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PERSPECTIVA

Ethical and epidemiological dilemmas in the treatment of dogs for visceral leishmaniasis in Latin America

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In the Americas there are between 4,500 and 6,800 annual cases of severe visceral leishmaniasis, and mortality is estimated to range between 7 and 10%. However, underreporting and subclinical infections mask the real epidemiological importance of visceral leishmaniasis. Control efforts, which have typically focused on insecticide spraying of sand fly vectors and dog culling, have yielded disparate results. Nevertheless, thousands of dogs are sacrificed each year in countries endemic for visceral leishmaniasis. Additionally, current guidelines of leishmaniasis control programs have banned dog treatment with drugs of human use while therapy with other drugs resulted in high rates of relapses. Society requires that control programs take a more humanitarian approach aimed at limiting dog culling. There is an urgent need to promote responsible dog-ownership and support research on: a) novel veterinary therapies, b) low-cost molecular diagnosis of canine visceral leishmaniasis, and c) determination of dog infectivity threshold for proper reservoir management.

Key words: Leishmaniasis, visceral/prevention & control; *Leishmania infantum*, dogs, therapy, Latin America.
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Dilema ético y epidemiológico sobre el tratamiento de perros para la leishmaniasis visceral en América Latina

En las Américas hay entre 4.500 y 6.800 casos anuales de leishmaniasis visceral grave y se estima que la mortalidad varía entre 7 y 10 %. Sin embargo, el subregistro y las infecciones subclínicas enmascaran la importancia epidemiológica real de la leishmaniasis visceral. Los esfuerzos de control que típicamente se han enfocado en la aspersión de insecticidas contra los flebotómicos vectores y el sacrificio de perros, han arrojado resultados dispares. No obstante, miles de perros se sacrifican cada año en países endémicos para leishmaniasis visceral. Además, los lineamientos actuales de los programas de control de la leishmaniasis han prohibido el tratamiento de perros con medicamentos de uso humano, mientras que otras drogas resultan en altas tasas de recaída. La sociedad requiere que los programas de control tengan un manejo más humanitario enfocado a limitar el sacrificio canino. Hay una necesidad urgente de promover la tenencia responsable de los perros y apoyar la investigación en: a) terapias veterinarias novedosas, b) diagnósticos moleculares de bajo costo y c) determinación de los umbrales de capacidad infecciosa canina para el manejo adecuado del reservorio.

Palabras clave: leishmaniasis visceral/prevencción & control, *Leishmania infantum*, tratamiento, perros, América Latina.

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Zoonotic visceral leishmaniasis caused by *Leishmania infantum* (= *L. chagasi* in Latin America) affects human populations in both the Old and New World with varied morbidity and mortality rates depending on the socio-economic level of

the country. In the Americas there are between 4,500 and 6,800 annual cases of full blown visceral leishmaniasis, and mortality is estimated to range between 7 and 10% (1). However, underreporting and subclinical infections tend to mask the real epidemiological importance of this parasitic disease in Latin America. Like many other vector-borne zoonotic diseases, spilling over of infection to human populations is linked to multiple social and economic factors that put humans in frequent contact with disease vectors. In the particular case of visceral leishmaniasis, the natural forest cycle of transmission between wild mammals and sand fly

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vectors has gradually shifted from enzootic to peri-urban and urban transmission. Vector adaptation to urban environments and migration of humans to the periphery of cities are considered the principal reasons for this epidemiological change in which dogs have become the main reservoir host (2).

Diverse and even opposing conclusions have been reported on the epidemiological impact that control efforts have had in regions where *L. infantum* is the etiological agent (2,3). In Latin America, Brazil has pioneered the implementation of visceral leishmaniasis control, targeting both the sand fly vector by means of insecticide spraying and the canine population through dog culling (4). The fact that control efforts focused on vectors and reservoirs have yielded disparate results points to the existence of multiple additional factors that have influenced intervention outcomes. Not only have control measures failed in many regions in which transmission is historically endemic, but ineffective control has also allowed the geographical expansion to newer areas of Southern Brazil and other South American countries (Paraguay and Argentina) (4,5).

A series of reasons why dog culling was less effective than expected to curb visceral leishmaniasis transmission has been proposed. A variety of serological methods has been used to identify infected dogs yet an ill-defined proportion of seronegative dogs could still be infected with *L. infantum* as determined by molecular methods (6). The prolonged lapse between diagnosis and actual culling of dogs was considered to be another factor that negatively impacted control efforts (3). In resource-limited villages of Brazil endemic for visceral leishmaniasis, which were characterized by a lack of responsible dog-ownership, the high rate of dog culling (60.9% in two years, not all related to canine visceral leishmaniasis) did not translate to reduction in canine visceral leishmaniasis incidence, which was attributed to the rapid replacement of infected dogs with susceptible puppies (7). On the other hand, the potential spillover of *L. infantum* from rural (forested) to periurban/urban environments represents a continuous influx of parasites to uninfected canine populations that remain after removal of infected dogs (2). Less frequent mechanisms of infection such as sexual and transplacental transmission also could contribute to sustained *L. infantum* circulation in the absence of sand fly vectors (8,9).

