Medical and Veterinary Entomology (1997) **11**, 105–111

Protection of dogs from bites of phlebotomine sandflies by deltamethrin collars for control of canine leishmaniasis

R. KILLICK-KENDRICK,[1] M. KILLICK-KENDRICK,[1] C. FOCHEUX,[2] J. DEREURE,[3] M-P. PUECH[4] and M. C. CADIERGUES[5] [1]Department of Biology, Imperial College at Silwood Park, Berkshire, U.K.; [2]Hoechst Roussel Vet, Romainville, France, [3]Laboratoire d'Ecologie Médicale et Pathologie Parasitaire, Faculté de Médecine, Montpellier, France, [4]23 rue Saunier, 34190 Ganges, France, and [5]Unité de Dermatologie, Ecole Nationale Vétérinaire de Toulouse, Toulouse, France

Abstract. Dog collars made of PVC plastic impregnated with the pyrethroid insecticide deltamethrin at 40 mg/g were investigated for their protective efficacy against phlebotomine sandflies. Collared dogs were kept separately (two untreated control dogs lived together) in outdoor enclosures, each with a kennel, in the Cévennes, southern France. To measure sandfly mortality and anti-feeding effects due to the deltamethrin-impregnated collars worn continuously by the dogs for up to 8 months, each dog was periodically sedated and exposed for 2 h to 150–200 laboratory-reared *Phlebotomus perniciosus* females (plus *c.* 25 males) inside a net (1.2 m square, 1.8 m high) indoors. After dogs were removed from the nets, allowed to recover and returned to their kennels, any dead sandflies were collected from inside the net and counted. Surviving flies were kept overnight, then scored according to whether they were still alive or dead, unfed or blood-fed.

From tests 2, 3, 4, 13, 20, 26 and 34 weeks after the dogs began wearing collars, the overall numbers of blood-fed female sandflies recaptured were 75 from two dogs with collars, compared with 1911 from two collarless dogs. Thus, for every 100 flies which fed on collarless dogs, only 4 fed on collared dogs, i.e. the collars protected dogs from 96% of the bites and this activity was maintained for up to 34 weeks. During the same period, the percentage of recaptured female sandflies that had fed on collared dogs was 0–12% compared to 55–95% on collarless dogs. Immediately after dogs were taken out of the nets, 21–60% of flies confined with the collared dogs were found dead, compared to 0–12% with the controls.

It is concluded that, at least in the Mediterranean subregion, this insecticidal collar would protect a dog from the majority of sandfly bites and retain a killing effect for a complete sandfly season. Moreover, it seems likely that the use of collars on all dogs in a focus of *Leishmania infantum* would reduce contact between sandfly vectors and canine reservoir hosts sufficiently to diminish the risk of infection for humans as well as dogs.

Key words. *Leishmania infantum*, *Phlebotomus perniciosus*, sandflies, dogs, dog collars, canine leishmaniasis, visceral leishmaniasis, sandfly control, deltamethrin, France.

Introduction

Canine leishmaniasis caused by *Leishmania infantum* Nicolle (Trypanosomatidae: Kinetoplastida) is highly prevalent in all countries of the Mediterranean subregion and many Latin American countries.* In some foci it is probable that all dogs are bitten by an infected sandfly in the first transmission season (Dye, 1996). After an incubation period of up to 1 year, or even more (Rioux *et al.*, 1979), some dogs develop clinical signs of leishmaniasis and rising titres of antibodies, whereas others mount a cell-mediated immune response with low, often transitory, titres of antibodies and no sign of disease (Cabral *et al.*, 1992; Pinelli *et al.*, 1994; Killick-Kendrick *et al.*, 1994). Around the Mediterranean, immunofluorescence tests show that prevalence

Correspondence: Professor R. Killick-Kendrick, Imperial College at Silwood Park, Ascot, Berkshire SL5 7PY, U.K.

* Assuming that *Leishmania chagasi* Cunha & Chagas, 1937 is a junior synonym of *Le.infantum* Nicolle, 1908, see Rioux *et al.* (1989).

rates of serologically positive dogs are commonly *c.* 10% (Bettini Gradoni, 1986) but may exceed 33% (Mansueto *et al.*, 1982). Since infected dogs do not necessarily have circulating antibodies (Killick-Kendrick *et al.*, 1994), these figures are underestimates of the true prevalence of infection. This is confirmed by a study in Marseilles in which leishmanial DNA was demonstrated by PCR in the skin or conjunctiva of 24/30 (80%) asymptomatic dogs (Berrahal *et al.*, 1996). Once signs of the disease are apparent, untreated dogs normally die. Treatment (with quinquevalent antimonials) is expensive and usually followed by recurrence. No vaccines are available.

