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EPIDEMIOLOGICAL INDICATORS AS PROPHYLAXIS TOOLS FOR CANINE VISCERAL LEISHMANIASIS IN BRAZIL

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Abstract: Canine Visceral Leishmaniasis (CVL) is a disease caused by the protozoan genus *Leishmania* and transmitted by the bite of the sandfly mosquito. It is considered a zoonosis and its control is based on the diversity of urban vector reservoir agents, where dogs are the main reservoirs of the parasite. This article aimed to investigate the prevalence and incidence of CVL in different regions of Brazil, based on a literature review. An analysis of scientific articles was carried out with a focus on epidemiological, clinical, diagnostic, prophylaxis and disease control aspects. The prevalence of CVL in Brazilian regions is determined using clinical, epidemiological and laboratory diagnostic methods. Laboratory confirmation is necessary to avoid false positive results, whose consequences can be fatal, such as euthanasia in uninfected animals. The study is based on the survey of transmission areas with the evaluation of the effectiveness of actions to control and progression of the disease, such as the reduction of prevalence and lethality, with intervention measures and environmental sanitation that can reduce its incidence. It was found that knowledge of the epidemiological indicators of CVL is essential to implement health actions to promote control and prevention in susceptible populations.
Keywords: Control, prevalence, incidence, *Leishmania*.

INTRODUCTION

Canine Visceral Leishmaniasis (LVC), also known as Kala-azar, is an important zoonosis transmitted between animals and humans. In humans, Leishmaniasis presents itself in four clinical forms, such as visceral, cutaneous, mucocutaneous and diffuse cutaneous. Of the presentation forms of the disease, the visceral form is the most serious because, generally, it can be fatal if not treated. In animals, the visceral and cutaneous forms are mainly observed (Marcondes & Rossi, 2013).

the *canis lupus familiaris* is identified as a reservoir of the disease, as it is configured as a domestic host, being the main natural reservoir related to cases in humans (Silva et al., 2003). CVL is a pathology caused by a protozoan of the genus *Leishmania,* which affects dogs. Dogs are considered, in the urban transmission cycle, the main reservoirs through which humans can become infected. However, wild animals such as wolves, coyotes and foxes can also act as reservoirs for this pathogen (Costa, 2011).

In Brazil, CVL is transmitted through the bite of a mosquito belonging to the Phlebotomidae family, *Lutzomyia genus and Lutzomyia* species. *longipalpis.* This vector is popularly known as mosquito, birigui or tatuquiras, being the main Brazilian vector. The sand fly is a very small insect that tends to reproduce in places with a lot of decomposing organic matter (Costa, 2011).

The infected animal can be clinically healthy for a long period, but remains as a reservoir of the disease and with the capacity to infect the vector and continue with the dissemination of the cycle (Gontijo & Melo, 2004). The most observed clinical signs include skin lesions, such as alopecia, desquamation, nasal hyperkeratosis, ulcers and hyperpigmentation, in addition to anorexia, onychogryphosis and alterations ophthalmic (Koutinas & Koutinas, 2014). Prevalence studies of canine disease in several cities in Brazil have detected rates of 9.7% in Montes Claros, in the state of Minas Gerais, and 40.3% in Paulista, in the state of Pernambuco (Silva et al., 2003).

Given its magnitude, transcendence and low vulnerability to control measures, CVL is a serious public health problem in Brazil (World Health Organization, 1990). From

1984 to 2002, 48,455 cases of CVL were reported in Brazil, 66% of them in the states of Bahia, Ceará, Maranhão and Piauí. In the 1990s, approximately 90% of the notified cases occurred in the Northeast Region, because, as the disease spread, it spread to other regions of the country. Another point to be emphasized about the disease is its correlation with predisposing factors. It is still important to consider the knowledge about the indicators of CVL in Brazil, which are formed from the notified cases, since a control and prevention program is an adequate measure for the eradication of this pathology.

