Evaluation of 65% Permethrin Spot-On for Prevention of Canine Visceral Leishmaniasis: Effect on Disease Prevalence and the Vectors (Diptera: Psychodidae) in a Hyperendemic Area*

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ABSTRACT

A study was designed to examine the effect of 65% permethrin spot-on on the prevalence of canine visceral leishmaniasis and the abundance of sand flies in two neighborhoods in Corumbá, Mato Grosso do Sul, Brazil known to have a high prevalence of visceral leishmaniasis. An enrollment survey was conducted to determine the prevalence of visceral leishmaniasis. Area residents were provided with information about the project; the study area was defined, and all dogs (160 in Cristo Redentor and 230 in Popular Velha) identified in the study area were enrolled. Three 65% permethrin spot-on treatments (June 15–25, July 13–15, and August 10–12) were administered to 150, 110, and 99 dogs, respectively, in Popular Velha, according to label recommendations. Dogs in Cristo Redentor were untreated controls. Visceral leishmaniasis was diagnosed periodically by indirect immunofluorescence assay. A reduction in canine visceral leishmaniasis prevalence was observed at the Popular Velha site. The infection rate for treated dogs 1 month following the final treatment was approximately 50% reduced from that observed before treatment (19.3% vs 9.6%). Conversely, the infection rate at the control site was more than 80% higher at the September sampling than that observed pretreatment (4.1% vs 7.4%). Similar numbers of sand flies were captured and identified from both sites throughout the study. The results suggest that regular use of 65% permethrin during months of high risk for canine visceral leishmaniasis can be a useful strategy for reducing the prevalence of this disease in hyperendemic areas. It should be stressed, however, that the success of this strategy depends not only on the efficacy of the product itself but also on the adoption of other control measures and on economic variables, considering the low purchasing

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power of the populations living in higher-risk neighborhoods.

INTRODUCTION

Visceral leishmaniasis is a severe systemic disease that is fatal in 90% of all untreated cases.[1] The etiologic agent, *Leishmania chagasi*, a flagellate protozoan, invades the human mononuclear phagocytosis system after being transmitted by the bite of hematophagous sand flies (phlebotomines).[2] Visceral leishmaniasis is zoonotic, and dogs are considered the primary domestic reservoir. The disease is widely distributed and occurs in both the Old World and in the Americas.[3] It is endemic in Brazil, where it is found in 17 of the 27 states in the country.

During the previous 10 years, several foci were found in Corumbá, a city in Mato Grosso do Sul, a region considered hyperendemic for visceral leishmaniasis.[4] The Ministry of Health has set 1% as the acceptable prevalence level in endemic areas. In 1998, the National Health Foundation (Fundação Nacional de Saúde; FNS) reported a 4.5% prevalence of leishmaniasis among dogs in Corumbá. Previously, Nunes and colleagues[5] reported the prevalence of leishmaniasis to be 8.7% in the area. The Corumbá FNS diagnosed 41 human cases in 1998, 38 of which were in Corumbá, two in Ladário (an adjacent city), and one in Bolivia (on the border), with eight deaths reported.

Control measures adopted in Corumbá include a canine survey and elimination of seropositive animals, residual spraying inside houses with residual insecticides, and insecticide spraying of property in neighborhoods with known positive cases. Stray dogs are captured and euthanized. At the same time, campaigns are held to educate residents about prevention and control measures being carried out. Even with these measures, however, the disease is not under control in the region. It is clear that further studies and measures that are more effective are needed to eliminate the vectors and reduce the incidence of visceral leishmaniasis in dogs.

Pulvex (Schering-Plough Animal Health) is a 65% permethrin spot-on formulation for dogs with a 30-day residual effect. Although originally used to control fleas and ticks in dogs,[6] the product also has some activity against hematophagous diptera. This action would be very valuable if a similar effect could be demonstrated against phlebotomines, allowing it to be used against visceral leishmaniasis in urban areas. When compared with other control measures, the advantages of using 65% permethrin spot-on include its ease of application and relatively low cost. A study was designed to examine the effect of 65% permethrin spot-on treatment on the prevalence of canine visceral leishmaniasis and the abundance of sand flies in neighborhoods known to have a high prevalence of the disease.

MATERIALS AND METHODS

Enrollment Survey

An enrollment survey to determine the prevalence of visceral leishmaniasis was conducted from April 5 to April 30, 1999 in Cristo Redentor and Popular Velha. Area residents were provided with information about the project; the study area was defined, and all dogs (160 in Cristo Redentor and 230 in Popular Velha) identified in the study area were enrolled. At enrollment, a nylon collar with a unique identification number (which was recorded on an individual reporting form) was placed on each dog.

