Deltamethrin-impregnated Dog Collars Have a Potent Anti-feeding and Insecticidal Effect on *Lutzomyia longipalpis* and *Lutzomyia migonei*

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Deltamethrin-impregnated PVC dog collars were tested to assess if they were effective in protecting dogs from sand fly bites of Lutzomyia longipalpis *and* Lu. migonei. *A protective effect against Old World species* Phlebotomus perniciosus *was demonstrated before. Four dogs wearing deltamethrin collars and three dogs wearing untreated collars (not impregnated with deltamethrin) were kept in separate kennels for over eight months in a village on the outskirts of Fortaleza in Ceará, Brazil. Periodically, a dog from each group was sedated, placed in a net cage for 2 h in which 150 female sand flies had been released 10-15 min before.* Lu. longipalpis *were used 4, 8, 12, 16, 22, 27, and 35 weeks after the attachment of the collars.* Lu. migonei *were used 3, 7, 11, 15, 22, 26, and 36 weeks after attachment. During 35 weeks, only 4.1% (81 of 2,022)* Lu. longipalpis *recovered from the nets with the deltamethrin collared dogs were engorged, an anti-feeding effect of 96%. Mortality initially was over 90% and at 35 weeks was 35% with half of the sand flies dying in the first 2 h. In contrast, 83% of the 2,094* Lu. longipalpis *recovered from the nets containing the untreated collared dogs were engorged and the mortality ranged from zero to 18.8% on one occasion with 1.1% dying in the first 2 h. Similar findings were found with* Lu. migonei*: of 2,034 sand flies recovered over this period, only 70 were engorged, an anti-feeding effect of 96.5%, and mortality ranged from 91% initially to 46% at 36 weeks. In contrast, engorgement of controls ranged from 91 to71% and a mortality ranged from 3.5 to 29.8%.*

These studies show that deltamethrin impregnated collars can protect dogs against Brazilian sand flies for up to eight months. Thus, they should be useful in a program to control human and canine visceral leishmaniasis.

Key words: sand flies - deltamethrin - anti-feeding effect - *Leishmania* - control - visceral leishmaniasis - dog collars

Visceral leishmaniasis (VL) is one of the most important vector-borne diseases in the world with 400,000 new cases and 50,000 deaths occurring each year worldwide (Murray & Lopez 1996). In 1990, more than 2 million Disability Adjusted Life Years (DALYs) were estimated for VL in the world, second only to malaria for parasitic infections (Murray & Lopez 1996).

VL is a serious health problem in the northeastern states of Brazil and in the State of Minas Gerais (Grimaldi et al. 1989, Marzochi & Marzochi 1994). There is considerable evidence that the domestic dog is an important reservoir host (Nicolle & Comte 1908, Adler & Theodore1932, Bettini et al. 1986, Bettini & Gradoni 1986, Shaw & Lainson 1987, Ashford et al. 1998). The sand fly *Lutzomyia longipalpis* is the vector of the parasite, *Leishmania chagasi*, which is essentially indistinguishable from *Le. infantum* in the Old World (Mauricio et al. 2000a,b). Control of VL has been attempted by the destruction of serologically positive dogs and by spraying insecticides in peridomestic areas.

The cost of these control activities from 1984-1996 in Brazil exceeded $96 million. In 1995 alone, the staff involved in the control program totaled 1,839 (Akhavan 1996). In seven years,

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nearly 1 million houses were sprayed and blood samples were taken from 6.5 million dogs for serology; 153,819 seropositive dogs were destroyed (Akhavan 1996). Despite this effort, morbidity and mortality in the human population remains unacceptably high, and the possibility of reducing the risk of infection to a negligible level is remote by the methods currently in use.

In 1997, Killick-Kendrick et al. reported that plastic dog collars impregnated with the insecticide deltamethrin protected dogs from 96% of *Phlebotomus pernicious* bites for up to eight months. This sand fly transmits *Le. infantum*, the parasite causing canine leishmaniasis (CanL) and VL in France and some other countries of the Mediterranean Basin. If the deltamethrin-impregnated collars can be shown to be effective in preventing sand flies from biting dogs in Brazil, we would have a simple procedure to control this animal reservoir host. In contrast to killing seropositive dogs, collaring dogs would be simpler and more acceptable to owners of the dogs at risk.

