Effect of insecticide-impregnated dog collars on incidence of zoonotic visceral leishmaniasis in Iranian children: a matched-cluster randomised trial

A S Mazloumi Gavgani, M H Hodjati, H Mohite, C R Davies

Summary

Background Deltamethrin-impregnated dog collars reduce sandfly bite rates on dogs, and are effective in killing sandflies that attempt to feed. Because domestic dogs are the principal reservoir hosts of zoonotic visceral leishmaniasis, we tested whether community-wide application of dog collars could protect children against infection with *Leishmania infantum*, the parasite that causes the disease.

Methods 18 villages were paired, matched by preintervention child prevalence of *L infantum* infection. Within pairs, villages were randomly assigned to either control or intervention. All domestic dogs in intervention villages were provided with collars for the transmission season. The main outcome measure was incidence of *L infantum* infection after 1 year measured by seroconversion. Secondary outcomes were leishmanin skin test (LST) conversion and seroconversion in dogs.

Findings The seroconversion rate in children was 1·49% (17/1141) in the intervention villages and 2·41% (26/1078) in control villages (odds ratio 0·57, 95% CI 0·36–0·90, p=0·017). LST conversion was also lowered, but not significantly (odds ratio 0·66, 0·41–1·08, p=0·096). The seroconversion rate in dogs in intervention villages was also significantly reduced (0·46, 0·30–0·70, p=0·0003).

Interpretation Community-wide application of deltamethrin-impregnated dog collars not only protects domestic dogs from *L infantum* infections, but might also reduce the risk of *L infantum* infection in children. These dog collars could have a role in control of visceral leishmaniasis and replace controversial dog culling programmes in some countries. However, the effectiveness of dog collars will depend on the importance of wild versus domestic canids as reservoir hosts of *L infantum*.

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Introduction

There are up to half a million new cases of visceral leishmaniasis worldwide every year.[1] Most of these cases are attributable to the virulent *Leishmania donovani* bacteria, but *L infantum* (also known as *L chagasi*) is the most widespread and is endemic in at least 70 countries in Latin America, Africa, Europe, and Asia.[2] Visceral leishmaniasis caused by *L infantum* is zoonotic, and domestic dogs act as the principal reservoir host throughout the world. Transmission is by the bite of a phlebotomine sandfly. In human beings, zoonotic visceral leishmaniasis is characterised by fever, hepatospenomegaly, anaemia, leucopenia, thrombocytopenia, hypergammaglobinaemia, and hypoalbuminaemia, and is generally fatal if untreated.[3] However, most *L infantum* infections in people are subclinical, and cause the development of a transient humoral response followed by a protective cell-mediated immune response, but no clinical symptoms.[4] The ratio of clinical to subclinical infections drops with age, so that nearly all clinical cases are children,[4] unless the disease is an opportunistic infection in HIV-infected patients.[5]

Strategies to control zoonotic visceral leishmaniasis tend to rely on early diagnosis and treatment with expensive and potentially toxic pentavalent antimonial drugs. In some countries with endemic disease, control programmes are designed to prevent infections in human beings by vector control (house spraying with residual insecticides) and reservoir control (culling of infected dogs). Infected dogs are identified either by their clinical signs, such as hair loss, weight loss, and extended nails, or by a serological diagnosis. Although dog culling seems to have been effective in reduction of infection in people in China,[6] whether it has worked in other countries is unclear. In Brazil for example, zoonotic visceral leishmaniasis has increased steadily during the past 10–20 years despite the spraying of 200 000 houses and killing of 20 000 dogs per year.[7]

Dog culling programmes have been questioned on logistical and theoretical grounds,[8,9] and they often fail for several reasons. First, a substantial proportion of infected and infectious dogs are not culled, either because diagnosis is not absolutely sensitive, or because of non-compliance of dog owners. Second, leishmania-positive dogs are identified long after they have been infected, and because surveys are infrequent, the time between surveying, diagnosing, and culling of dogs is long (up to 6 months in Brazil).[7] Third, owners quickly replace their dogs with puppies, which are susceptible to infection. In view of the seriousness of the disease, the unwillingness of dog owners to allow their dogs to be culled, and the inconsistent results of trials designed to test the effectiveness of culling,[10,11] there is a clear need to identify alternative sustainable strategies to reduce the burden of zoonotic visceral leishmaniasis.