The importance of canine leishmaniasis as a veterinary problem is overshadowed by the fact that dogs are reservoirs of *Le.infantum* that causes visceral leishmaniasis for the human population. Therefore attempts to control canine disease are usually aimed at reducing the risk of infection to man, rather than simply to protect dogs. However, except in western China where canine leishmaniasis and the human disease were eradicated by insecticide spraying and destroying all dogs (Shao *et al.*, 1982), the results of control campaigns have been disappointing. Attempts in Brazil to eliminate the reservoir of infection by culling serologically positive dogs (Vieira *et al.*, 1990) have met with only limited success. Dye's (1996) mathematical models of the options for control strongly suggest that culling is less likely to reduce the incidence of infection than either vaccination, if and when it becomes available, or insecticide spraying in American foci where the vector, *Lutzomyia longipalpis* (Lutz & Neiva), is peridomestic. As vectors in the Mediterranean subregion, e.g. *Phlebotomus ariasi* Tonnoir and *P.perniciosus* Newstead, are mainly exophagic and exophilic, insecticide spraying of houses in this part of the world is not effective and it is recognized that an alternative is urgently needed (Dye, 1996). Gradoni *et al.* (1987) showed that infection rates in sandflies fell after they had fed on leishmanial dogs treated with Glucantime® and suggested that diagnosis and treatment of infected dogs should be considered as a means to control transmission. The disadvantages of this are (a) infected dogs without clinical signs (therefore likely to escape diagnosis and treatment) are capable of infecting sandflies (Molina *et al.*, 1994); (b) costs of surveying and treating dogs are high; and (c) there is risk of selecting drug resistant strains of the parasite (Gramiccia *et al.*, 1992).

We therefore investigated the impact of insecticidal dog collars against leishmaniasis vectors, using a potent lipophilic pyrethroid insecticide that would spread readily over the dog's fur and skin. In this paper we present results of experiments showing that deltamethrin-impregnated collars protect dogs from sandfly bites for at least 34 weeks after attachment. Furthermore, high proportions of flies confined with dogs wearing collars are killed. Our observations suggest that, under natural conditions in a focus of visceral leishmaniasis with a canine reservoir, the use of insecticidal collars on dogs for at least two seasons (spanning the long incubation period of *Le.infantum*) might so interfere with the cycle of transmission that the risk of infection to both human and canine populations would be significantly diminished.

Materials and Methods

Dog collars consisted of a 48 cm strip of white polyvinyl chloride (PVC) weighing 20 g impregnated with deltamethrin (Hoechst Roussel Vet) 40 mg/g. The dogs had no visible reactions where collars were in contact with the skin during the 8 months of the experiments.

Experimental procedure. 1, 2, 3, 4, 13, 20, 26 and 34 weeks after attachment of deltamethrin-impregnated collars, two experimental dogs were sedated and put inside separate nets for 2 h with 160–200 female (and about 25 male) sandflies *Phlebotomus perniciosus*. Two other dogs without collars were similarly exposed as controls. At the end of the 2 h exposure period the dogs were removed and all dead flies were collected, kept at room temperature until the following day (in case they recovered after knock-down) and then counted, examined with a dissecting microscope and recorded as engorged or unengorged. Even a trace of a bloodmeal was considered important because it confirmed that the fly had been in contact with a dog. Live flies were collected and maintained for ~20 h at 19–24°C in suspended gauze 16 cm cubic cages, to allow for the possibility that some flies with the collared dogs might have received a low dose of insecticide causing delayed mortality. After the holding period, they were scored as either (i) live and engorged, (ii) live and unengorged, (iii) dead and engorged, or (iv) dead and unengorged.

Cubic nets (1.8 m high, 1.2 m square), in which dogs were exposed to sandflies, were suspended on frames of copper tubing hanging from wires attached to the walls of a large room. The upper parts and ceiling of the nets were made of fine gauze; the complete floor and lower 55 cm of walls were made of cotton cloth to avoid draughts which interfere with sandfly biting behaviour. Sleeves were fitted on the sides of the nets to introduce sandflies. Access for dogs was by openable zip fasteners fitted along two sides of the net base. Sedated dogs were insulated from the floor by foam cushions wrapped in plastic sheeting under the nets and two disposable napkins were laid inside the nets to absorb urine. Plastic curtains were hung across the room between nets containing collared and control dogs. When necessary, the room was heated to prevent the temperature falling below 20°C; humidity was kept high with damp towels. All experiments were done in the dark, after sunset. A preliminary test with four dogs before collars were attached gave no significant differences between them in the proportions of sandflies that engorged or died.