The present study aimed to investigate the prevalence of Canine Visceral Leishmaniasis in different regions of Brazil based on a literature review. Secondarily, we sought to raise the incidence of CVL in certain periods and in different locations, present the CVL epidemiological chain and identify the risk factors for the animal population and for public health in the national territory and in the different regions of Brazil.

METHODOLOGY

To obtain the results and answers regarding the purpose of the article, a literature review on Canine Visceral Leishmaniasis in Brazil was carried out. The methodology used is based on studies and analyzes to identify, select and evaluate research considered relevant that contribute as theoretical support on the topic addressed in this review.

The search, analysis, study and preparation of this article took place from February 26, 2021 to May 21, 2021. The keywords used were dogs, incidence, Leishmania spp and *zoonosis*. Preference was given to articles published in the last ten years.

For the research, usual databases in reviews in the field of veterinary medicine were used, such as SciELO, LILACS, MEDLINE, CAPES and PubMed. The institutional repositories of ‘’Universidade Federal de Pelotas”, ‘’ Universidade Federal de Lavras” and ‘’ Universidade Federal do Rio Grande do Sul” were also used; in addition to the Google Scholar search tool.

Some mixed articles of qualitative and quantitative methodology were selected for analysis. The works were selected based on the reading of the titles, abstracts and relevance to the objective of the present research, without any restriction regarding the type of study and language. This way, articles that investigate CVL in Brazil, between the years 2003 and 2014, were selected.

RESULTS

According to Werneck et al. (2008), the environmental transformations associated with migratory movements and the urbanization process may explain, in part, the reason why LVC was restricted to rural areas of the country until the 1970s. in an endemic and epidemic form in large cities in the Brazilian Northeast and, subsequently, spread to other micro-regions of the country. From 1984 to 2002, there were 48,455 reported cases of CVL in Brazil, 66% of them in the states of Bahia, Ceará, Maranhão and Piauí. In the 1990s, approximately 90% of reported cases of CVL occurred in the Northeast Region.

From the expansion of the disease to other regions between 2000 and 2002, more than 25% of cases in Brazil occurred outside the Northeast Region. Thus, it can be observed that it occurs in the large cities of Bahia, such as in the Metropolitan Region of Salvador, where a large number of human and canine cases of CVL have been recorded (Frank et al., 2002; Julião et al., 2007).

Such data can be related to another piece of information provided by the Ministry of Health (2004). According to this reference, the LVC is distributed in 21 Federated Units.

In recent years, an annual average of 3,357 human cases and 236 deaths have been recorded. CVL is a disease that mainly affects low-income populations, being considered emerging due to its urbanization and co-infection with the Human Immunodeficiency Virus.

Due to the incidence of CVL in the country, an exposed population has a high risk of becoming infected, implying the need to implement control measures. In Brazil, in addition to the high incidence and wide distribution, what aggravates the risks of CVL is the possibility of this disease manifesting severe and lethal forms when the animal has immunosuppression or concomitant diseases (Santos et al., 2017).

According to Werneck et al. (2008), the rapid urbanization of the country brought with it a huge wave of precarious living conditions concomitant with environmental destruction. Such factors influence the emergence of the disease in urban areas, since *Leishmania* spp easily adapts to peridomestic conditions in impoverished areas, exploiting the accumulation of organic matter generated by domestic animals and poor sanitary conditions.

®Leishmania screening test. Two of these animals were diagnosed seropositive. However, the confirmatory ELISA test was performed, which confirmed the same reagents as positive and, therefore, considered sick. The observed apparent prevalence was 5.12%. The identification of confirmed cases of CVL is essential to find risk areas and, thus, adopt preventive measures (Reis et al., 2020).