Treatment of infected dogs with anti-leishmania drugs is not a practical control policy because of the high cost, and because of the very high relapse rates—up to 74%[12]—in treated and clinically cured dogs. In the absence of a

vaccine for leishmaniasis,[13,14] a novel approach to cut transmission by treatment of domestic dogs with topical insecticides has been proposed, either with lotions[15] or insecticide-impregnated dog collars.[16] The epidemiological effect of topical insecticide treatments on dogs will depend not only on a decrease in the number of sandflies feeding on dogs (ie, an antifeeding effect), but also on a reduction in the survival of those flies that do feed, so that they are less likely to transmit leishmania.

Results of trials with topical insecticide treatments[17] suggest that deltamethrin-impregnated dog collars (Scalibor, Intervet International, Boxmeer, Netherlands) are more effective than pour-on insecticides in reduction of the proportion of surviving bloodfed sandflies on dogs. The insecticidal effect of dog collars lasts longer than topical applications. For example, trials with *Phlebotomus perniciosus*, a vector of zoonotic visceral leishmaniasis in Europe, showed that collars can reduce the proportion of sandflies that bloodfeed and survive by over 90%, for at least 8 months after collar application.[18] On the basis of these findings, we have investigated the epidemiological effects of application of collars to all domestic dogs in a village.

Methods

Location and population

The study area consisted of nine treated and nine control villages in the provinces of Kalaybar and Meshkin-Shahr in northwest Iran. This area is the principal focus of zoonotic visceral leishmaniasis in Iran, with between 100 and 500 cases reported every year since 1987, but no cutaneous leishmaniasis, which is caused by *L major* or *L tropica* infections in other parts of the country. An immunoepidemiological prospective survey done in 38 villages from 1995 to 1996 showed that the mean incidence of leishmania infection in the focus area was 2·8% per year, as measured by leishmanin skin test (LST) conversion. All clinical cases were children, and in children 8% of all infections caused clinical symptoms.[4] The importance of domestic dogs as reservoir hosts in this focus area is suggested by associations between the village dog infection rate (measured by seroprevalence) and the village human infection rate; the number of dogs and the human infection rate; and dog ownership and household infection rate.[19] Transmission in the domestic environment is further indicated by the finding that all household members are at equal risk of infection, independent of age and sex. All parasites isolated from human beings in the region (n=12) and dogs (n=16) were characterised by isoenzyme analysis as *L infantum* zymodeme MON-1.[19] The sandfly vectors in the region have not been conclusively incriminated, but suspected vectors include *P kandelakii* which is active from July to September, suggesting that the transmission season is no more than 3 months.[20]

Trial design

The trial protocol was approved by the ethics committee of Tabriz University of Medical Sciences, Tabriz. Between Jan 18 and March 19, 2000, 21 villages were surveyed. 16 of these villages were previously surveyed in 1995 and 1996, and showed high transmission rates at that time. An additional five were identified as high transmission villages, on the basis that cases had been notified there for 3 consecutive years (1996–98). We explained the logistics and objectives of the intervention trial to community leaders in every village before the trial began. Then, with the informed oral consent of parents or guardians, children between ages 1 and 10 years were tested for leishmania infection; 58% of children were tested by LST and 80% by the direct agglutination test (DAT). Additionally, with the informed consent of their owners, all domestic dogs were tested by DAT between March 18 and April 4, 2000. Masked blood samples were analysed in Tabriz immediately after each field trip. In line with practice in Iran, all stray dogs were killed by village health workers.

Within each district, the trial villages were ranked and ordered into pairs on the basis of DAT prevalence in children, which should indicate present transmission rate, since DAT positivity persists for a maximum of 1–2 years, whereas LST positivity generally persists for life.[4] In the highest prevalence pair in each district, villages were randomly assigned to the intervention or control group by the toss of a coin. All subsequent pairs were then assigned alternately to either the intervention or control groups. Hence, the trial was planned with a matched-cluster randomised trial design.[21] Three villages were excluded before randomisation because we discovered that the populations were largely nomadic, leaving five matched pairs in Kalaybar and four in Meshkin-Shar. Villages were all separated by distances far greater than sandfly flight ranges.