In this paper, we show that deltamethrin impregnated dog collars (referred to as deltamethrin collars) are effective in preventing the neotropical sand flies *Lu. longipalpis* and *Lu. migonei* from biting dogs for at least six to eight months and, furthermore, the majority of the sand flies are killed.

MATERIALS AND METHODS

Dog collars - The collars were a gift from Hoechst Roussel Vet, Paris. They consist of a 48 cm strip of white polyvinylchloride (PVC) weighing 20 g impregnated with deltamethrin 40 mg/g and are called Scalibor™ ProtectorBands™. They caused no visible reactions on the skin of the dogs during the period of the experiments. PVC collars without deltamethrin were used as untreated controls. If the collars were large on the dogs, the loose ends were cut off.

Sand flies - *Lu. longipalpis* and *Lu. migonei* sand flies were reared in laboratory colonies at the Laboratory of Entomology at the Fundação Nacional de Saúde, Fortaleza, Ceará (CE), following a technique already described (Killick-Kendrick et al. 1977). The *Lu. longipalpis* colony originated from captures in the municipalities of Baturité and Sobral, CE, in July 1996, whereas the *Lu. migonei* colony originated from captures in the municipality of Maranguape, CE, in September, 1997. Briefly, larvae of both species were reared in plaster-lined pots of volume 200 ml and were fed on rabbit feces. Three days post-emergence, females of *Lutzomyia* of both species were fed on anaesthetised hamsters (Tiopental, 0.025 g/hamster), and were maintained in mesh cages (40 cm^3) in a B.O.D. incubator (Fanem®). Temperature inside the incubator was 25^{o}C and humidity was 80-90%. The sand flies were stored for a period of four days for complete digestion of blood, then were placed in rearing pots, consisting of bottomless plastic pots with the wall and bottom covered by a layer of plaster of Paris. After oviposition, the females died and were removed. The pots containing the hatched larvae were placed in plastic boxes and maintained in the B.O.D incubator at the same humidity (80-90%) and temperature (25^{o}C) as the adults. Adults were maintained in the Barraud cages (40 cm^3) with saturated sucrose solution until the experiments. Sand flies 3-4 days old were used in all experiments, since in preliminary observations, we found that females fed well at that age. On the day of the experiment, female sand flies were counted and transferred using a Castro aspirator (Sherlock & Pessoa 1964, Alexander 2000) into two separate larger plaster-lined pots measuring approximately 1500 cm^3 and kept in styrofoam containers with moist cotton wool to keep the humidity high for transport. The females were transferred into large cubic net cages 10-15 min before the anesthetised dogs were placed inside the nets. The females found dead inside the transport pots were counted and subtracted from the total number of sand flies released into the net cages.

Cubic nets - The net cages used in the experiment to expose the dogs to the sand flies were as described (Killick-Kendrick et al. 1997) with minor modifications. They measured 1.2 m^2, and were 1.8 m in height. The bottom and the lower 55 cm of the walls were made of cotton to prevent drafts while the rest of the net was made of fine netting. On one side, the net had a vertical double-headed zipper to adjust the size of the opening when releasing flies, adding the dogs or entering to capture the flies. The nets were suspended on PVC piping tied by cord to beams in the ceiling. A 6.5 cm thick sponge overlaid with plastic was laid under the net. The nets were placed in two indoor locations and separated by a canvas curtain. In preliminary tests these two locations were shown to give similar sand fly biting rates on dogs prior to the experiments with the collars. The temperature during the experiments varied between 24-28^{o}C, and humidity was 89-98%. In a series of 17 preliminary experiments using 10 dogs, we found no significant difference in the biting of sand flies in this temperature and humidity range. Atmospheric pressure was also measured and was approximately 1009 mb.