Dogs, identified by tattooed numbers, were female laboratory-bred beagles aged 7 months when the experiments started. Before the work began, they had been immunized against all common canine viral diseases, treated for worms five times and given prophylactic treatment against ear infections. Five collared dogs were separately housed outside in large enclosures with kennel shelters in the Cévennes area of southern France (Killick-Kendrick & Rioux, 1981). Two control dogs without collars were similarly housed together in another outside enclosure, 25 m from the collared dogs. They were all fed with the same proprietary dog food and given a constant supply of clean water. To avoid contamination of the control collarless dogs by the insecticide, different people handled the collared and collarless dogs, and they were transported to the experimental site in different vehicles in cages assigned to them. Handlers of the collared dogs wore protective clothing and surgical gloves. To ensure there was no variation in the responses of the dogs to sandfly bites that could disturb the flies, they were all sedated before each experiment with Ketamine® and Dormitor®

Table 1. Dates of birth and weights of dogs, dates collars attached, and post-attachment weeks of tests.

Dog	Date of birth	Weight on 21/11/95	Date collar attached	Week of test
296	24/07/95	6.4 kg	Control	1, 2, 3, 4, 13, 20, 26, 34
297	24/07/95	5.7 kg	27/01/96	26, 34
301	31/07/95	6.1 kg	Control	1, 2, 3, 4, 13, 20, 26, 34
302*	31/07/95	5.6 kg	27/01/96	2, 3, 4, 13, 20
306	24/07/95	5.7 kg	27/01/96	2, 3, 4, 13, 20, 26, 34
307†	22/07/95	5.7 kg	23/04/96	1
308	22/07/95	4.1 kg	23/04/96	1

* Dog 302 lost collar after week 20 and was replaced by dog 297.

† Dog 307 lost collar on 26/04/96: another collar attached on 27/04/96.

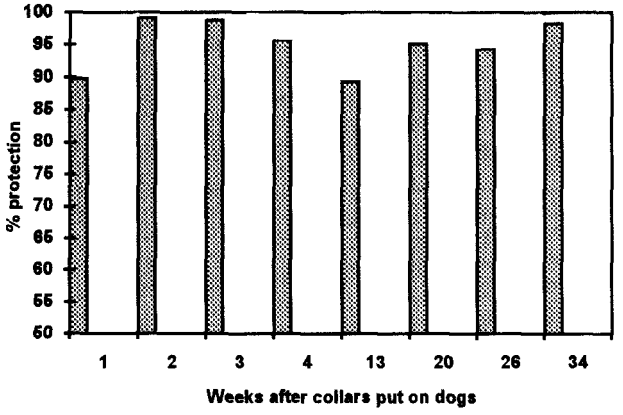

Fig. 1. Percentage protection of dogs from sandfly bites 1–34 weeks after deltamethrin collars attached.

(SmithKline Beecham) given intravenously as a mixture of 0.6–0.9 ml of each drug per 10 kg body weight (depending on the animal's reaction on a previous occasion). At these doses, sedation was satisfactory for the whole 2 h period of exposure.

Two dogs lost their collars during the period of the experiments: dog 307 had another collar attached, but later it was lost, and this dog was taken out of the series; dog 302 lost its collar after 20 weeks and was replaced by dog 297. The same control dogs (nos. 296 and 301) were used in all experiments. Table 1 gives the weeks after collars were attached when each dog was used.

Sandflies were from a closed laboratory colony of *Phlebotomus perniciosus* originating from females collected in Murcia, Spain, in June 1988. Larvae were reared in plastic pots lined with plaster of Paris and were fed on a composted mixture of equal parts of commercially available rabbit chow and rabbit faeces. Females were routinely blood-fed on a hamster or rabbit and transferred to pots for oviposition after complete digestion of the meal. The age of sandflies put into nets with dogs was 7–15 days, i.e. the age when we found that females of this species feed most readily in the conditions of our experiments. Flies of similar age were counted from stock cages (cubic 45 cm) and distributed with an aspirator into four small cages (cubic 16 cm). They were transferred to the nets 15–30 min before the experiments. Any flies dead in the small cages were counted and the number deducted from the total. When opening the zip fasteners at the