Collars were provided to all domestic dogs in the nine intervention villages between March 18 and April 4, 2000. Dog collars consisted of a 65 cm strip of white polyvinyl chloride weighing 25 g, impregnated with deltamethrin 40 mg per g. Spare collars were left with the village health workers to replace lost collars, and to apply to new dogs. Health workers visited all households in all 18 villages, every 2 weeks during the trial, to record changes in the dog population, and to identify any difficulties with the collars in the intervention villages. Dog owners did not report any trouble with collars. As soon as uncollared dogs were identified in the village, they were diagnosed by DAT and fitted with collars.

A second survey was done in all 18 villages from Jan 8 to March 12, 2001. Where possible, we did a second DAT on all dogs and children previously tested by this method, and a second LST on all children previously diagnosed as negative with this test (figure 1). The main outcome measure was DAT incidence in the 92% of DAT-negative

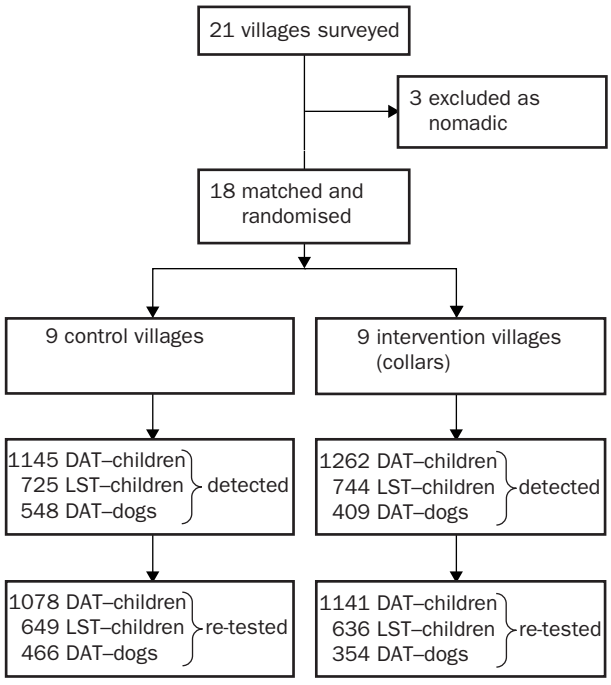

Figure 1: **Trial profile**

children retested. Secondary outcomes were DAT incidence in the 88% of DAT-negative dogs retested and LST rates in the 88% of LST-negative children retested.

Diagnostic methods

For LST, the leishmanin used was derived from *L major* (strain MRHO/IR/75/ER) and prepared at the Pasteur Institute of Iran.[22] The volar surface of one forearm was injected intradermally with 0·1 mL antigen (at a concentration of 10^7 promastigotes per mL). The LST response was measured 48 h later, and an induration width of at least 5 mm regarded as a positive response. The method for human serodiagnoses is standard, and has been described previously.[4] Briefly, fingerprick blood samples were spotted onto filter paper, allowed to dry, and stored at −20°C before elution in citrate saline solution, containing 1% fetal calf serum and 0·1 mol/L 2-mercaptoethanol. The DAT antigen was prepared from log-phase promastigotes of a Sudanese strain of *L donovani* (strain MHOM/SD/68/1S). The cutoff point to designate human infection with *L infantum*, 1 in 1600, was chosen by seeking the best correlations between the village incidence calculated by LST conversion, and the village incidence calculated by seroconversion (with use of a series of different DAT titres to define seroconversion). The specificity of DAT was previously confirmed by tests on healthy controls from non-endemic sites, and on patients with other diseases such as tuberculosis, toxoplasmosis, and malaria.[4]

For serodiagnosis in dogs, animals were first anaesthetised by intramuscular inoculation of acepromazin (2%). At least 1 mL blood was collected from a femur or foreleg vein, centrifuged on the same day, and the serum was stored at −20°C. The antibody titre in the serum was measured in Tabriz by DAT, as described above. The cutoff point to designate dog infections (1 in 800) was chosen to obtain greater sensitivity (96%) and specificity (97%) in comparison with the results from parasitological examination of 114 study site dogs, plus an additional 22 unexposed dogs from the non-endemic region, Tabriz city.