Dogs - The dogs were 7-9 month-old mongrels weighing between 6-9 kg from the kennel at the Núcleo de Medicina Trópical, Universidade Federal do Ceará, and from the city pound in Fortaleza. All dogs were vaccinated against rabies (Tec-Par), canine viruses (Duramune, Fort Dodge, Campinas, São Paulo), and de-wormed at least four times with ivermectin and mebendazole before the start of the experiment. The kennels were located in Gereraru, approximately 25 km from Fortaleza. All dogs were fed the same mixture of dog food and given continuous clean water. Ten dogs were initially tested with *Lu. longipalpis* and *Lu. migonei* to assess their variability in attractiveness to sand flies. Three dogs were eliminated before experiments with the collar started because of illness. The four deltamethrin-collared dogs and three untreated-collared dogs were housed in separate kennels made of chicken wire and a roof, and were approximately 37.5 m apart. To avoid contamination, the kennels were cleaned with a neutral soap twice a day by different people, and handlers of the deltamethrin-collared dogs always wore disposable gloves. Dogs were walked to the experimental site, approximately 600 m from the kennels, by separate handlers and entered the indoor test site by different doors.

On the days of the experiments dogs were not fed before sedation. To anesthetise the dogs, atropine (0.044 mg/kg dog's body weight, Aritson) was given intramuscularly 10 min before a mixture of Rompun^R (0.2 mg/kg dog's body weight, Bayer) and Ketalar^R (10mg/kg dog's body weight, Parke-Davis). A second dose, 70-80% of the first dose of Rompun and Ketalar, was administered after the dogs spent 1 h in the net to keep them sedated for a total of 2 h. Dogs wore disposable diapers to absorb urine during the 2-h exposure period in the net. Upon awaking, the dogs were carried back to the kennel in wheelbarrows, were allowed to recover, and were fed. The dogs in each group in the experiments were picked using a rotating schedule.

Exposure of dogs to sand flies - Pots containing 150 females of *Lu. longipalpis* or *Lu. migonei*, were used in the experiments. Male *Lu. longipalpis* exhibit a lek-like behavior and arrive at the source of the blood meal before the females (Jarvis & Rutledge 1992). In some preliminary observations, we therefore added 50 males to the females in the net cages assuming this might improve female engorgement. We found, however, that the females showed an average engorgement of 64% when males were present ($n = 20$) and 81% without males ($n = 5$), $p < 0.025$. Therefore all experiments with collared dogs were carried out only with female flies, which were released into the cubic nets at 6 p.m. (In preliminary studies we found there was no significant difference whether the experiments started at 5:30 p.m. or 7 p.m, sunset being at 5:30 p.m, data not shown). The dogs were placed in the cages 10-15 min after the sand flies and kept there for 2 h in total darkness and then removed. Details of the procedures are given below. The protocol for these studies was approved by the Standing Committee on Animals of the Harvard Medical Area.

Experimental procedure with the collars - At 3, 7, 11, 15, 22, 26 and 36 weeks post-attachment of the collars, dogs were sedated and put into nets with about 150 female *Lu. migonei*. On weeks 4, 8, 12, 16, 22, 27 and 35 weeks post-attachment of the collars, tests were performed with *Lu. longipalpis* using the same protocol. Each week, two sets of experiments were done; each night one deltamethrin-collared dog and one untreated-collared dog were tested, each group always using the same net. The pots were opened to release the flies into the net 10-15 min before the sedated dog was introduced. All experiments began at approximately 6 p.m. While the dog was added or removed, or while recapturing the flies, a fluorescent light was turned on to attract the flies to the top of the net. The dogs were exposed for a 2 h period and examined for sand flies on their fur before being removed. Dead flies knocked down in the net were collected and saved until the next day to see if they recovered, after which they were scored as engorged or non-engorged. Living flies were counted by inspection in the aspirator as they were replaced in the large pot. Accuracy of such inspection compared to inspection under the dissecting microscope was 99.5%. Essentially, throughout the study no sand flies had taken a small blood meal that was detectable only by microscopy. Live flies were scored as engorged and not-engorged. These flies were held in the stock cage for 18 h after which dead flies were counted and scored as engorged and not-engorged and subtracted from the previous night's live totals. We recovered almost every fly from the nets. Percent engorgement was calculated as the ratio of the sum of all dead engorged flies after 2 and 20 h plus live engorged flies over the total of recovered sand flies. Percent mortality was calculated as the ratio of the sum of all dead sand flies after 2 and 20 h over the total of recovered sand flies.