As mentioned before, control programs in developing countries focus on dog culling regardless of its varied efficacy, and only health authorities of developed countries consider that dogs should be treated. For example, between 2005 and 2010, almost 60,000 dogs were sacrificed as part of the canine visceral leishmaniasis control campaign in the city of Belo Horizonte, Brazil (2). Therefore, a positive serology in Latin America is equivalent to issuing a “death certificate” when dog owners act in accordance with public health recommendations. Current guidelines of leishmaniasis control programs in South America recommend sacrificing any infected dog and have banned treatment with drugs of human use (10-12). The latter recommendation is based on the poor clinical and parasitological results of antileishmanial therapy in dogs and the possibility that parasites could develop resistance by exposure to drugs that are used for patients. In fact, strains of *L. infantum* isolated from treated dogs demonstrated more resistance to pentavalent antimonials compared with the corresponding pre-treatment isolates (13). However, because dog sacrifice is not mandatory, many owners decide to keep their animals and according to their economic capabilities initiate the antileishmanial treatment. Anecdotal information suggests that dogs of affluent families may receive treatment with meglumine antimoniate which is still widely used to treat patients suffering from cutaneous or visceral leishmaniasis.

The social and epidemiological aspects of canine visceral leishmaniasis

According to phylogenetic analysis, dogs originated from wolves more than 100,000 years ago and domestication occurred 15,000-10,000 BCE (14,15). From that time on, dogs were increasingly integrated into societies, and although some of the human-dog interactions are based on utilitarian motives (guardian, hunting, cattle ranching, illicit-drug detection, etc.), companionship is the reason for the vast majority of interactions (16). Increasing educational level of the owner is associated with dogs playing a companion role, which is promoted by anthropomorphism (dogs being considered more as a person than animal) rather than being regarded as a “pet to be owned”. Interestingly, dog companionship in less educated people is characterized by a lesser tendency toward anthropomorphism and allowing dogs to move freely in the household with little restrictions (owners set few boundaries) (16).

The combination of the aforementioned human-dog interactions has different implications in an urban area where visceral leishmaniasis is endemic. On one hand, due to canine anthropomorphism there is an increasing societal pressure to find alternate solutions to euthanasia of infected companion dogs by seeking efficacious antileishmanial treatment. On the other hand, persons who do not establish boundaries most likely allow dogs to roam freely in suburban areas, complicating canine visceral leishmaniasis control. Several studies (mostly in the USA) have estimated that the home-range of free-roaming dogs varies between 1 and 10 hectares (2.5 and 25 acres), but could be larger depending on food availability (17). In areas endemic for visceral leishmaniasis, this behavior could put stray dogs in contact with different sand fly populations, increasing the chances of acquiring and dispersing *L. infantum*. A survey carried out in different sections of the city of Santiago, Chile (not endemic for visceral leishmaniasis), showed that 52.4% of unattended street dogs had owners, 21.9% were actual stray dogs and 8.9% were "protected-stray dogs" always living in the same vicinity and fed by neighbors (18). Assuming that a similar situation occurs in other Latin American cities, those results indicate that approximately 30% of dogs found in the streets are free-roaming animals with no health control including canine visceral leishmaniasis diagnosis. The importance of free-roaming dogs in *L. infantum* transmission was revealed during screenings carried in Posadas City, Argentina, at the beginning of an active canine visceral leishmaniasis control program. A higher infection prevalence was found in stray dogs (49%) collected by the Municipal Institute of Animal Health compared with 27% infection prevalence in dogs screened at households (L. Tartaglino, personal communication).

The general public accepts the slaughtering of domestic animals for food consumption, while the sacrifice of household pets has a different connotation. As mentioned before, canines play a special societal role and the sole idea of considering dog killing as the only alternative to solve a public health problem is considered unacceptable in most places. However, protecting dogs in canine visceral leishmaniasis endemic areas is not an easy undertaking. A vaccine showing acceptable immunogenicity and protection ($\approx 70\%$) has recently been approved for phase III trial (Leish Tec™, Brazil), but still needs to demonstrate high efficacy and logistic feasibility before it could be considered

a sound component of visceral leishmaniasis control (19,20). Similarly, despite the fact that field trials using deltamethrin-impregnated dog collars may be more effective in reducing visceral leishmaniasis transmission than dog culling, its utilization on a large scale over the course of many years makes it difficult to implement (21).