In the epidemiological survey of CVL cases in the municipality of Jaguaribe, state of Ceará, 194 dogs were examined. 31 of them were positive and 163 were negative. In the study, it was demonstrated that, of the 31 animals positive for CVL, 12 animals lived in the urban area (38.70%) and 19 animals in the rural area (61.30%). The samples had been sent to the Central Laboratory to carry out the Immunoenzymatic Test (ELISA) and, in case of a positive reaction, the Indirect Immunofluorescence Reaction (IFAT) test, a confirmatory test for the disease according to the Ministry of Health, was carried out. Brazil Health. Thus, the disease has several diagnostic methods, and it is important to evaluate the clinical, epidemiological and, mainly, laboratory diagnosis, interpreting the results so that a false positive result is avoided.

At the Unimes Veterinary Hospital, in the city of Santos, state of São Paulo, eleven positive samples were obtained in the *Dual Path-Plataform* DPP ® LVC test, and two positive samples were confirmed by the ELISA immunoenzymatic test. Thus, it was noted that it is necessary to increase the use of serological methods in the Baixada Santos to increase the detection of the disease. From the works selected in the literature review, it was evident that the place with the highest prevalence of CVL in Brazil is the Northeast region, with the state of Alagoas being the place with the highest prevalence and highest lethality due to the disease (Reis et al., 2017). It is emphasized, therefore, that CVL has a high prevalence and importance due to the high incidence and consequences for public health.

According to Baneth and Shaw (2002), treatment of CVL in animals, it results in temporary clinical improvement and a decrease in anti- *Leishmania antibody titers*. However, this treatment does not prevent a recurrence of clinical manifestations and does not prevent the dog from remaining infective for the vector, that is, it still works as a reservoir, potentially being able to transmit the disease. In August 2007, a discussion forum on the treatment of dogs with CVL was held. Researchers in the field from various research institutions participated in this Forum.

Among them, representatives of the Ministry of Health (MS), Federal Council of Veterinary Medicine and the National Association of Small Animal Veterinary Clinics.

According to the Ministry of Health, the treatment of animals with leishmaniasis may be carried out through controlled clinical trials after authorization from the Ministry of Agriculture, Livestock and Supply (MAPA) and approval of the report on the conclusion of clinical trials through a joint technical note prepared by MAPA and the MS.

The appearance and dissemination of resistant strains of the parasite have been alerted in communities where the treatment of dogs is practiced, which can have irreversible character and unpredictable consequences. According to Ribeiro et al. (2004), if the treatment is authorized and approved by MAPA and MS, it can be carried out. It is extremely important that the animal being examined is accompanied by a veterinarian. From this follow-up, it is essential that the animal be clinically, serologically and biochemically checked every three months. Sirtoli (2009) adds that the cost of treatment will depend on the type of protocol, ranging from R$ 600 to R$ 2,000 reais. Below are some of the existing drugs that may be used in the treatment of Leishmaniasis if treatment for CVL is authorized by MAPA and MS: Antimonials, Allopurinol, Aminosidine, Pentamidine, Amphotericin B, Marbofloxacin, Miltefosine and Nimodipine (Ribeiro et al., 2004).

According to Amaral (2009), Leishmaniasis control actions in Brazil include diagnosis and adequate treatment of human cases; chemical vector controls; improvement of hygienic-sanitary conditions; and control of the canine reservoir through sample or census serological surveys followed by euthanasia of seropositive dogs. These are efficient measures to control this disease. It is also recommended the use of natural and/or chemical repellents on animals, the spraying of domestic and peridomiciliary environments with insecticides and, in the future, the vaccination of non-infected animals.

Pour-on products and the use of a collar based on Deltamethrin 4% - Scalibor ® are effective ways to repel sandflies in dogs. It is also recommended to use screens on windows and kennels. For humans, it is recommended to use a repellent and avoid exposure during the hours when the vector is active, as well as avoiding environments where the vector can usually be found. In addition, it is recommended to carry out a serological survey of animals in endemic areas, as well as to encourage health education among the population and the cleaning of vacant lots (Fortes, 2004).

The theoretical basis that supports the use of vector control and reservoirs as intervention strategies on CVL is the conjecture that the incidence of infection in humans is directly related to the number of infected dogs and the capacity of the sandfly population to transmit infection from the dog for man. Both vector control and dog elimination, in theory, can be considered effective measures (Werneck et al., 2004).