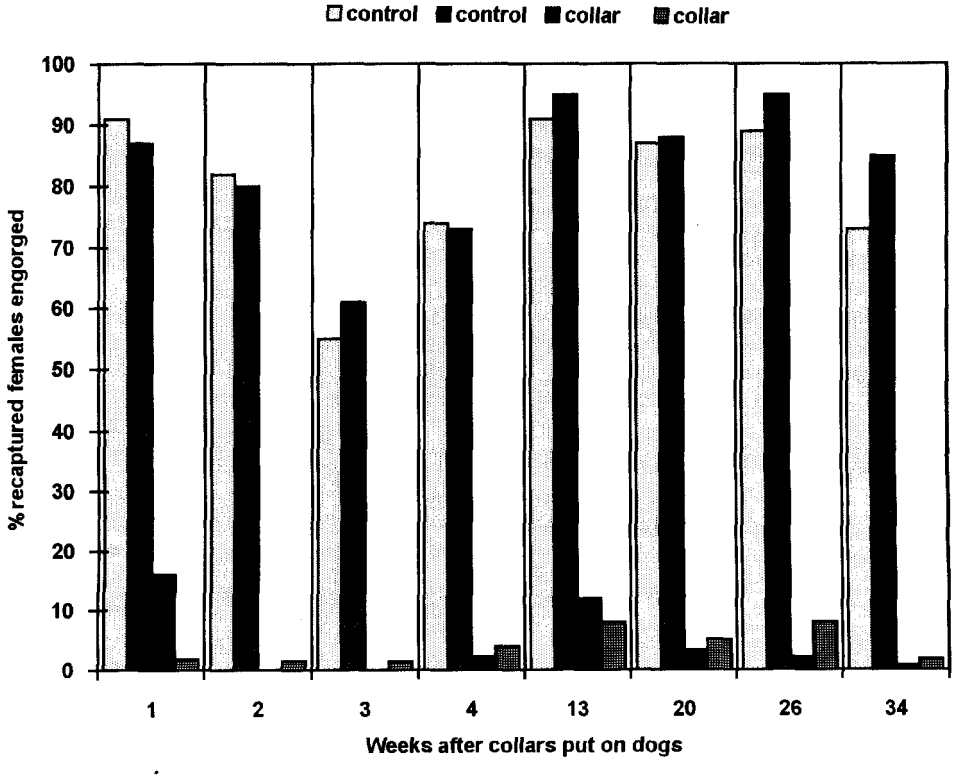

Fig. 2. Percentages of *P.pernicious* females that engorged on control or collared dogs during 2 h exposure under a net.

Table 2. Results of experiments on the anti-feeding and/or lethal effects to sandflies (*Phlebotomus perniciosus*) of deltamethrin collars 1–34 weeks after collars put on dogs. *Phlebotomus perniciosus* rates of blood-feeding and survival from 2 h exposure to dogs with or without (control) deltamethrin collars worn for number of weeks specified.

		No. of sandflies					
Dog	Collar (weeks)	Put with dogs	Collected at 2 h	Dead at 2 h	Dead at 20 h[1]	Total dead	Fed on blood
296	0	196	189	1 (0.5%)	2 (1.1%)	3 (1.6%)	166 (88%)
301	0	190	173	2 (1.2%)	1 (0.6%)	3 (1.7%)	151 (87%)
307[2]	1	191	140	29 (21%)	12 (11%)	41 (29%)	22 (16%)*
308	1	194	168	42 (25%)	32 (25%)	74 (44%)	3 (1.8%)*
296	0	158	152	1 (0.7%)	0	1 (0.7%)	124 (82%)
301	0	157	120	5 (4%)	7 (6%)	12 (10%)	96 (80%)
302	2	151	96[3]	61 (64%)	15 (43%)	76 (79%)	0
306	2	157	130	66 (51%)	13 (20%)	79 (61%)	2 (1.5%)
296	0	165	130	4 (3%)	7 (6%)	11 (8%)	71 (55%)
301	0	165	149	9 (6%)	12 (9%)	21 (14%)	91 (61%)
302	3	167	158	80 (51%)	15 (19%)	95 (60%)	0
306	3	167	146	77 (53%)	6 (9%)	83 (57%)	2 (1.4%)
296	0	200	166	6 (4%)	19 (12%)	25 (15%)	123 (74%)
301	0	197	173	4 (2%)	4 (2.4%)	8 (5%)	127 (73%)
302	4	189	131	58 (44%)	7 (10%)	65 (50%)	3 (2.3%)
306	4	196	152	57 (38%)	26 (27%)	83 (55%)	6 (4.0%)
296	0	199	183	1 (0.5%)	2 (1.1%)	3 (1.6%)	166 (91%)
301	0	195	187	1 (0.5%)	2 (1.1%)	3 (1.6%)	177 (95%)
302	13	192	172	43 (25%)	12 (9%)	55 (32%)	20 (12%)
306	13	194	179	54 (30%)	23 (18%)	77 (43%)	14 (8.0%)
296	0	196	189	0	7 (3.7%)	7 (3.7%)	165 (87%)
301	0	197	196	2 (1.0%)	4 (2.1%)	6 (3.1%)	172 (88%)
302	20	191	177	77 (44%)	20 (20%)	97 (55%)	6 (3.4%)
306	20	?[4]	117	56 (48%)	9 (15%)	65 (56%)	6 (5.1%)
296	0	198	178	2 (1.1%)	8 (4.5%)	10 (5.6%)	159 (89%)
301	0	192	168	2 (1.2%)	10 (6.0%)	12 (7.1%)	159 (95%)
297	26	196	138	47 (34%)	17 (12%)	64 (46%)	3 (2.2%)*
306	26	199	125	55 (44%)	2 (1.6%)	57 (46%)	10 (8.0%)*
296	0	193	174	3 (1.7%)	4 (2.3%)	7 (4.0%)	127 (73%)*
301	0	194	182	5 (2.7%)	3 (1.7%)	8 (4.4%)	154 (85%)*
297	34	197	129	71 (55%)	5 (58%)	81 (63%)	1 (0.8%)
306	34	193	108	65 (60%)	13 (30%)	78 (72%)	2 (1.9%)

