO, 2009). The indicated dosage is 10 to 20 mg/kg/day, intramuscularly, between 14 and 30 days, according to the animal response to the treatment. However, because it is highly nephrotoxic and ototoxic, its use should be interrupted after this period. It has a high rate of remission close to 100%, but recurrences after treatment interruption are between 50 and 100 days (PINHÃO, 2009).

TREATMENT PROTOCOLS

As described in the reviews on the main drugs used to treat *leishmania spp*, therapies do not guarantee a complete cure and there are considerable recurrence rates, especially when used alone. Given the above, the topics below exemplify some of the main protocols administered in Brazil. The choice of the best therapy depends on the clinical evaluation, disease staging, and conditions of the tutors to assist and dedicate themselves to it. The tutors' commitment is needed as the treatment must be followed until the end so that the animal does not suffer from recurrence of the disease to a greater degree, and to avoid the manifestation of resistance of the parasite to the drugs previously used.

I. Leishmaniostatics: Allopurinol; Immunomodulators - Marbofloxacin; Leishmanicidal such as Miltefosine (NISHIDA, 2017).

II. Antifungal: Ketoconazole associated with Antiprotozoan, Metronidazole and Leishmaniostatic - Allopurinol, (NERY, 2017).

III. Antifungal: Amphotericin B and liposomal Amphotericin B (PINHÃO, 2009).

IV. Antifungal: Amphotericin B associated with leishmaniostatic Allopurinol with Prednisone (NOGUEIRA, 2007).

V. Leishmanicidal: Miltefosine with Leishmaniostatic: Allopurinol (PINHÃO, 2009)

VI. Leishmanicidal: N-metil glucamine antimoniate with Leishmaniostatic (Allopurinol) (PINHÃO, 2009).

VII. Leishmanicidals: Miltefosine in combination with Meglumine Antimoniate and Allopurinol (SILVA, 2016).

In addition to these protocols, doses of therapeutic vaccines have been administered, helping to control clinical signs, preventing the evolution of the disease to polysymptoms and death (SILVA, 2009). In addition, it reduces the parasitic load on the animal's skin, thereby reducing the continuity of the transmission cycle through the phlebotomine sand flies' bite (SILVA, 2009).

PROPHYLAXIS

Given the importance, severity, and difficulties demonstrated on the diagnosis, treatment, and complications due to the infection, the best option to be taken by tutors, community, and government officials is to reduce the contagion of animals and humans, avoiding the contact of the phlebotomine sand flies both with sick and healthy individuals, minimizing the spread of the protozoan.

The control measures adopted by the Ministry of Health of Brazil, according to the 2006 Visceral Leishmaniasis Control and Surveillance Manual (MCVLV), aim to reduce lethality rates and degree of morbidity through early diagnosis and treatment of cases, as well as to decrease transmission risks by controlling the population of reservoirs and the disease agent (BRASIL, 2006).

The preventive measures are different depending on the area and are listed in the Manual (MCVLV. 2006):

I. **Individual protection measures for humans,** which encourages the use of fine mesh mosquito nets on doors and windows, repellents, and the non-exposition of people to the vector's activity hours (dusk and night) in environments where it can usually be found;

II. **Directed to the Vector,** through an environmental management by cleaning backyards, land, and public squares in order to change the conditions of the environment that favor the establishment of breeding sites of immature forms of the vector. Simple measures such as urban cleaning, elimination of moisture source, non-permanence of domestic animals inside the house, among others, will certainly help to prevent or reduce the proliferation of the vector;

III. **Control of the wandering canine population,** which is an important disseminator of several diseases, including *Leishmania spp*.;

IV. **Control of animals donation** previously tested for leishmaniasis and appropriate measures taken in negative and positive cases;

V. **Anti-Leishmaniasis vaccine.** In Brazil, there are vaccines registered with MAPA, but its use has not yet proven its effectiveness, which difficult the adoption of this measure by tutors due to its high cost;

VI. **Use of mosquito nets in individual or collective kennels,** to avoid contact of the phlebotomine sand flies with these animals, especially at times when it is most active, such as at dusk;

VII. **Dog collars impregnated with 4% Deltamethrin,** proven to repel the phlebotomine sand flies' contact with the animal, in addition to shortening the sand flies' life span, resulting in decreased protozoan propagation. Its use must be both in healthy animals and those affected by the disease;

VIII. **Elimination of the seropositive canine population,** a controversial measure for reasons of animal welfare, sentimental by their guardians, and the proven ineffectiveness in reducing cases of canine and human leishmaniasis, mainly in already endemic regions.

DISCUSSION

The worldwide concern to control the expansion of leishmaniasis in humans and animals every day promotes research on the best measures for the prevention and treatment of the disease. It is well known that the reduction of vectors' contact with susceptible individuals is of outstanding importance. However, the vectors' of this disease have been adapting to large urban centers, making it difficult to control, which unfortunately justifies the adoption of more drastic and not proven to be more efficient measures for the reduction of leishmaniasis cases. One of these is to reduce the presence of domestic reservoirs closer to man, which in this case is the

dog, by adopting the instructions contained in DECREE 51.838/1963, to euthanize seropositive animals for leishmaniasis based on test results in suspicious animals or coming from an endemic region.