Statistical analysis - Data used to produce Figs 1 and 2 were fitted on a repeated measure model, Stata (StataCorp 1999). In other statistical analysis Student's t test for unpaired samples was used.

RESULTS

The deltamethrin collars had a dramatic anti-feeding effect on both species of sand flies. For *Lu. longipalpis* (Fig. 1A) inhibition of feeding was 99.3% when tested 4 weeks after the application of the collars, 100% after 8 and 12 weeks and over 96% at 16 and 20 weeks. The anti-feeding effect went below 90% in one of two dogs tested at 27 weeks, but was still 94% at 35 weeks. Fitting the data on a repeated measure model (StataCorp 1999), the difference between the anti-feeding on deltamethrin collars and the untreated collars was highly significant ($p < 0.0005$). The anti-feeding effect of the deltamethrin collars was still present after more than eight months (Table I).

The deltamethrin collars also caused mortality of *Lu. longipalpis* (Fig. 1B). This ranged from 96% at 4 weeks to 35% at 35 weeks, gradually falling after 22 weeks. When the data were fitted in the model as above, the difference in the mortality of *Lu. longipalpis* with dogs with the deltamethrin collars compared to dogs with the untreated collars was highly significant ($p < 0.0005$). The mortality of *Lu. longipalpis* was recorded at 2 h and after 20 h. In early experiments almost 100% of the sand flies with the dogs wearing deltamethrin collars were killed in the first 2 h (Table II). Throughout the study an average of 50% of the sand flies died within 2 h. In contrast, over the whole study, only 1.1% of the sand flies with the untreated-collared dogs died at 2 h. With time, the sand fly killing effect diminished considerably faster than the anti-feeding effect (Fig. 1). The majority of the sand flies that engorged on the deltamethrin collared dogs also died. This was 100% during the first 16 weeks, falling to 35% at 35 weeks. In contrast, the mortality of sand flies engorged on dogs with untreated collars ranged from 3.8% to 0.7% with an average of only 1.6%. This difference was significant ($p < 0.01$).

The deltamethrin collars also had a dramatic anti-feeding effect on *Lu. migonei* (Fig. 2A). Inhibition of feeding was 98.3% when tested 3 weeks after the application of the collars, 100% after 7 and 11 weeks, 99.3% at 15 weeks, 94.3% at 26 weeks and 91.8% at 36 weeks. Except for one test on dog # 1 at 26 weeks which showed 86.7% inhibition of feeding, the deltamethrin collars inhibited feeding by more than 90% throughout the 36 weeks of the study. We have no explanation for this single lower value except for biological variation. Fitting the data on a repeated measure model (StataCorp 1999) the difference between the deltamethrin collars and the untreated collars was highly significant ($p < 0.0005$).

The deltamethrin collars also caused mortality of *Lu. migonei* (Fig. 2B). This ranged from 91% at 3 weeks to 46% at 36 weeks. Except for one test at 11 weeks, mortality gradually fell after 22 to 26 weeks. When the data were fitted in the model as above, the mortality of *Lu. migonei* with dogs with the deltamethrin collars compared to the untreated collars was highly significant ($p < 0.0005$). As with *Lu. longipalpis*, the sand fly killing effect diminished considerably faster than the anti-feeding effect. The mortality ranged from 100% to 50% and was still 86% at 22 weeks. In contrast, the mortality of sand flies engorged on dogs with untreated collars ranged from 0 to 17%. This difference was significant ($p < 0.025$). As with *Lu. longipalpis*, the majority (55%) of *Lu. migonei* placed with the deltamethrin collared dogs over the 36 weeks were dead when recovered at 2 h. In contrast, over this period only 0.8% of *Lu. migonei* with untreated collared dogs were dead at 2 h.