[1] Of female flies alive after 2 h exposure with dogs.
[2] Collar seen to be lost on day 3 and replaced by another on day 4.
[3] Some flies lost when sedated dog vomited in the net, so net had to be opened.
[4] Some flies lost when sleeve of net accidentally left open *before* dog put in net.
* Significantly different pairs of replicates.

bottom of the nets to put in and take out dogs, or recapture the flies, lights were put on to attract the flies to the top of the nets. As the dogs were taken out at the end of the exposure, they were examined for dead or feeding flies (which were seldom present). The people who entered the nets to recover the flies wore overalls to prevent being bitten.

Results

The numbers of sandflies used, engorged and dead in each experiment are given in Table 2. Numbers recovered were always less that the number put in the nets, and the figures for engorged or dead females are based on the numbers recaptured.

Anti-feeding effect. Of the sandflies recaptured from tests 2–34 weeks after the dogs began wearing deltamethrin-impregnated collars, 1911 females had engorged on the collarless (control) dogs and 75 on the dogs with collars (Table 2), i.e. the collars protected dogs from 96% of the bites. The percentage protection for each experiment is shown in Fig. 1.

More than 70% of female flies engorged on the control dogs at weeks 2–34 (excluding week 3) compared to < 13% on the collared dogs (Fig. 2). The comparatively low percentages biting even collarless dogs at week 3 (55% and 61%) were perhaps

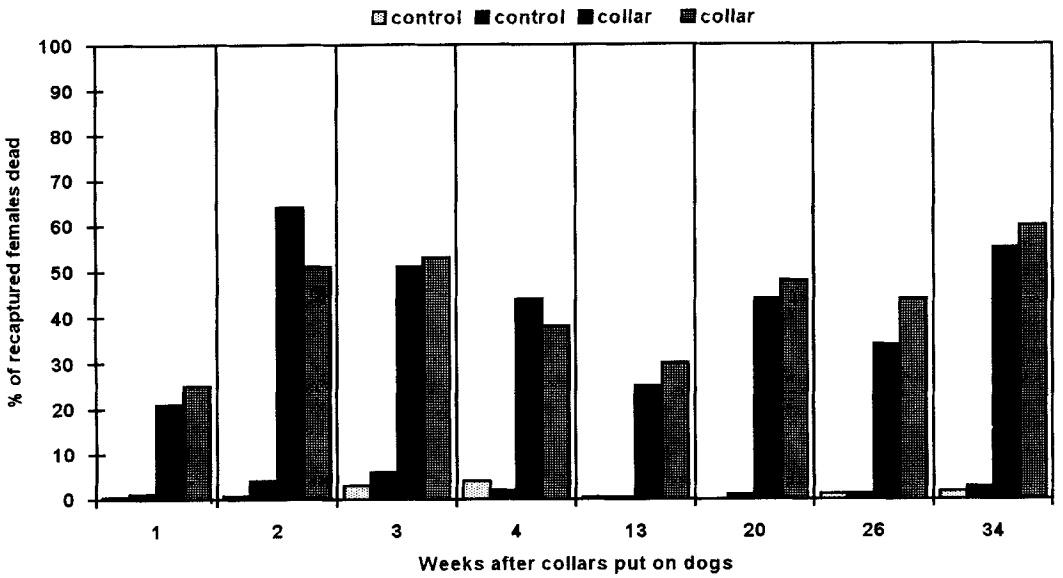

Fig. 3. Percentage of *P.perniciosus* females dead at the end of 2 h exposure to control or collared dogs.

due to the prevailing weather which was stormy with falling atmospheric pressure.

Lethal effect. For sandflies exposed to the dogs 1 week after collars were fitted, before the insecticide from the collars had diffused well over the fur pelt of the dogs, the mortality-rate of sandflies following 2 h exposure was, as expected, the lowest (21% and 25%). From tests in weeks 2–34 the proportions of *P.perniciosus* dead in nets with collared dogs were 25–64%, consistently of much higher statistical significance than 1.1–12.0% dead in nets with collarless dogs (Table 1, Fig. 3). Knock-down followed by recovery was not recorded. For each pair of replicate dogs, there was no statistically significant difference in the proportions of flies dead in the nets at the end of 2 h confinement.