The existing forms of prevention such as vaccines and repellent collars are not 100% effective and are little encouraged by the government, which raises their cost and opens a risk window for both animals and humans. This issue could be avoided if agreements were made between the government and the industries of these products to promote the greatest number of protection against vectors and immunization of animals. This would generate, according to ORLANDI (2011), the herd immunity effect, even in animals not collared and not immunized, reducing the force of infection by the barrier imposed by the collar and increasing antibodies against the parasite. Besides, it reduces the spraying of insecticides, which are harmful to the environment, in addition to representing much lower costs than those spent with the objectionable elimination of animal life (SCHIMMING, 2012).

Treatments with different drugs for the leishmaniasis are authorized in Brazil only for humans, so that in this way, the selection of strains resistant to them is avoided, which would hinder the effectiveness of the treatment of the human population. Even with the release and authorization of miltefosine, the only veterinary medicine available for the treatment of dogs, its high cost for being an imported product prevents its wide use by the population most affected by the disease, residents in peripheries with precarious sanitation where the vector is most prevalent and leishmaniasis endemic. The result of this difference in social settings implies a greater severity of the disease in the affected animals, decreasing the effectiveness of the treatment that may be carried out, which would lead to the euthanasia of animals to minimize and end their suffering.

However, if the animals that could be treated were in fact submitted to treatment, together with other forms of control and prevention, the chances of the phlebotomine sand flies ingesting the contaminating form of *Leishmania spp.* would decrease. The available protocols, despite not healing the animals, decrease the parasitic load on them, leaving their skin and attachments, parts of the body accessible to the vector, practically free from the parasite, thus interrupting the biological cycle. With the adoption of these measures at a national level, it would be possible to reduce the incidence of new cases of the disease and effectively promote its control.

In his study, SILVA (2016) demonstrates that the efficacy of euthanasia in dogs with leishmaniasis does not decrease the incidence of new cases of the disease. Also, the euthanasia is a measure in which no statistical difference was observed in the incidence of cases of human leishmaniasis, when compared an area where dog euthanasia was adopted and another area without the use of this measure.

According to the Brazilian Visceral Leishmaniasis Surveillance and Control Programme (VLSCP), epidemiological surveillance actions aimed at dogs include alerting veterinary services and the medical profession to the risk of transmission of visceral leishmaniasis. Besides, the VLSCP actions include making the population aware of the occurrence of the disease in the region, the clinical signs, and methods for diagnosis, as well as preventive measures to eliminate the likely breeding sites of the vector (BRASIL, 2010).

However, the disorderly growth of peripheral cities towards rural and wild areas, usually accompanied by basic sanitation and precarious garbage collection, and a lack of public awareness of the risks to which it is exposed increases the rates of spread of leishmaniasis. This demonstrates that the program is not practiced with the frequency, commitment, and seriousness that it should.

FERRER (2002) states that in endemic areas, many cases of leishmaniasis arise secondary to immunosuppressive factors, such as certain medications and other chronic diseases. This leads to an imbalance in the host's immunity, which then presents the clinical

symptoms of the disease (PINHÃO, 2009), factors that are noticed in regions such as those mentioned above.

Given the above, why then are the control and prevention measures the same, when it is more than proven that, instead of decreasing the spread of leishmaniasis, the opposite has been seen? Which laws and projects should be reviewed? And what real attention should be given to the least favored and most susceptible communities?

These questions are repeated every day by several animal protection entities, with the support of veterinarians, sympathizers of the cause, and members of groups that aim at unique health more broadly, understanding that the environment in which humans live is directly linked to their well-being and health. In this way, the animals inserted in it are also important for the maintenance of this state, and, if the forms of control entrusted to them do not have the proper effect, the same is likely to happen with humans living in the area.

Fortunately, in 2016, with the institution of the Working Group for the revision of the guidelines for the control of Leishmaniasis by Ordinance 2,684, it is expected that these questions will be considered as fundamental factors for new decision-making on the activities that must be performed by the bodies and professionals involved with the cause, respecting both human and animal health and safety.

FINAL CONSIDERATIONS

After the research carried out in this project on the etiology, pathogenesis, epidemiology, treatment, and forms of control and prevention of leishmaniasis, it was possible to see that much remains to be done about it. The forms of diagnosis must be more precise, avoiding false results, both positive and negative. Encouraging research to produce effective vaccines, without interfering with the results of tests that can be performed later, as well as that of drugs and safe protocols for treatment that guarantee the maintenance of the animal's clinical status and infective capacity, without the risk of interfering in the effectiveness of treatment in humans.

The government's role in endemic regions, with greater activity by health agents in poor communities, ensuring that they assist in the diagnosis and prevention of leishmaniasis has to be enhanced, as it is clear that in these places control programs are failing to reach their goals.

Finally, to entrust the veterinarian the autonomy and knowledge about the best measure to be taken when treating a *Leishmania spp.* case, always acting ethically and responsibly towards public and unique health.