Treatments

Dogs in Popular Velha were assigned to treatment with 65% permethrin spot-on because of the high incidence of dogs positive for visceral leishmaniasis in this area. Dogs in Cristo Redentor were untreated controls.

Technicians were trained and supervised by a veterinarian to administer three 65% permethrin spot-on treatments (June 15–23, July 13–15, and August 10–12) according to label recommendations to 150, 110, and 99 animals, respectively. Dogs weighing up to 3 kg were treated with one-half of a tube (0.5 ml); dogs weighing 3 to 15 kg received the full contents of one tube (1.0 ml); and dogs weighing more than 15 kg received two tubes (2.0 ml). The product was applied directly on the dry skin of the animals between the scapulas (one-half or one tube) or between the scapulas and at the base of the tail (two tubes). Treatment was postponed for animals that had been wet 2 days or less before the visit. All owners were instructed to prevent dogs from becoming wet for 3 days following the treatment. Gender, breed, and age were not considered as inclusion, exclusion, or treatment criteria in the study.

Sampling for Antibodies to *Leishmania*

Four serologic surveys were conducted in the selected populations. A blood sample was collected from a small cut in the tip of the ear with disposable microlancets and filter paper (3 × 10 cm) interspersed with cellophane. Each sample, identified by the name and number assigned to the animal, was refrigerated until testing. All blood samples were sent to the Leishmaniasis Laboratory of the Entomology and Vectors Technical Management of the FNS in Campo Grande for testing, except those collected at the first treatment, which, were analyzed by the Zoonosis Control Center in Corumbá. Visceral leishmaniasis was diagnosed by indirect immunofluorescence assay.

At enrollment (May 3–June 4), samples were collected from all dogs identified at the two locations. Dogs at both sites were next sampled concurrently with the first application of 65% permethrin at the Popular Velha site (June 15–23). A total of 107 dogs (animals positive for visceral leishmaniasis in the first sampling, those with skin disease, dogs treated with an insecticide product, or any not located after three visits) in Popular Velha were excluded from the project during this sampling period. However, 27 new animals from this town were introduced into the study. The same criteria excluded 72 animals from the Cristo Redentor site, and 58 new animals were introduced during this sampling period.

Blood samples were next collected from September 13 through 17, approximately 30 days after the last treatment with 65% permethrin spot-on (i.e., the end of the residual period for the 65% permethrin). The final sampling was November 10 and 11, approximately 90 days after the last 65% permethrin was applied (i.e., 2 months after the end of the product's residual period).

Trapping of Vectors

Vector traps were set up after dogs were enrolled in the study. Two centrally located houses in each neighborhood were selected as vector capture sites.

Sand flies were captured using Centers for Disease Control and Prevention (CDC) light traps placed 1.5 m above the ground in trees close to the four houses from May 25 to October 27. Although planned to coincide with the start of treatments, problems with the function of the traps led to a 3-week delay in starting this procedure. Vector captures took place between 5 PM and 8 AM 3 days per week for 5 months using two traps separated by 10 to 17 meters at each house. All material collected in the traps was stored in 70% alcohol and sent to the Leishmaniasis Laboratory of the Entomology and Vectors Technical Management of the FNS in Campo Grande with the capture form for taxonomic classification and counting of captured insects.[7]

RESULTS

Popular Velha Site (Treated Dogs)

Results of antibody testing for *Leishmania* spp are presented in Table 1. The enrollment survey (approximately 1 month before treatment) and the initial sampling (at the first treatment) provided baseline information about the visceral leishmaniasis prevalence in the population. Of the 230 blood samples initially collected at the Popular Velha site (treated dogs) during enrollment, 65 (28.3%) were positive for visceral leishmaniasis. By the next sampling (at the first treatment), 22 dogs had died and 20 had moved or run away. However, 27 new animals were identified, tested, and entered into the study at the first treatment. Therefore, 123 of the original animals tested plus 27 new subjects (total: 150) were treated and evaluated in June. Of these 150 animals treated with 65% permethrin in June, 29 (19.3%) were positive for visceral leishmaniasis and subsequently excluded from further testing. In addition, 15 died and 12 could not be located by the next sampling in September. Therefore, 94 dogs were tested in September. Nine of the 94 dogs sampled (9.6%) were positive for visceral leishmaniasis and were eliminated from further testing. Eleven other dogs at the site also were unavailable for further testing at that sampling, including three that had died and eight that could not be located. In November, eight of 74 dogs sampled (10.8%) were positive for visceral leishmaniasis.