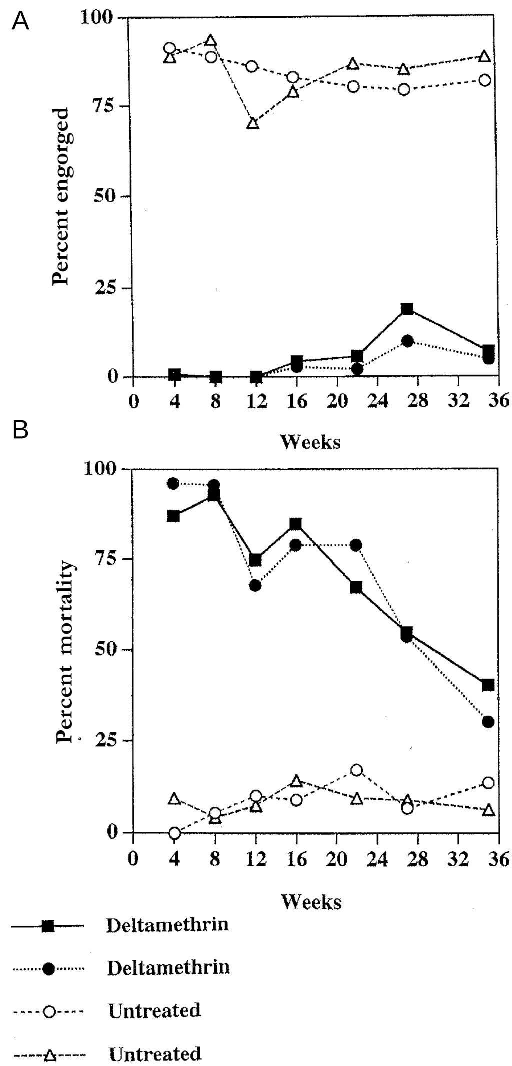

Fig. 1: deltamethrin collars prevent biting by *Lutzomyia longipalpis* and cause mortality. A: the anti-feeding effect is seen by the diminished engorgement in the dogs with the deltamethrin collars. Two of the four deltamethrin-collared dogs and two of the three untreated-collared dogs were tested each week thus the two lines. The identification of the dogs for any given week can be found on Table I; B: the mortality as assessed at 20 h includes sand flies that died after 2 h.

TABLE I

Results of experiments with *Lutzomyia longipalpis* exposed to dogs wearing a deltamethrin-impregnated collar

		Dead flies after 2 h		Dead flies after 20 h		Live flies after 20 h				
Week	Dog	Engorged	Not engorged	Engorged	Not engorged	Engorged	Not engorged	Total[a]	Percent engorged	Percent mortality
4	9	1	124	0	1	0	19	145	0.6	86.9
4	10	1	142	0	2	0	6	151	0.6	96
8	9	0	124	0	13	0	11	148	0	92.6
8	10	0	141	0	6	0	7	154	0	95.5
12	9	0	72	0	26	0	33	131	0	74.8
12	10	0	78	0	13	0	43	134	0	67.9
16	9	4	62	2	53	0	22	143	4.2	84.6
16	10	3	103	1	8	0	31	146	2.7	78.8
22	9	4	49	3	41	1	46	144	5.6	67.4
22	10	1	78	0	36	2	29	146	2.1	78.8
27	9	3	18	7	51	17	48	144	18.8	54.9
27	10	1	34	4	39	9	58	145	9.6	53.8
35	9	0	22	2	35	8	79	146	6.9	40.4
35	10	3	28	1	12	3	98	145	4.8	30.3
Total	-	21	1,075	20	336	40	530	2,022		

a: total = 2 h + 20 h

TABLE II

Results of experiments with *Lutzomyia longipalpis* exposed to dogs wearing a control collar