REFERENCES

BRASIL. DECRETO Nº 51.838, DE 14 DE MARÇO DE 1963. Presidência da República Casa Civil Subchefia para Assuntos Jurídicos. Brasília, D.F., 14 de março de 1963; 142º da Independência e 75º da República.

BRASIL. Manual de Vigilância e Controle da Leishmaniose Visceral Brasília. Ministério da Saúde - Secretaria de Vigilância em Saúde Departamento de Vigilância Epidemiológica, 2006.

BRASIL. PORTARIA INTERMINISTERIAL Nº 1.426, DE 11 DE JULHO DE 2008. Ministério da saúde. Brasília. 2008.

BRASIL. NOTA TÉCNICA Nº 11/2016/CPV/DFIP/SDA/GM/MAPA. Coordenação de Fiscalização de Produtos Veterinário -DFIP-SDA – CPV - Ministério da Agricultura Pecuária e Abastecimento - MAPA - Brasília. 2016

BRASIL. PORTARIA Nº 2.684, DE 9 DE DEZEMBRO DE 2016. Ministério da Saúde. Brasília. 2016.

BRASIL. INSTRUÇÃO NORMATIVA Nº 35, DE 11 DE SETEMBRO DE 2017- Secretaria de Defesa Agropecuária - Ministério da Agricultura Pecuária e Abastecimento – MAPA. 2017

MAIA, L.S. Leishmaniose visceral canina: Aspectos clínicos e hematológicos de casos

suspeitos e confirmados atendidos no Hospital Veterinário da Universidade de Brasília em 2011, Brasília, 2013.
MORAES, A.R.D.P. de. "Avaliação da combinação da oxigenação hiperbárica com óleos de *Copaifera sp* e *Carapa guianensis* no tratamento da leishmaniose experimental" - Universidade Estadual de Campinas - Instituto de Biologia. Campinas, 2016.
MOURA, L.D. Proteínas recombinantes e seu uso no diagnóstico de leishmaniose visceral canina – Programa de Pós-Graduação em Ciência Animal (PPGCA), da Universidade Federal do Piauí – Teresina, 2017.
NERY, G., BECERRA, D.R.D., BORJA, L.S., MAGALHÃES-JUNIOR, J.T., SOUZA, B.M.P.S, FRANKE, C.R., VERAS, P.S.T., LARANJEIRA, D.F., BARROUIN-MELO, S.M. Avaliação da infectividade parasitária a Lutzomyia longipalpis por xenodiagnóstico em cães tratados para leishmaniose visceral naturalmente adquirida – 2017.
NISHIDA, L.H.G & DELMASCHIO, I.B. Leishmaniose Visceral Canina – Revisão de - Revista Científica de Medicina Veterinária-UNORP. 2017.
NOGUEIRA, F.S. "Avaliação Clínico-Laboratorial de Cães Naturalmente Infectados por Leishmaniose Visceral, Submetido à Terapia com Anfotericina B". Tese (doutorado) – Universidade Estadual Paulista, Faculdade de Medicina Veterinária e Zootecnia, Botucatu, 2007.
PAYANO, V.J.H. Avaliação dos achados Clínicos, Hematológicos e Bioquímicos Séricos em Cães naturalmente infectados por *Leishmania infantum,* submetidos a Tratamento Experimental – UNIVERSIDADE FEDERAL RURAL DE PERNAMBUCO – Programa de Pós-Graduação em Biociência animal. Recife. 2018.
PINHÃO, C.P.R. Leishmaniose Canina – Estudo de 158 Casos da Região de Lisboa – Universidade Técnica de Lisboa. Faculdade de Medicina Veterinária. Lisboa. 2009.
SANTANA, M.A. Estresse Oxidativo em Cães (*Canis familiaris)* Linnaeus, 1785, naturalmente infectados com *(Leishmania) infantum chagasi* (CUNHA E CHAGAS, 1937) SHAW, 2002, submetidos a Tratamentos Experimental. Recife. 2017.
SANTOS, L.R. Tese de Doutorado – Avaliação de Protocolos para Indução de Resposta Imune Contra Antígenos Recombinantes de - *Leishmania chagasi*. Salvador. Bahia. 2007.
SCHIMMING, B.C.Leishmaniose Visceral Canina – Revisão de Literatura. Revista Científica Eletrônica de Medicina Veterinária. Ano X. Nº 19. Periódico semestral. Julho de 2012
SILVA, K. R. Avaliação Hematológica, Bioquímica e Clínica do uso de Dietilditiocarbamato (DECT) no Tratamento In Vitro e In Vivo da Leishmaniose Visceral Canina Natural e Experimental. Salvador. Bahia. 2016.
VARGAS, E. & PINTO, L. – "Leishmania" Reflexos Sobre o Manejo do Mundo em Contexto de Risco Sanitário. Vivência – Revista de Antropologia. – Nº. 49.2017.
VIOL, M.A., AQUINO, M.C.C, NETO, L.S., OLIVEIRA, B.C.M., KANETO, C.N., ZUCATTO, A.S., BRESCIAN, K.D.S. Aspectos Epidemiológicos da Leishmaniose Visceral Canina no Brasil – Revisão de Literatura. UNIMAR CIÊNCIAS. 2014.