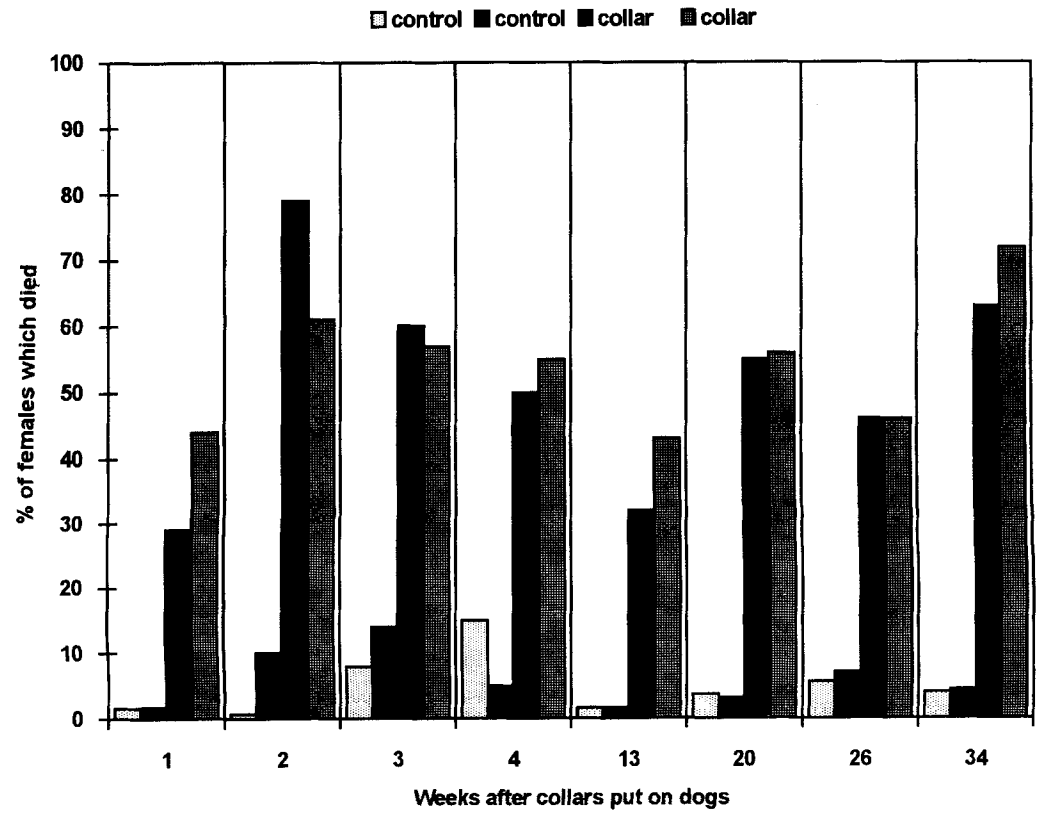

Fig. 4. Percentage mortality of *Phlebotomus perniciosus* females during and ~20 h after 2 h exposure to control or collared dogs.

The death rate of *P.perniciosus* kept for ~20 h post-exposure was generally higher among females confined with the collared dogs than the controls but, since comparatively few survived confinement with the collared dogs, comparisons are misleading. Numbers and percentages of flies dead (in the nets and after ~20 h combined) are shown in Table 2 and Fig. 4. By then, the flies had been handled three times in sucking tubes and some deaths were probably due to damage. Overall mortality of sandflies was <16% with control dogs and >45% with collared dogs (omitting tests at weeks 1 and 13).

Discussion

For this evaluation of deltamethrin-impregnated dog collars, dogs were kept under natural climatic conditions in kennels and outdoor enclosures in southern France. The weather was at first exceptionally wet: 538 mm of rain fell between 25 January, 2 days before the collars were put on, and 20 March 1966. Hence the dogs were subjected to weather similar to that commonly experienced by hunting or guard dogs living outside. On the day when collars were attached, it was raining heavily and the coats of the dogs were saturated with water.

The major limitation of the bioassay was the number of sandflies required. As 600–800 female *P.perniciosus* were used for each test, it was necessary to rear over 12,000 flies (males and females) for eight tests. In preliminary experiments, we found that reducing the number of flies to 100 per net gave unacceptably low proportions of females engorging on the control dogs (<40%): evidently the density of sandflies is an important factor stimulating *P.perniciosus* females to engorge in the laboratory.