		Dead flies after 2 h		Dead flies after 20 h		Live flies after 20 h				
Week	Dog	Engorged	Not engorged	Engorged	Not engorged	Engorged	Not engorged	Total[a]	Percent engorged	Percent mortality
4	7	0	0	0	0	135	13	148	91.2	0
4	5	0	1	2	11	131	5	150	88.7	9.3
8	5	0	0	2	6	129	11	148	88.5	5.4
8	6	0	0	1	6	156	5	168	93.5	4.2
12	6	0	2	1	12	127	7	149	85.9	10.1
12	6	0	0	3	9	110	39	161	70.2	7.5
16	7	0	2	4	7	116	16	145	82.8	9
16	5	0	3	5	13	111	15	147	78.9	14.3
22	5	1	0	2	22	114	7	146	80.1	17.1
22	6	0	2	0	12	128	6	148	86.5	9.5
27	7	1	0	2	7	115	24	149	79.2	6.7
27	5	0	2	2	9	121	11	145	84.8	9
35	5	0	3	0	17	119	7	146	81.5	13.7
35	6	0	2	2	5	125	10	144	88.2	6.3
Total	-	2	17	26	136	1,737	176	2,094		

a: total = 2 h + 20 h

Examining all the data to 35 weeks, of the total of 2,022 *Lu. longipalpis* recovered from the nets with the deltamethrin collared dogs, 81 or 4% were engorged (Table I). Further, only 2% of the *Lu. longipalpis* that became engorged in the nets with the deltamethrin-collared dogs during the 35 weeks remained alive. In contrast, of the 2,094 *Lu. longipalpis* recovered from the nets with the untreated collared dogs, 1,737 or 83% were engorged and 98.4% of these engorged flies remained alive. During this period of study, 54% of the sand flies with the deltamethrin-collared dogs died during the first 2 h and only 2% of these dead flies were engorged. In contrast, only 10% of the sand flies in the nets with the untreated- collared dogs died at 2h. Of the 2,094 sand flies in these nets, only 19 sand flies (1.1%) died after 2 h and only 2 of the these flies were engorged.

Similarly, all the data to 36 weeks showed that 21 out of the 2,034 (1%) *Lu. migonei* recovered

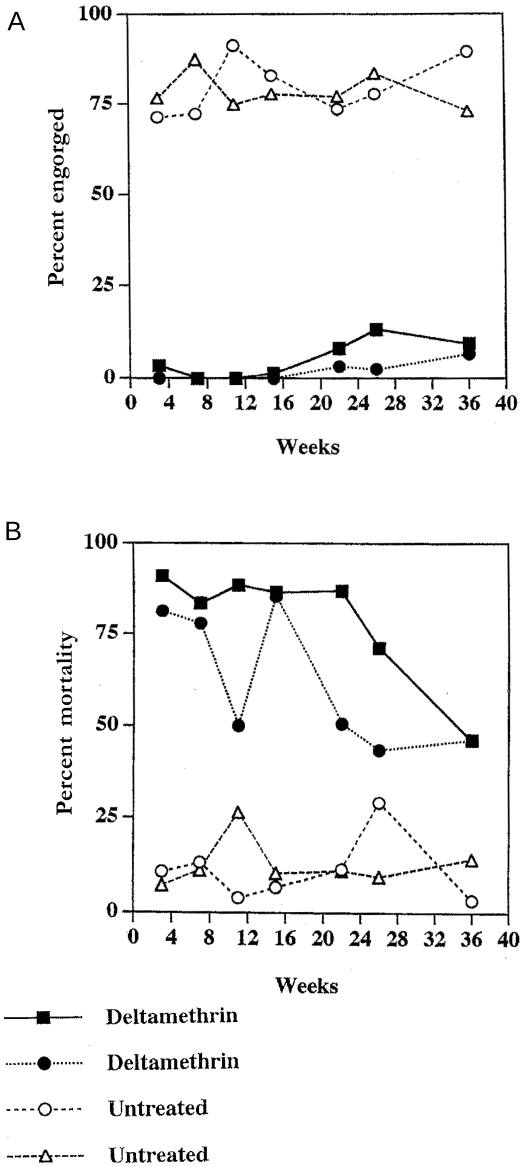

Fig. 2: deltamethrin collars prevent biting by *Lutzomyia migonei* and cause mortality. A: the anti-feeding effect is seen by the diminished engorgement in the dogs with the deltamethrin collars. Two of the four deltamethrin-collared dogs and two of the three untreated-collared dogs were tested each week. The identification of the dogs for any given week can be found on Table II; B: the mortality as assessed at 20 h includes sand flies that died after 2 h.

from the tents with the deltamethrin- collared dogs were engorged and living compared to 1555 out of 2,035 (76%) from the tents with the untreated collared dogs (Table III). Furthermore, 69% of the 68 sand flies that engorged on the deltamethrin-collared dogs died during this period (Table IV).