Current status of dog treatment and its epidemiological and individual implications

As previously discussed, dogs are at the susceptible end of the spectrum of *L. infantum* infection. Progression from the asymptomatic to the polysymptomatic stage is characterized by an increasing number of signs such as lymphadenopathy, dermatitis, onychogryphosis, anemia, weight loss, ocular pathologies, hepatosplenomegaly, diarrhea and kidney dysfunction, all of which lead to multi-organ failure and death (22). There are several reviews on the treatment of canine visceral leishmaniasis (23,24) and thus this viewpoint only summarizes the principal observations. Only a few of the >120 articles regarding dog treatment were controlled studies in which infection status before therapy was thoroughly evaluated (24). Nevertheless, these studies indicated that treatment of canine visceral leishmaniasis is cumbersome and characterized by high rates of relapses regardless of the antileishmanial drugs used, either as a single drug or in combination regimens. Long-term follow up of treated dogs revealed that true clinical and parasitological cure is rarely achieved, and underscores the exquisite susceptibility of most canines to *L. infantum*.

Pentavalent antimonials: Meglumine antimoniate (Glucantime®) or sodium stibogluconate (Pentostam®) have been the first-line drugs to treat patients suffering cutaneous or visceral leishmaniasis, and these compounds are still used in many countries. Glucantime treatment protocols are based on the antimony (Sb^V) concentration which is approximately 85 mg/mL. Meglumine antimoniate was utilized to treat canine visceral leishmaniasis principally in countries of the Mediterranean basin (50-150 mg/kg, subcutaneous or intramuscular for 3-4 weeks). Initially promising results were consistently followed by persistent parasites in spleen, bone marrow, liver and lymph nodes, including 70-100% clinical relapses at one year post-treatment, all of which indicated the inadequacy of meglumine antimoniate as monotherapy (24,25).

Allopurinol: This drug, which is given primarily to treat patients with hyperuricemia that leads to chronic gout, is the compound most widely prescribed by veterinarians to treat canine visceral leishmaniasis. It has been proposed that the antileishmanial activity of allopurinol is based on the blockade of the purine salvage pathway of amastigotes, producing purine starvation and parasite death (26). Although *in vivo* results suggested that allopurinol acts as a parasitostatic drug, it is feasible that its actual parasitocidal effect is masked by the short half-life of the parent drug (2 hours) and lack of antileishmanial activity of the major metabolite (oxypurinol) (27). The drug has been used at doses of 15-30 mg/kg/day *per os*, divided in two or three daily doses over 4 to >12 months (24). This drug became well accepted in spite of the marginal efficacy because of its low cost and the ease of household administration. Nevertheless, it is not uncommon that dog owners become discouraged with the protracted therapy and incomplete clinical and parasitological cure and, therefore, tend to abandon the treatment. Consequently, relapses are the norm once treatment is suspended (23,24).

Allopurinol combined with pentavalent antimonials: The debatable convenience and efficacy of allopurinol regimens prompted researchers to evaluate its inclusion in combination therapies. The co-administration of meglumine antimoniate and allopurinol showed initial clinical and parasitological remission. The treatments were initiated by subcutaneous, intramuscular or intravenous administration of meglumine antimoniate (100 mg/kg for 20-30 days) and oral allopurinol (15 mg/kg *bid*) for ≥8 months. These results suggest that meglumine antimoniate given at the beginning of treatment sharply reduced the parasite burden which was then kept at bay by chronic administration of allopurinol. Nevertheless, parasite elimination was not achieved in spite of clinical remission (23).

More recently, allopurinol was evaluated in combination with liposomal meglumine antimoniate in naturally infected dogs at different stages of clinical canine visceral leishmaniasis. Although positive results were obtained compared with these drugs used as monotherapy, the weakness of the study was the small number of dogs per group and the heterogeneity of parasite burden between groups before treatment (28). From the epidemiological standpoint, there was no clear benefit of the combination therapy vs. allopurinol alone because reduction of dog infectivity to sand fly vectors (xenodiagnoses) at 140 and 200 days

post-treatment was similar in both groups of dogs. Besides, like other therapeutic schemes, allopurinol was administered daily for over 4 months, a scheme that is likely to affect adherence to treatment. Two additional factors that would prevent its large-scale implementation in Latin America are the utilization of liposomes, which are costly and involve parenteral administration, and the use of pentavalent antimonials which are banned for veterinary use according to public health guidelines (10,12).

