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An Epidemic Outbreak of Canine Cutaneous Leishmaniasis in Colombia Caused by *Leishmania braziliensis* and *Leishmania panamensis*

Iván D. Vélez,* Lina M. Carrillo, Liliana López, Erwin Rodríguez, and Sara M. Robledo

Program for Study and Control of Tropical Disease – PECET, School of Medicine, University of Antioquia, Medellín, Antioquia, Colombia; School of Agrarian Sciences, University of Antioquia, Medellín, Antioquia, Colombia; Health Office at the Army, Republic of Colombia

Abstract. The largest recorded outbreak of cutaneous leishmaniasis in Colombia's history occurred during 2005–2009 in soldiers of the Colombian Army, with ~40,000 cases. This outbreak was caused by the influx of military personnel into the jungle with the mission of combat illicit crops and the guerrilla. The soldiers remain for long periods within the rainforest and are exposed to the bite of infected sand flies. During the military activities, soldiers work with dogs specially trained to detect landmines, and therefore, dogs are also exposed to the infected sand flies and show high incidence of cutaneous leishmaniasis (CL). This work describes an epidemic outbreak of canine CL caused by *Leishmania braziliensis* and *Leishmania panamensis* in Colombia, South America. The clinical features of the disease and the response to treatment with pentavalent antimonials observed in 72 guard dogs from the Colombian Army are described. A program for prevention and control of canine CL is also discussed.

INTRODUCTION

Leishmaniasis is a vector-borne disease of human beings and animals caused by protozoan parasites of the genus *Leishmania*. The dogs are susceptible to visceral and cutaneous leishmaniasis and the most important reservoirs of *Leishmania infantum*, the causative agent of visceral leishmaniasis (VL) in humans.[1] Canine VL is a chronic infection that leads animals to death. This disease is endemic in many countries of Latin America, the Mediterranean basin, and West Africa,[2] where it constitutes a serious public health and veterinary problem.[3] Canine VL has a worse prognosis than in humans, because there is no effective treatment and the recommendation is the sacrifice of infected dogs, especially in countries where there is a high number of human cases and few alternative therapies.[4] The early diagnosis and sacrifice of the infected dogs allows more effective control of the transmission of the infection to other dogs and humans.[1]

Less common than the visceral form is the cutaneous leishmaniasis (CL). Species of *Leishmania* such as *Leishmania braziliensis* and *Leishmania panamensis*, among others, can infect dogs and produce symptoms and signs similar to CL in humans. Dogs can be infected when the sandflies bite them. Females sandflies need blood meal for oviposition. With biting, the sand fly inoculates promastigotes of *Leishmania*, which invade the macrophages in the skin and after a variable incubation period (from 2 weeks to 2 months) the signs of the disease appear. The signs and symptoms of canine CL include skin lesions that begin with the formation of a nodule in the place where the bite occurred. This nodule increases in size and then may ulcerate. Lesions may also look as plaques. The lesions usually occur on the nose, ears, and perineal region that are the body regions where dogs have less hair (or have more exposed skin).

Although the dog may be considered as a potential reservoir for CL, its role as reservoir has not been demonstrated. Indeed, there are very few studies describing canine CL in South America.[5–14] These studies offer little information regarding clinical, parasitological, or immunological aspects in the infected dogs. On the other hands, despite the high prevalence of *L. braziliensis* and *L. panamensis* in Colombia, information about the canine CL is poor. In both Colombia and other American countries cases of canine CL caused by *L. braziliensis* but not by *L. panamensis* have been reported.

Since 2003 human CL has increased in Colombia. From about 10,000 cases reported in 2003, the number of cases in 2010 was 15,000. Thus, Colombia occupies the second place in number of cases of CL in Latin America after Brazil. Different factors have contributed to the increase in the number of cases in Colombia. Thus, the appearance of new foci of transmission is caused by the colonization of vectors to new geographic areas, the rural domestic transmission process, and the urbanization of the disease.[15–19] Nevertheless, the main factor that has influenced this outbreak is the high number of military members who are entering the forest areas because of the armed conflict in the country and in the fight against drugs; therefore, they are the population group with the highest incidence of the disease.[20] The soldiers enter the jungle areas accompanied by dogs specially trained to detect landmines or illicit crops. This study aimed to identify and characterize the presence of CL in dogs belonging to the Colombian Army personnel who are monitoring the jungle areas of Colombia where no previous studies have been conducted. This work describes the clinical features and the response to treatment with pentavalent antimonials in 72 dogs with CL in Colombia. A program for prevention and control of canine CL is also discussed.