According to Amaral (2009), the transmitting mosquito proliferates in areas of shade and with decomposing organic matter, mainly in areas under trees and places that accumulate leaves. Cleaning the environment, environmental management, raising awareness among the population is also a control measure. The fact that the transmitting insect does not need water to reproduce, but decomposing organic matter, combined with the fact that its behavior is not fully known, makes it difficult to control it. Due to this, the euthanasia of dogs with Leishmaniasis is a complementary measure in the control of this disease.

Euthanasia of seropositive dogs for CVL was recommended as a control measure by the World Health Organization (W.H.O.) and the Pan American Health Organization (PAHO) (Jericó et al., 2015). However, studies have stated that this methodology is ineffective, as transmission occurs through sandfly vectors, and there are several species of wild animals that can act as reservoirs (Greene, 2015).

The World Health Organization recommends sacrifice as an ideal control measure. However, she recognizes the limitations of this practice when dogs of high emotional and economic value are infected. In Brazil, the embarrassment caused by this measure is reported by professionals linked to the public sector and responsible for controlling CVL when looking for a positive dog for elimination. According to Feijão et al. (2001), this moment, crossed by a strong emotional component, means, given the importance of the dog in the family environment, the determination of the "death sentence" for a "family member". The occurrences of refusal to deliver the animal are increasing, which, consequently, maintains the chain of transmission.

According to Tesh (1995), the lack of an effective treatment for the total cure of the canine disease and the controversy about eliminating infected dogs led to the idea of a new strategy centered on the production of a vaccine. Mendes et al. (2003) complements that the published results of experiments carried out in Brazil state that the vaccine blocks the transmission of the agent to other animals and humans, provided that the vaccination protocol is followed: 3 doses are given at intervals of 21 days, and revaccination must be performed one year after the first dose, and must be repeated annually.

For dogs, collars impregnated with 4% Deltamethrin under experimental conditions have shown good efficacy as a sandfly repellent (Alves et al., 2018). Natural repellents based on citronella and neem extract can also be used, as well as topical insecticides in the form of sprays based on permethrin (Jericó et al., 2015). In Brazil, there is the Leish-Tec® vaccine, which is the only one licensed by public health authorities in the country for sale and administration by veterinarians (Matias et al., 2020). According to studies carried out by Fernandes et al. (2008), this vaccine was able to induce immunity against the infection caused by a high intravenous dose of *L. chagasi* in beagle dogs. In addition, it was also tested in heterogeneous populations of dogs, demonstrating to be safe and well tolerated by the animals (Testasicca et al., 2014).

No studies were found in Brazil that evaluated the knowledge that veterinarians have about the disease and their conduct in relation to it, but only studies that evaluated the knowledge and conduct of health professionals in general, such as that of Luz et al. (2005). In this study, the effectiveness of informative materials aimed at veterinarians was evaluated, in which low effectiveness was observed regarding the knowledge added to these professionals.

The diagnosis of CVL represents a problem given the variety of clinical signs and the similarity of symptoms with other diseases. The choice of diagnostic technique is based on the likely area of transmission. It is investigated whether this is an endemic area only for CVL and immune to its main differential diagnoses, such as the Tegumentary Leishmaniasis and American Trypanosomiasis. In addition, the diagnosis also involves the sensitivity and specificity of the method used, its limitations and clinical interpretation. Currently, the Ministry of Health recommends the use of the immunochromatographic test as a screening test and the ELISA test as a confirmatory procedure (Ministry of Health, 2011).

Peridomestic prevention were routine cleaning of the backyard and caution in raising other domestic or wild animals, in addition to the use of citronella in pots that are in the surroundings. According to the Ministry of Health (2011), controlling the transmission of the disease requires permanent measures, such as environmental management, through cleaning backyards, not allowing domestic animals to remain close to housing, the disposal of solid and organic waste, in addition to the destination suitable for them.