Cristo Redentor Site (Untreated Controls)

At the Cristo Redentor site, 25 of 160 blood samples (15.6%) tested in the enrollment survey were positive for visceral leishmaniasis. These 25 dogs were not included in the June sampling. In June, six of 146 dogs sampled (4.1%) were positive for visceral leishmaniasis and another 47 dogs that had either died or could not be located were not included in the September sampling. In September, nine of 121 dogs sampled (7.4%) were positive and 11 other dogs were lost from the study. In November, 17 of 101 dogs sampled (16.8%) were positive for visceral leishmaniasis.

Phlebotomine Populations

The results of phlebotomine captures were divided into three periods to permit evaluations of insect counts relative to treatment periods (Table 2).

Eight phlebotomine species in two genera were found, including seven in the *Lutzomyia*

TABLE 1. Results of Immunofluorescence Assays for Detection of Antibodies to Canine Visceral Leishmaniasis in Neighborhoods in Corumbá, Mato Grosso do Sul, Brazil with and without Treatment of Dogs with 65% Permethrin Spot-On

	Number of Dogs Positive for Leishmania Antibodies/Total Sampled (% Infected)			
Neighborhood	*Enrollment Survey*	*First Treatment (June 15–23)*	*1 Mo after Last Treatment (Sept 13–17)*	*3 Mo after Treatment (Nov 10–11)*
Popular Velha*	65/230 (28.3%)	29/150 (19.3%)	9/94 (9.6%)	8/74 (10.8%)
Cristo Redentor†	25/160 (15.6%)	6/146 (4.1%)	9/121 (7.4%)	17/101 (16.8%)

*Dogs at this location were treated monthly (June, July, August) with 65% permethrin spot-on. All dogs positive for visceral leishmaniasis at any sampling were removed from the study before the next blood sampling.
†Dogs at this location were not treated with an insecticide. All dogs positive for visceral leishmaniasis at any sampling were removed from the study before the next blood sampling.

TABLE 2. Relative and Absolute Abundance of Phlebotomines Captured in CDC Light Traps in Neighborhoods in Corumbá, Mato Grosso do Sul with and without Treatment of Dogs with 65% Permethrin Spot-On

		Popular Velha			*Cristo Redentor*		
Period	*Species*	*Female*	*Male*	*Total*	*Female*	*Male*	*Total*
May 25–June 11*	*Lutzomyia cruzi*	2 (33.3%)	4 (66.7%)	6	1 (20.0%)	4 (80.0%)	5
June 6–Sept 8†	*Lu. cruzi*	4	41	45	4	35	39
	Lu. corumbaensis	0	1	1	4	3	7
	Lu. oliveirai	0	1	1	0	1	1
	Lu. forattinii	1	0	1	0	0	0
	Lu. sordellii	0	0	0	1	0	1
	Lu. goiana	0	0	0	1	0	1
	Subtotal	5 (10.4%)	43 (89.6%)	48	10 (20.4%)	39 (79.6%)	49
Sept 13–Oct 27‡	*Lu. cruzi*	10	13	23	10	18	28
	Lu. corumbaensis	1	2	3	6	6	12
	Lu. oliveirai	0	0	0	0	1	1
	Lu. sordellii	0	1	1	0	0	0
	Lu. trinidadensis	0	0	0	0	1	1
	Brumptomyia spp	2	0	2	1	0	1
	Subtotal	13 (44.8%)	16 (55.3%)	29	17 (39.5%)	26 (60.5%)	43
	Grand total	**20 (31.7%)**	**63 (75.9%)**	**83**	**28 (28.9%)**	**69 (71.1%)**	**97**

*Period before the first treatment with 65% permethrin.
†Period from the first treatment until 1 mo after the last treatment with permethrin (end of the residual effect).
‡Period between 1 and 3 mo after the last treatment with 65% permethrin.
Numbers in parentheses represent the abundance of each sex in relation to the total number trapped in each neighborhood.

genus and one in the genus *Brumptomyia*. Six species were found at the treated site and seven species were collected from traps at the control site. *Lutzomyia forattinii* was only found in the treated neighborhood and *Lutzomyia trinidadensis* only in the control area. The most abundant species overall was *Lu. cruzi* (81.1%), followed by *Lutzomyia corumbaensis* (12.7%) (Table 2).

During the study, 180 phlebotomines were captured, including 83 (46.1%) at Popular Velha and 97 (53.9%) from Cristo Redentor. Approximately 26.7% of the total sand flies captured at both sites were females and 73.3% were males. The distribution of male and female insects was relatively similar between the two sites (i.e., male sand flies generally outnumbered females by a large margin). At both sites, the ratio of males to females was greatest from June to September. The distribution of sand flies shifted after this sampling such that

numbers of male and female sand flies were relatively similar from September to late October at both locations (Table 2).