MATERIALS AND METHODS

Study population. A total of 72 dogs belonging to the Colombian Army, having cutaneous lesions compatible with CL were included in this study. All dogs having visited endemic areas of CL in Colombia as guard dogs of soldiers.

Ethical consideration. The management of experimental animals was performed according to the regulations in the National State for the Protection of Animals in Colombia (Act 84 of 1989). For sampling and treatment administration, dogs were anesthetized with a intramuscular (IM) mixture of ketamine at a dose of 8–10 mg/kg body weight (bw) + xylazine at a dose of 1 mg/kg bw.

* Address correspondence to Iván D. Vélez, Program for Study and Control of Tropical Disease – PECET, School of Medicine, University of Antioquia, Carrera 53 No. 61-30, Lab 632, Medellín, Colombia. E-mail: id_velez@yahoo.com

Animal sampling and clinical examination. A clinical form was filled out for each dog and a photographical record of the lesions was done. For sampling, the animals were restrained and in each lesion samples were taken for direct examination, culture, serology, and/or biopsy using the techniques described for the parasitological diagnosis of CL in humans.[21]

Parasitological diagnosis. The parasitological diagnosis was made by microscopic visualization of amastigote forms of *Leishmania* in smears from the edge of lesions on glass slides. The slides were stained with Giemsa and examined with a 10× eyepiece and a 100× oil objective. Additionally, tissue obtained by needle aspirates from the edge of the skin lesion, bone marrow, or popliteo lymph node were inoculated into a tube of NNN medium and kept at 26°C. Cultures were examined by microscopic visualization of promastigotes every week for 1 month. After initial growth of promastigotes in culture tubes, the parasites were mass cultivated for analyses with monoclonal antibodies to identify the *Leishmania* species responsible for the infection as described.[22]

Serological diagnosis. The presence of *Leishmania*-specific antibodies was determined by indirect immunofluorescent-antibody test (IFAT) in blood samples. The IFAT was carried out according to an established method[21] using promastigotes of *L. panamensis* (MHOM/CO87/UA140) cultured in modified NNN medium as the antigen. Briefly, the parasites were exposed to 2-fold serial dilutions of sera, from 1:8 to 1:128, in phosphate-buffered saline, pH 7.2, in a moist chamber, washed, and then exposed to FITC-rabbit anti-dog immunoglobulin G (diluted 1:500); both incubations took place at 37°C for 30 min. Samples scored positive when they produced promastigote fluorescence, with a cut-off dilution of 1:32. Each test included *L. panamensis*-positive and -negative canine sera as controls.

Histopathology. Skin biopsies were taken from the edge of the lesion (avoiding ulcerated and heavily crusted parts) with a 4-mm disposable punch and 2% xylocaine as an anesthetic. The material was fixed in 10% formalin, routinely processed, and embedded in paraffin. Sections were stained with hematoxylin and eosin for visualization of intracellular amastigotes by microscopy.

Treatment. Dogs with clinical or parasitological diagnoses of CL were treated empirically with one ampoule (14–16 mg/kg bw) of meglumine antimoniate IM every day during 10 to 30 days. Most of the animals received a total of 30 ampoules. Dogs were followed up through an average period of 180 days to verify the occurrence of relapses. Food and water ingestion was evaluated and the animals were submitted daily to a physical examination.

Statistical analysis. Because this was an exploratory study, the analysis of data was descriptive.

RESULTS

In total 72 dogs were evaluated. All dogs were Labrador-Retriever. Forty-four (61%) were males and 28 (39%) were females. The age ranged from 8 to 48 months old (31 months in average). Thirty-five dogs (49%) came from the SE Amazonian region (Guaviare, Putumayo, Meta, and Caquetá departments) and the other 37 dogs (51%) came from the Urabá region (NW Colombia, near Panama Border).