Other drugs previously or currently used in humans (amphotericin B, pentamidine, miltefosine) have shown varying results, usually characterized by clinical remission and parasitological persistence (23). In conclusion, none of the drugs used so far can ensure parasitological cure in spite of clinical improvement, and almost all therapeutic regimens have a high rate of relapse at different times post-treatment. As mentioned before, in resource-limited countries of Latin America, the drugs commonly used to complement allopurinol treatment would be costly and not recommended for veterinary use, leaving no therapeutic alternative.

Treatment of canine visceral leishmaniasis can be viewed from two different standpoints, i.e., the health of the individual animal and the epidemiological impact the treatment may have. Most dog owners regard clinical cure or remission as the sole goal of treatment and this is likely to continue unless leishmaniasis control programs can change the community's individualistic view of the problem. On the other hand, the epidemiological viewpoint considers that interrupting transmission is the key result of treatment regardless of the clinical outcome of therapy. Assessing clinical improvement of dogs is fairly simple, while determining their infectivity to sand fly vectors is cumbersome. There is no established threshold of tissue parasitism that could be reliably associated with infectivity to sand flies and, therefore, any parasitologically positive dog is considered to be potentially infectious under public health guidelines. This assumption may be closer to reality in the Old World where sand fly species seem to be more efficient vectors than those of Latin America (29,30). However, recent studies in Spain indicated that treatment, either with allopurinol-meglumine antimoniate or any of these drugs as monotherapy, significantly reduced dog infectivity to *Phlebotomus perniciosus* (60-67% pre-treatment down to 0-11% post-treatment) as determined by xenodiagnosis at 60 days post-treatment (31). Consequently, it would be useful to determine whether in the absence of the "magic bullet", sub-curative treatments in which

L. infantum is detected only by DNA amplification could be of clinical and epidemiological benefit in the American continent where a comparatively less efficient vector (*L. longipalpis*) predominates.

The road to a more efficacious and humane control of visceral leishmaniasis

It is imperative to realize that despite current control measures, several thousands of dogs are or will be infected with *L. infantum*. Under the present circumstances these dogs will die of canine visceral leishmaniasis, or will be unsuccessfully treated or will be euthanized to eliminate the reservoir host. Society requires that dog management in *Leishmania*-endemic areas of Latin America take a more humanitarian turn.

The strategies used so far in Latin America to control visceral leishmaniasis include: a) insecticide spraying of entire neighborhoods or focusing on houses where visceral leishmaniasis patients are detected, b) removal of potential sand fly breeding sites around houses, c) serological identification of infected dogs followed by euthanasia, d) canine neutering, and e) intensification of stray dog removal. Other complementary measures such as community education on visceral leishmaniasis prevention and responsible dog ownership only recently have begun to be adopted and their long-term efficacy requires further evaluation (32).

As described in this viewpoint, control measures are only partially efficacious and therefore there is an urgent need to develop and adopt additional strategies that could improve visceral leishmaniasis prevention and control. The following approaches, ranging from short-term application to mid-term development and implementation, should be considered:

a) Mandatory use of deltamethrin-impregnated collars on infected dogs would decrease the epidemiological risk that these animals represent. This strategy would be more feasible than providing deltamethrin-impregnated collars to the whole canine population of the city over a period of several years. Voluntary use of deltamethrin-impregnated collars in household pets could also be recommended as part of the community education.

b) Community education about visceral leishmaniasis could be complemented by dog ownership accountability, enforced by implementing subcutaneous microchips that identify free-roaming dogs. Since this approach is currently not feasible, university research groups in bioengineering and government should work together to develop

low-cost microchips. This tool should be made available mostly in urban areas where human and dog densities and transmission rates are higher compared with rural areas.

c) Field diagnosis of canine visceral leishmaniasis relying only on serology will miss an unknown proportion of infected dogs. Therefore, in the short term, control programs will require the development of low-cost molecular amplification methods capable of detecting dogs at early stages of infection before they become more infectious to sand flies. Research should make emphasis on isothermal amplification assays, which appear to be the next generation of field-applicable diagnostic methods. With these tools in place, infected stray dogs could be efficiently removed from the canine visceral leishmaniasis cycle or subjected to treatment provided newer efficacious therapies become available.

d) Substantial support should be given to research aimed at the identification of new antileishmanial molecules specifically developed to treat dogs.

The establishment of canine infectivity thresholds using well-designed multidisciplinary studies will determine the epidemiological risk, helping health authorities in their decision making. The difficulty of the latter studies would be compensated by the possibility of identifying antileishmanial molecules and treatment regimens that in addition to seeking animal well-being will fulfill epidemiological requirements in Latin America.

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Conflict of interest

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