During development of the impregnated PVC plastic collar it was found that deltamethrin is released slowly for several months and, as it is lipophilic like all synthetic pyrethroids (Miller & Salgado, 1985), it spreads in the dermal secretions over most of the body of the dog. There is therefore a short delay in reaching maximum efficacy, demonstrated by the test at week 1 when the anti-feeding and lethal effects were less than during the ensuing weeks.

The relatively low numbers of female *P.perniciosus* that fed on the collared dogs compared to the control collarless dogs show that the collars gave a high degree of protection against the bites of sandflies from week 2 until at least week 34. Of the few sandflies that fed on the dogs with collars, most survived until they were scored 20 h later, suggesting that they had not been in contact with the insecticide. It is likely they had engorged on the dog's tongue which was deliberately left out of the mouth during sedation to ensure there was no impediment to breathing.

Deltamethin has previously been shown to protect dogs from the bites of sandflies. In China, a study was made of its effect on *P.chinensis* Newstead. Fifteen dogs bathed in water containing 25 mg of deltamethrin (unspecified concentration) were caged overnight (23.00–07.00 hours) with an unstated number of sandflies in a mosquito net 1, 2 and 'over 2' months after treatment. Only 8/282 (2.8%) fed on treated dogs compared to 198/322 (61.5%) on control dogs. For 70 days the majority of sandflies confined with treated dogs died within 4–5h or exposure (Xiong *et al.*, 1990). Repellent effects of deltamethrin have also been reported with other haematophagous insects, but for shorter times than with *P.perniciosus*, e.g. stable flies >4 days (Escuret & Scheid, 1982) and sheep keds 23 weeks (Hervé, 1985).

Our tests did not include control collars without deltamethrin and we have no evidence of the possible contribution of excipients to the anti-feeding effects, as has been shown with mosquitoes and bednets treated with emulsifiable concentrates consisting of ionic and nonionic aromatic hydrocarbon solvents (Lindsay *et al.*, 1991). However, since the PVC collars did not contain these solvents, it is reasonable to conclude that long-term anti-feeding effects on sandflies were due to deltamethrin absorbed into the fatty secretions of the skin rather than substances used in the manufacture of the collars.

A collar that simply killed sandflies after they had fed on a dog would give no individual protection and, as the vectors feed on a wide range of animals (Guy *et al.*, 1984), would be unlikely to significantly reduce the population of sandflies. In contrast, the strong anti-feeding effects of deltamethrin dog collars could be expected to break the contact between the reservoir host and the vector, thus diminishing the circulation of the parasite.

Deltamethrin collars could be used in at least two ways. Firstly, they offer owners a means of reducing the risk of their dogs acquiring leishmaniasis. In the Mediterranean subregion the sandfly season starts in May and continues until September, or, if the weather remains warm, October. As the sandfly anti-feeding effect of the collars lasts for more than 6 months, an owner should be able to give considerable protection to his dog for a whole sandfly season with only one collar.

Secondly, it is probable that widespread use of the collars on dogs in areas where canine leishmaniasis is prevalent would interfere with the transmission cycle of the parasite to such a degree that the incidence of canine disease would be reduced, or perhaps disappear. As this would also reduce the risk of infection to man, breaking contact between dogs and sandflies with deltamethrin collars is a new option in public health campaigns to control human visceral leishmaniasis with a canine reservoir. This would not necessarily eradicate the disease of man and dogs because the parasite might still be maintained at a low prevalence in foxes (except in Malta where foxes are absent).

Acknowledgments

We thank Mrs Josette Bastide and Mr Patrick Lami for technical assistance, Mr Bill Bell for meteorological data, and Dr John Baker for critically reading the manuscript. We acknowledge with thanks the support of Hoechst Roussel Vet, Paris, the Medical Research Council, London, and the Leverhulme Trust, London.

References

Berrahal, F., Mary, C., Roze, M., Berenger, A., Escoffier, F., Lamouroux, D. & Dunan, S. (1996) Canine leishmaniasis: identification of asymptomatic carriers by polymerase chain reaction and immunoblotting. *American Journal of Tropical Medicine and Hygiene*, **55**, 273–277.

Bettini, S. & Gradoni, L. (1986) Canine leishmaniasis in the Mediterranean area and its implications for human leishmaniasis. *Insect Science and its Application*, **7**, 241–245.

Cabral, M., O'Grady, J. & Alexander, J. (1992) Demonstration of *Leishmania* specific cell mediated and humoral immunity in asymptomatic dogs. *Parasite Immunology*, **14**, 531–539.

Dye, C. (1996) The logic of visceral leishmaniasis control. *American Journal of Tropical Medicine and Hygiene*, **55**, 125–130.

Escuret, P. & Scheid, J.P. (1982) Control of arthropods in veterinary medicine. *Deltamethrin Monograph*, pp. 273–283. Roussel-Uclaf.