Clinical lesions were nodules or ulcers but no indurate border (Figure 1). None of the cases presented systemic symptoms. Lesions varied in sizes from 0.4 to 10 cm in diameter. The evolution time of lesions ranged from 2 to 12 months. Forty-one dogs (57%) had only one lesion; 22 (30.6%) had two lesions, and the other 9 (12.5%) had three or more lesions. Lesions were located mainly in the muzzle (62.5%) and scrotum (41.7%). Other anatomical regions affected were legs, vulva, ears, back, and prepuce (Figure 1). Fifty-nine dogs in total (82%) were positive for *Leishmania* infection by one or more laboratory tests: 31 of 58 (53.4%) was positive by direct examination, 24 of 50 (48%) by culture, 37 of 37 (100%) by IFAT, and 14 of 14 (100%) for biopsy.

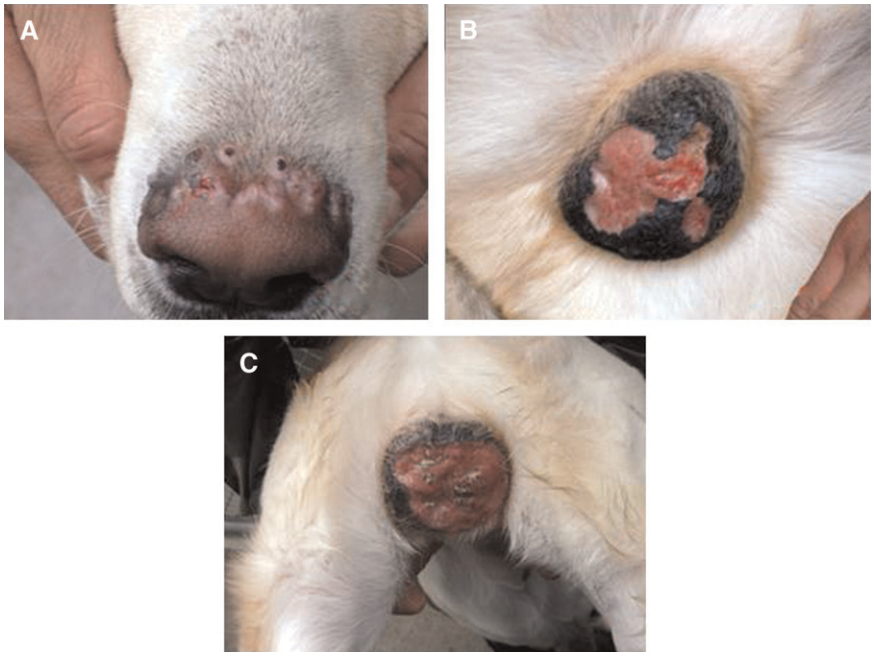

FIGURE 1. Typical lesions in canine cutaneous leishmaniasis. (**A**) Multiple nodules with or without crust; (**B** and **C**) ulcers varying in size.

Leishmania panamensis was isolated in 12 of the dogs with CL and *L. braziliensis* was isolated in 8 dogs. The remaining four dogs were just identified as *Leishmania Viannia* "sensu lato." *Leishmania braziliensis* was predominant in the SE region, whereas *L. panamensis* was more frequent isolated from dogs in the NW region.

Because CL was confirmed in only 59 of 72 dogs, it was assumed that the remaining dogs also had leishmaniasis (based on clinical features) and hence they were treated with antimonials. Eighteen of the 72 dogs had received prior treatment with pentavalent antimony, which consisted of the empirical application at varying doses of meglumine antimoniate and without a specific scheme. All dogs showed a good response to the treatment with meglumine antimoniate (Figure 2).