DISCUSSION

The objective of the survey in September (1 month after the last treatment) was to obtain information about the prevalence of the disease after the product had been used for 3 consecutive months. Similarly, the objective of the sampling in November (3 months after the last treatment) was to evaluate the disease prevalence after a longer incubation period. The transmission of canine visceral leishmaniasis by insect bite is associated with high infection rates and existence of a significant number of healthy, asymptomatic carriers that never develop clinical signs of the disease.[8] One researcher[9] evaluated three conventional measures used to control canine visceral leishmaniasis, including elimination of phlebotomines with effective insecticides, euthanasia of affected dogs, and treatment of dogs and humans with antimonial drugs. The conclusion from that study was that the use of insecticides is the most efficient means to control phlebotomines. Vaccination of dogs is still being studied and could provide a higher protection level for a longer period; however, presently, the best method for control of visceral leishmaniasis seems to be the control of vectors.

A reduction in the prevalence of canine visceral leishmaniasis was observed at the Popular Velha site in the present study. Although the actual prevalence of infected dogs was marginally greater among the treated population than for controls 1 month after the final treatments were given, the infection rate was reduced by approximately 50% from that at the pretreatment sampling (19.3% vs 9.6%). Conversely, the infection rate at the control site was more than 80% higher at the September sampling than that observed pretreatment (4.1% vs 7.4%). A laboratory study conducted in Spain with 65% permethrin product showed that it was an effective repellent and toxicant for sand fly vectors of leishmaniasis.[10]

The most abundant species at both sites, *Lu. cruzi*, is considered the primary vector for *L. chagasi* in Corumbá.[11] Ironically, *Lu. longipalpis*, known as the primary visceral leishmaniasis vector in Brazil,[12] was not found in this study. The number of phlebotomines captured during the study (particularly females) was relatively low, and insects were not dissected to determine the prevalence of infection among these vectors. Therefore, it was not possible to determine patterns or effects of treating dogs with the 65% permethrin product on the vector populations at these locations. The low capture rate is probably related to the project being carried out in a period that did not coincide with peak populations of phlebotomines, such as would be found during the rainy season (October through March). The known high density of vectors in this region, however, increases the risk of visceral leishmaniasis and dengue transmission, and this situation has led to emergency control measures by FNS, such as spraying with residual insecticides in houses located within a 200-m radius of each house with an identified case of visceral leishmaniasis and area-spraying with ultra–low-volume insecticides. Designing the present study to be conducted between April and November took into consideration the interference that could result from the application of other insecticides. As such, it is recognized that the present study alone does not provide adequate statistical evidence of the benefits of treating dogs with 65% permethrin for controlling visceral leishmaniasis through a reduction of phlebotomine populations. It is important that further studies be carried out in different sites and at different times. It will also be essential to gather information on other local control measures used at the test sites.

The presence of several species of domestic animals (horses, cattle, poultry, dogs, swine, and cats) and wild animals (armadillos and opossums) was recorded for both test sites. The observation of unsanitary conditions for particular residences was also noted. The existence of domestic and wild animals close to a residence is likely to attract a large number of phlebotomines, and unsanitary conditions on the property create a favorable habitat for the aggregation of several phlebotomine species,[13,14] increasing the risk of parasite transmission. Although not considered *Leishmania chagasi* reservoirs, horses, calves, and armadillos attract phlebotomines that can feed on dogs and humans.[14] All owners of dogs that tested positive for visceral leishmaniasis were instructed to surrender the animals to authorities for euthanasia; however, several dogs at both locations were not euthanized. Therefore, these animals were sources of infection for others in the area.

CONCLUSION

The monthly application of 65% permethrin spot-on on dogs for 3 consecutive months reduced the prevalence of canine visceral leishmaniasis in a population of dogs living in a hyperendemic area in the city of Corumbá. One month following the three treatments, the infection rate was reduced by 50% from the rate observed before initiation of treatments. In contrast, the infection rate for untreated control dogs in another neighborhood increased more than 80% during the same period. The results suggest that the regular use of 65% permethrin spot-on during months of high risk for canine visceral leishmaniasis can be a useful strategy in reducing the prevalence of this disease in hyperendemic areas. It should be stressed, however, that the success of this strategy also depends not only on the efficacy of the product itself but also on the adoption of other control measures and on economic variables, considering the low purchasing power of the populations living in higher risk neighborhoods.

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