DISCUSSION

Killick-Kendrick et al. (1997) showed that deltamethrin-impregnated collars prevented the feeding of laboratory reared *P. perniciosus* on beagles by an average of 94% over 34 weeks. Only 0-12% of the recaptured sand flies had fed on deltamethrin collared dogs compared to 55-95% that had fed on the collarless dogs. Furthermore, immediately after the dogs were removed from the nets, 21-60% of the flies from the collared dogs were dead compared to 0-12% from the controls. Lucientes (1999) has confirmed these findings also using laboratory-reared *P. perniciosus* and beagles. He found that 85-98% of the deltamethrin-collared dogs were protected from bites of sand flies compared to dogs with untreated collars lacking deltamethrin. The lethal effect of the deltamethrin collars was about 50%. His study was carried out over 26 weeks. Recently, Halbig et al. (2000) reported on a study in Iran using the same deltamethrin collars and wild-caught *P. papatasi* confined with large guard dogs. They tested the anti-feeding and killing effect before attachment and after the collars had been on the dogs for eight days. It rose from 11 to 51%. Sand fly mortality, however, did not differ, being 17% before and 18% eight days after attaching the collars.

During the development of the impregnated PVC collars, it was found that the deltamethrin was released slowly over several months from the collars (Killick-Kendrick et al. 1997). As deltamethrin is lipophilic like all synthetic pyrethroids (Miller & Salago 1985), it spreads in the dermal secretions over most of the body of the dog. This explains the delay in reaching its maximum efficacy demonstrated by the tests in Killick-Kendrick's study at one week, and probably explains the lower efficacy and lack of lethal effect of the deltamethrin collars in the study on *P. papatasi* done on day 8. Studies by the producers of the collars have also shown that their activity resists both rain and sun. Indeed, they were effective on dogs that were exposed to rain (Killick-Kendrick et al. 1997).

As different species of sand flies, as well as mosquitoes, show different behavior and insecticide susceptibility (Killick-Kendrick 1999), it was important to determine whether if neotropical *Lutzomyia* spp. were as susceptible as *Phlebotomus* spp. to the deltamethrin from the collars. Indeed, whereas pyrethroid impregnated clothing was found to protect against *Phlebotomus* spp. in the Sinai desert (Dees et al. 1985), it protected poorly against *Lutzomyia* spp. in Panama (Schreck et al. 1982). Our studies show that *Lu. longipalpis* and *Lu. migonei* are at least as susceptible as the two previously tested phlebotomines to the anti-feed-

TABLE III

Results of experiments with *Lutzomyia migonei* exposed to dogs wearing a deltamethrin-impregnated collar

		Dead flies after 2 h		Dead flies after 20 h		Live flies after 20 h				
Week	Dog	Engorged	Not engorged	Engorged	Not engorged	Engorged	Not engorged	Total[a]	Percent engorged	Percent mortality
3	1	0	50	3	79	2	11	145	3.5	91
3	8	0	114	0	4	0	27	145	0	81.4
7	1	0	106	0	6	0	22	134	0	83.6
7	8	0	96	0	17	0	32	145	0	77.9
11	1	0	102	0	22	0	16	140	0	88.6
11	8	0	49	0	23	0	72	144	0	50
15	1	2	118	0	9	0	20	149	1.3	86.6
15	8	0	96	0	33	0	22	151	0	85.4
22	1	8	86	2	31	2	17	146	8.2	87
22	8	2	50	1	23	2	72	150	3.3	50.7
26	1	11	79	6	0	1	38	135	13.3	71.1
26	8	2	44	0	22	2	86	156	2.6	43.6
36	1	2	43	3	20	9	70	147	9.5	46.3
36	8	7	54	0	7	3	76	147	6.8	46.3
Total	-	34	1,087	15	296	21	581	2,034		

a: total = 2 h + 20 h

TABLE IV

Results of experiments with *Lutzomyia migonei* exposed to dogs wearing a control collar