DISCUSSION

This is the first description of an outbreak of canine CL caused by *L. braziliensis* and *L. panamensis* in Colombia and, to our knowledge, in the world. Although *L. braziliensis* has been involved in canine CL,[14,23,24] this is the first report of *L. panamensis* affecting dogs. Canine CL is not a frequent event. Despite the active search for infected dogs carried out in multiple foci of American cutaneous leishmaniasis (ACL) in Colombia performed by our group in the last 25 years, only two dogs showing plaque-type lesions of ~3 cm in diameter had been found in Cúcuta (north east Colombia), where the causative agent was *L. braziliensis* (Velez ID, personal communication). In another study, six dogs were diagnosed with CL caused by *Leishmania guyanensis* in Villavicencio, Meta.[25]

The *Leishmania* species isolated from dogs with CL in this study were *L. panamensis* and *L. braziliensis*, which are the two species most widely distributed in Colombia as causative agents of CL in humans, being responsible for ~50% and 50% of human CL cases. *Leishmania panamensis* was isolated in 30% and *L. braziliensis* was isolated from 11 dogs (46%). Thirty-five dogs included in this study were from the SE, where according to our studies 80% of human cases are caused by *L. braziliensis* and the other 20% are caused by *L. panamensis*, and 37 dogs were from the NW region of Colombia (on the border with Panama), where the proportion of cases by species is 80% of cases are caused by *L. panamensis* and 20% are caused by *L. braziliensis.* The proportion of dogs by species, in each of these regions, was similar to that found in humans. This is consistent with the ratio of humans and the fact that the dogs are an accidental host that are infected in the forest when they accompany soldiers patrolling and where they share similar risks of infection.

The fact that *L. panamensis* and *L. braziliensis* are causing canine and human CL is an important finding for the epidemiology and distribution of CL in Colombia, because the same *Leishmania* species isolated in humans are being isolated in dogs sharing the same areas. This implies that both humans and dogs are exposed to the vector bites and are an accidental host for *Leishmania* parasites. If the dog is an accidental host, as humans, and its epidemiological and clinical behavior is similar to humans, it should be discussed if dogs become a reservoir for domestic cycles of leishmaniasis transmission and able to participate in the dissemination of the parasites to urban and peri-urban areas where vectors are present. In the case of humans, there is good evidence that the man can play as a reservoir of CL, and the association with epidemic outbreaks of CL in the valleys after the arrival of people with active lesions.[18] Some studies have discussed the possible role of dogs in the transmission of the CL and suggest that dogs may play an important role as reservoirs of CL in endemic areas.[26] However, the results provide no evidence that dogs are reservoirs, mainly because of the high rates of infection in dogs and the increased transmission of CL in rural areas,[27,28] and the ability to transmit the parasite to sand fly vectors should be demonstrated. In a previous study, dogs with CL caused by *L. braziliensis* were exposed and bitten by *Lutzomyia trapidoi*, *Lutzomyia gomezi*, *Lutzomyia longipalpis*, and *Lutzomyia youngy*, which are proven vectors of ACL.[24] After feeding,