CVL reservoirs are infected through the bite of female sandflies. Initially, the parasites are at the site of the bite and, after that, infection of the viscera occurs with eventual distribution through the dermis (Salzo, 2008) . The amastigote form of CVL is present within leukocytes and cells of the Mononuclear Phagocytic System (SFM) in definitive hosts. Inside these cells, the parasite divides by simple fission, destroying them. The intermediate host, which are the female sandflies, become infected through the ingestion of the amastigotes present in the blood of the vertebrate. In the mosquito's intestine, the amastigotes transform into promastigotes and, by simple splitting, multiply rapidly. Then, the protozoa are inoculated into vertebrates through the bite of the sandfly (Fortes, 2004).

After inoculation of promastigotes in dogs, they are phagocytosed by SFM cells, which are mainly represented by macrophages. Within the leukocytes, the promastigotes transform into amastigotes, which multiply rapidly through successive binary divisions. According to Cimerman and Cimerman Neves (2005), when macrophages are densely parasitized, they rupture, releasing several amastigotes that are phagocytosed again by other macrophages. CVL is a chronic disease. Clinical signs appear between three months and seven years after infection. There is proliferation of B lymphocytes, plasmocytes, histiocytes and macrophages, resulting in lymphadenopathy, splenomegaly and hyperglobulinemia (Salzo, 2008) .

Clinical symptoms include locomotor difficulty, weight loss, polydipsia, apathy, anorexia, vomiting, diarrhea, polyphagia, epistaxis and melena. On physical examination, lymphadenopathy, cachexia, dermatological changes, pale mucous membranes, hyperthermia, splenomegaly, uveitis and conjunctivitis can be observed (Salzo, 2008) . Cutaneous manifestations may be present in 50 to 90% of infected dogs (Salzo, 2008) .

Clinical diagnosis is difficult due to the variety of disease symptoms or their absence. In addition, laboratory changes in the blood count, or in tests of renal or hepatic function, may be nonspecific. The confirmation of the diagnosis can be through parasitological, serological and molecular methods. The parasitological diagnosis is considered, by some authors, the main exam. Another form of diagnosis is through aspiration cytology, an easy-to-perform method (Ikeda-Garcia & Marcondes, 2007).

The observation of parasites in cytological impressions or through the aspiration of skin nodules is also used. It is also possible to perform skin biopsies collected from macroscopically normal areas. The serological diagnosis occurs through the detection of circulating anti-Leishmania antibodies. The serological tests recommended by the Ministry of Health to investigate the disease are indirect immunofluorescence (IFAT) and ELISA (Ministry of health, 2003).

DISCUSSION

Among the main hypotheses to justify the change in the pattern of transmission of CVL, one points out the migration of people

from rural areas to cities, with deforestation and the construction of works with great environmental impact. In our work, it was observed that the increase in the prevalence of the disease in dogs is consistent with the growth in the incidence of the disease in humans. The presence of the vector is necessary for the dissemination of the parasite, since transmission does not occur from vertebrate host to vertebrate host. After the multiplication cycle, there is hematogenous and lymphatic dissemination to tissues rich in cells of the phagocytic mononuclear system. The infection spreads to the spleen, lymph nodes, and bone marrow within the first few hours.

Freitas et al. (2012) state that protozoa of the genus Leishmania spp. are considered hemoparasites, as they can be transmitted through blood transfusion from infected donor animals. In a study carried out with 215 dogs with CVL in the city of Araçatuba, São Paulo, it was observed that there was no sexual or age predisposition for the occurrence of the disease, as observed in our research (Feitosa et al., 2000).

Peters et al. (2008) carried out an experiment with females of the insect *Phlebotomus duboscqi* carrying *Leishmania major* to infect laboratory animals through the ear of mice. Using a microscope, the authors observed the fight against the parasites, and that the rodents' immune system identified their invasion, with the neutrophils directing themselves to the site of the bite. After half an hour, the neutrophils had engulfed most of the parasites and were trying to destroy them with digestive enzymes. It was verified by the research that, after the death of the neutrophils, the parasites approached the macrophages to lodge and reproduce. These authors believe that this disguise allows *Leishmania chagasi* to penetrate macrophages, causing damage to the spleen, liver and bone marrow, weakening the defense system of the infected mouse, data that corroborate the results of this research.