		Dead flies after 2 h		Dead flies after 20 h		Live flies after 20 h				
Week	Dog	Engorged	Not engorged	Engorged	Not engorged	Engorged	Not engorged	Total[a]	Percent engorged	Percent mortality
3	5	0	1	3	11	96	28	139	71.2	10.8
3	6	0	1	0	9	105	22	137	76.6	7.3
7	6	0	0	2	17	102	23	144	72.2	13.2
7	7	0	2	2	12	123	4	143	87.4	11.2
11	7	0	1	0	5	136	7	149	91.3	4
11	5	0	2	8	32	111	6	159	74.8	26.4
15	5	0	0	2	8	120	17	147	83	6.8
15	6	0	0	6	9	106	23	144	77.8	10.4
22	6	1	5	3	8	104	26	147	73.5	11.6
22	7	0	0	0	16	111	17	144	77.1	11.1
26	5	0	0	26	16	86	16	144	77.8	29.8
26	6	0	1	2	11	120	12	146	83.6	9.6
36	7	0	0	1	4	128	11	144	89.6	3.5
36	5	0	2	1	18	107	20	148	73	14.2
Total		1	15	56	176	1,555	232	2,035		

a: total = 2 h + 20 h

ing effects of the deltamethrin collars and much more susceptible to the lethal effect. Furthermore, as we used mongrel dogs, our studies show that the anti-feeding and killing effect of the deltamethrin collars are not limited to beagles.

The anti-feeding effect appears to need less pyrethroid than the killing effect as is seen by the more rapid decrease in the lethal effect compared to anti-feeding effect with time in our study, and to the absence of a lethal effect despite an anti-feeding effect in the study after only eight days with *P. papatasi*. This is consistent with the findings of Wu et al. (1991) who showed strong anti-feeding effects of deltamethrin on impregnated gauze on *Culex quinquefasciatus* well below doses recommended to kill the mosquito.

The anti-feeding effects we observed (determined by the lack of engorgement), is probably due to the neurotoxic effects of pyrethroids (Soderlund & Bloomquist 1989). Alterations of the voltage-sensitive sodium channel is the principle molecular target site for all pyrethroids in insects and mammals, although it is probably not the only site involved in intoxication. Insect neurosecretory neurons are sensitive to low concentrations of pyrethroids and disruption of the neuroendocrine systems has also been implicated as contributing to pyrethroid intoxication.

The finding in our study that only 1% of the few sand flies that have fed live after the first bite should significantly reduce the circulation of the *Leishmania* parasite when the dog is the major reservoir host. This is especially true since flies have to bite twice to transmit the *Leishmania* parasite to the reservoir host and the collars should greatly reduce the passage of the parasite from dog to dog as well as from dog to man. Indeed, it is probable the risk of infection to man where the dog is the predominant reservoir host would be reduced significantly or fall to zero (Killick-Kendrick 1999).

These deltamethrin collars should not have the untoward effect of causing sand flies to bite humans instead of dogs. Once having gone to the dogs, the majority of flies would die before getting a blood meal and thus very few of the remaining living sand flies would be infected.

A field trial is about to start to find out whether the collars are effective on dogs in their natural habitat. If they are, the deltamethrin collars would appear to be an excellent and cost effective strategy for the control of leishmaniasis where the dog is the main animal reservoir. Use of collars should be much more acceptable to the population than the current strategy of killing seropositive dogs. The deltamethrin collars, however, must first be shown to be effective in the field, i.e. they should dramatically decrease the seroconversion of seronegative dogs in the endemic areas. The effect of collaring dogs on the incidence of human cases of VL must be also assessed. The collars could also be used to determine how important the dog is in the transmission of the parasites in various other clinical forms of leishmaniasis where dogs have been postulated to be the natural reservoir host.

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