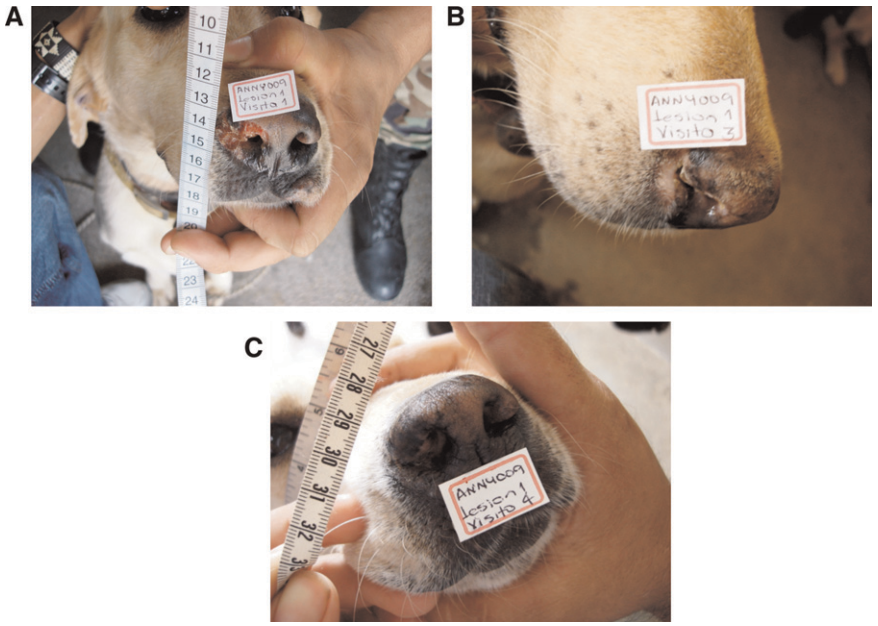

FIGURE 2. Response of canine cutaneous leishmaniasis to meglumine antimoniate. (**A**) Photographs of lesion in the muzzle before treatment, (**B**) 5 weeks after beginning of treatment, and (**C**) 3 months after treatment.

there was no development of the parasites in the vectors gut, suggesting that dogs infected with *L. braziliensis* are not good reservoirs for CL. However, the ability of infected dogs with *L. panamensis* to serve as a reservoir remains to be determined.

All dogs showed only ulcer type lesions mainly in areas deprived of hair such as the muzzle or the scrotum that are easily accessible to the vector bite. These results differ from those observed by Pirmez and others[23] in dogs naturally infected with *L. braziliensis* where the majority of the dogs had ulcerated single lesions on the ears. Fifty-nine dogs in total (82%) had a diagnosis of CL by parasitological, serological, or both methods. The positive results obtained with the smear and culture methods were lower than those obtained by serology or biopsy (100%). This could be caused by the long time of evolution of the lesions or the previous treatment some dogs had.

Although all dogs showed a good response to the treatment with meglumine antimoniate, most of them had relapses at 1–2 months after cure. Nevertheless, because dogs returned to the endemic areas and were exposed again to the sand flies biting, the possibility that these new lesions were new infections rather than relapses was not determined. Thus, it would be very interesting to study how long these dogs remain infected even after a clinical "cure" in response to treatment are reached.

The symptoms and clinical signs demonstrated by dogs can differentiate the canine CL and canine VL as two different clinical entities. Although canine VL is characterized by several symptoms such as poor appetite, weight loss, diarrhea, lymphadenopathy (popliteal), cachexia, splenomegaly, and a poor response to any other treatment, in the canine CL the sick animals show an optimal physical appearance and their response to treatment with pentavalent antimonials is adequate. Therefore, although the sacrifice of animals with VL is recommended even though the sacrifice of dogs with positive serology does not decrease the incidence of the disease,[27] in canine CL the treatment with pentavalent antimonial is a possible strategy.

Up to now, the strategies for control of the canine VL have included: 1) use of collars impregnated with deltamethrin, a strategy that has shown good results in regions such as Italy[29] and Tunis[30]; 2) treatment of infected dogs that although commonly used in the European countries, this approach is not recommended due the poor results and the possibility to generate resistance to the antiparasitic drugs; and 3) sacrifice of infected animals.

We conclude that there was an outbreak of canine CL simultaneously with the outbreak of CL in humans in Colombia because dogs stay with their owners during patrolling in the jungle. Because of the poor evidence that the dog is acting as a reservoir of ACL and dogs suffering CL may respond to treatment with pentavalent antimonials, we recommend that treatment of dogs with CL is a promising strategy for control of canine CL. Moreover, the use of insecticide-impregnated collar could be a promising control measure for avoiding infection of sand flies from infected dogs. However, the impact of these measures should be determined.

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Authors' addresses: Iván D. Vélez, Liliana López, and Sara M. Robledo, Program for Study and Control of Tropical Disease – PECET, School of Medicine, University of Antioquia, Medellín, Colombia, E-mails: id_velez@yahoo.com, lililop14@yahoo.com, and sara_robledo@yahoo.com. Lina M. Carrillo, Program for Study and Control of Tropical Disease – PECET, School of Agrarian Sciences, University of Antioquia, Medellín, Colombia, E-mail: linacarrillo@gmail.com. Erwin Rodríguez, Health Office at the Army, Republic of Colombia, E-mail: erroga@yahoo.com.

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