Classification according to clinical signs in infected animals can be as follows: asymptomatic dogs, which do not present suggestive clinical signs; oligosymptomatic dogs, which have lymphoid adenopathy and reduced weight and dull hair loss; and symptomatic dogs, which present cutaneous alterations (alopecia, furfuraceous eczema, ulcers, hyperkeratosis), onychogryphosis, weight loss, keratoconjunctivitis and hindlimb paresis. The disease occurs slowly, being systemic and severe, affecting the spleen according to the host's immune response (Luvizotto, 2006).

For the diagnosis of the disease, it is necessary to assess whether the animal comes from an endemic area or has visited such areas. To confirm the serological diagnosis, it is necessary to carry out a second test. The parasitological exam is the exam of choice, being the gold standard. However, some of the procedures, despite their simplicity, are invasive methods, offering risks to the animal. However, the parasitological examination is a safe diagnostic method, since the positive result is given through direct observation of amastigotes. As observed in the research in Jaguaribe, state of Ceará, present in our work, the authors performed two tests to confirm the infection, since the diagnostic test must be reliable to avoid false positives.

The specificity of the parasitological method is approximately 100%, and its sensitivity depends on the degree of parasitemia, the type of biological material collected and the time taken to read the slide. As observed in the studies of our research, in areas with low socioeconomic status, there may be difficulties in confirming the clinical diagnosis, mainly of dermatoses and malnutrition, which mask the clinical picture of CVL. The owner of the animal

may require a counter-test, which must be a serological test carried out by an accredited laboratory. In 2012, the diagnostic protocol was changed to use the newly validated DPP test as screening and ELISA for confirmation (Ministry of health, 2011; Grimaldi, 2012). Canine leishmaniasis is more resistant to treatment than human leishmaniasis. Only a few animals are considered fully cured.

According to Spina (2009), the choice of treatment protocols is based on the general condition of the patient, always taking into account that, in order to determine their general condition, in addition to clinical evaluation, laboratory tests that can evaluate renal function, liver function, serum proteins, in addition to specific serology for Visceral Leishmaniasis confirmed by parasitological diagnosis. Although there are many drugs for this disease, the combination of Glucantime® and Zyloric® are the most common.

As a way to prevent the spread of CVL, it is necessary to prevent contact between the infected vector and the dog. Prevention measures in large urban centers are directed at dogs. The lack of effective treatment for the total cure of the disease makes it urgent to practice new strategies, such as the Leishmune® vaccine against CVL, already approved by MAPA. Even vaccinated, dogs, because they become seroreactive, may be indicated for euthanasia when, by chance, canine surveys are being carried out in transmission areas.

Furthermore, the Ministry of Health does not recognize the vaccination of dogs as an effective measure for reservoir control (Ministry of Health, 2006). At the *13th Symposium of the Companion Vector-Borne Diseases World Forum,* held in 2018 in the United Kingdom, the concept was established that euthanasia is not an effective control measure.

Information from the Ministry of Health reports that, historically, chemical control is one of the measures that has proven to guarantee the reduction of diseases transmitted by vectors, especially in times of epidemics.

CONCLUSION

From the study, it was concluded that CVL is a public health problem because it is a zoonosis that leads to a considerable number of deaths in Brazil, being identified as one of the parasitic diseases that kills the most in the world. These alarming data are intimately related to the uncontrolled advance of urbanization, which results in inadequate sanitary conditions, among other factors. It is necessary to combine the efforts of public health authorities to try to reverse the epidemiological situation of CVL, which is so critical in developing countries. The region with the highest prevalence of CVL is the Northeast. However, this serious disease has spread throughout Brazil, to places without previous notifications, becoming a worrying factor that requires adequate public policies for its prophylaxis.

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