Gradoni, L., Maroli, M., Gramiccia, M. & Mancianti, F. (1987) *Leishmania infantum* infection rates in *Phlebotomus perniciosus* fed on naturally infected dogs under antimonial treatment. *Medical and Veterinary Entomology*, **1**, 339–342.

Gramiccia, M., Gradoni, L. & Orsini, S. (1992) Decreased sensitivity to meglumine antimoniate (Glucantime) of *Leishmania infantum* isolated from dogs after several courses of drug treatment. *Annals of Tropical Medicine and Parasitology*, **86**, 613–620.

Guy, M.W., Killick-Kendrick, R., Gill, G.S., Rioux, J-A. & Bray, R.S. (1984) Ecology of leishmaniasis in the south of France. 19. Determination of the hosts of *Phlebotomus ariasi* Tonnoir, 1921 in the Cévennes by bloodmeal analyses. *Annales de Parasitologie Humaine et Comparée*, **59**, 449–458.

Hervé, J.J. (1985) Agricultural, public health and animal health usage. *The Pyrethroid Insecticides* (ed. by J. P. Leahey), pp. 343–425. Taylor & Francis, London.

Killick-Kendrick, R., Killick-Kendrick, M., Pinelli, E., del Real, G., Molina, R., Vitutia, M.M., Canavante, M.C. & Nieto, J. (1994) A laboratory model of canine leishmaniasis: the inoculation of dogs with *Leishmania infantum* promastigotes from midguts of experimentally infected phlebotomine sandflies. *Parasite*, **1**, 311–318.

Killick-Kendrick, R. & Rioux, J.-A. (1981) The Cévennes focus of leishmaniasis in Southern France and the biology of the vector, *Phlebotomus ariasi*. *Parasitological Topics* (ed. by E. U. Canning), pp. 136–145. Society of Protozoologists, Special Publication No. 1. Allen Press, Lawrence, Kansas.

Lindsay, S.W., Adiamah, J.E., Miller, J.E. & Armstrong, J.R.M. (1991) Pyrethroid-treated bednets effects on mosquitoes of the *Anopheles gambiae* complex in The Gambia. *Medical and Veterinary Entomology*, **5**, 477–483.

Mansueto, S., Leo, R., Miceli, M.D. & Quartararo, P. (1982) Canine leishmaniasis in three foci in Western Sicily. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **76**, 565–566.

Miller, T.A. & Salgado, V.L. (1985) The mode if action of pyrethroids on insects. *The Pyrethroid Insecticides* (ed. by J. P. Leahey), pp. 43–97. Taylor & Francis, London.

Molina, R., Amela, C., Nieto, J., San-Andres, M., Gonzalez, F., Castillo, J.A., Lucientes, J. & Alvar, J. (1994) Infectivity of dogs naturally infected with *Leishmania infantum* to colonized *Phlebotomus perniciosus*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **88**, 491–493.

Pinelli, E., Killick-Kendrick, R., Wagenaar, J., Bernardina, W., del Real, G. & Ruitenberg, E.J. (1994) Cellular and humoral immune response in dogs experimentally and naturally infected with *Leishmania infantum*. *Infection and Immunity*, **62**, 229–335.

Rioux, J-A., Killick-Kendrick, R., Leaney, A.J., Young, C.J., Turner, D.P., Lanotte, G. & Bailly, M. (1979) Ecologie des leishmanioses dans le sud de la France. 11. La leishmaniose viscérale canine: succès de la transmission expérimentale 'chien-phlébotome-chien' par la piqûre de *Phlebotomus ariasi* Tonnoir, 1921. *Annales de Parasitologie Humaine et Comparée*, **54**, 401–407.

Rioux, J.-A., Lanotte, G., Serres, E., Pratlong, F., Bastien, P. & Perières, J. (1989) Taxonomy of *Leishmania*. Use of isoenzymes. Suggestions for a new classification. *Annales de Parasitologie Humaine et Comparée*, **65**, 111–125.

Shao, Q.F., Wang, Z.H., Han, S.L. & Zhang, P.J. (1982) The surveillance of kala-azar after its disappearance in the old epidemic area [in Chinese]. *Chinese Journal of Epidemiology*, **3**, 35–37.

Vieira, J.B., Lacerda, M.M. & Marsden, P.D. (1990) National reporting of leishmaniasis: the Brazilian experience. *Parasitology Today*, **6**, 339–340.

Xiong Guan-Hua, Jin Chang-Fa, Chen Xin-Zhong & Su Zhong-Wei. (1990) Chemical insecticide bath of domestic dog in the prevention of sandfly bite. (Abstract). *Proceedings of a Symposium on Medical Parasitology, Shanghai*, pp. 38–39.

Accepted 15 December 1996