	Frequency % (number positives/number tested)		
	Children		Dogs
	DAT	LST	DAT
Control villages*			
1 Kalalag (K)	10·5% (11/105)	24·8% (26/105)	12·5% (5/40)
2 Galeh-kandi (K)	10·5% (21/200)	23·0% (42/183)	10·2% (5/49)
3 Khomarloo (K)	9·5% (14/148)	21·2% (28/132)	8·6% (3/35)
4 Bashab (K)	7·4% (7/94)	26·0% (19/73)	8·9% (5/56)
5 Abdorrazzag (K)	5·2% (5/97)	23·7% (23/97)	9·4% (3/32)
6 Mejandi (MS)	7·3% (19/260)	23·3% (28/120)	12·1% (20/165)
7 Urkandi (MS)	5·4% (7/130)	17·6% (15/85)	11·8% (11/93)
8 Andarzag (MS)	4·2% (5/118)	17·1% (12/70)	11·8% (12/102)
9 Kevij (MS)	3·5% (3/85)	11·7% (7/60)	7·0 (3/43)
Total	7·4% (92/1237)	21·6% (200/925)	10·9% (67/615)
Treated villages			
10 Kalantar (K)	11·7% (19/163)	26·6% (37/139)	12·1% (4/33)
11 Haddadan (K)	9·2% (13/141)	24·0% (31/129)	14·1% (10/71)
12 Molan (K)	9·1% (24/264)	25·9% (59/228)	7·5% (1/46)
13 Zarbil (K)	7·0% (3/43)	22·0% (9/41)	5·0% (2/40)
14 Mardaneh-gom (K)	6·8% (12/176)	22·9% (39/170)	7·1% (2/28)
15 Doshanloo (MS)	6·7% (9/135)	23·1% (15/65)	13·0% (10/77)
16 Mizan (MS)	6·3% (10/159)	18·6% (13/70)	6·1% (2/33)
17 Hyagh (MS)	6·1% (12/197)	18·7% (14/75)	13·3% (11/83)
18 Kohkanar (MS)	5·5% (5/91)	12·0% (6/50)	14·9% (7/47)
Total	7·8% (107/1369)	23·1% (223/967)	10·7% (49/458)

K=Kalaybar, MS=Meshkin-Shahr.

Table 1: **Preintervention frequency of leishmania infection in children and dogs**

	DAT			LST
	Observed	Expected	Ratio O/E	Observed
Control villages				
1	3·23% (3/93)	2·22%	1·45	1·54% (1/65)
2	3·01% (5/166)	2·48%	1·22	2·92% (4/137)
3	2·65% (3/113)	2·00%	1·33	3·23% (3/93)
4	2·47% (2/81)	2·00%	1·24	0 (0/46)
5	3·41% (3/88)	1·61%	2·12	1·64% (1/61)
6	2·17% (5/230)	1·70%	1·28	2·38% (2/84)
7	3·48% (4/115)	1·61%	2·16	0 (0/67)
8	0 (0/112)	1·63%	0	0 (0/49)
9	1·25% (1/80)	1·58%	0·79	2·13% (1/47)
Average	2·41% (26/1078)	1·9	1·29	1·85% (12/649)
Treated villages				
10	1·49 (2/134)	2·44%	0·61	1·35% (1/74)
11	0·81 (1/123)	2·39%	0·34	1·35% (1/74)
12	2·17 (5/230)	2·23%	0·96	1·31% (2/153)
13	0 (0/34)	1·79%	0	0 (0/26)
14	2·63 (4/152)	2·03%	1·29	1·61% (2/124)
15	0·95 (1/105)	1·61%	0·59	2·56% (1/39)
16	0·76 (1/132)	1·57%	0·48	0 (0/49)
17	1·95 (3/154)	1·58%	1·23	1·89% (1/53)
18	0 (0/77)	1·84%	0	0 (0/44)
Average	1·49% (17/1141)	1·9	0·61	1·26% (8/636)

O/E=observed/expected ratio. For village names, see table 1.

Table 2: **Post intervention incidence of leishmania infection in children**

Statistical analysis

Logistic regression with robust standard errors (ie, clustering on village to control for within-village correlations) was used to test the effect of dog collars on the odds of a child seroconverting, the odds of a child skin-test converting, and the odds of a dog seroconverting. All analyses controlled for preintervention village transmission rate (child seroprevalence). The analyses of DAT incidence in dogs and children also controlled for age (treated as a categorical variable), and the analysis of DAT incidence in children additionally controlled for previous LST status. The planned sample size of nine clusters per treatment type was calculated on the basis of an average of 120 children and 50 dogs per village, followed up for DAT conversion with an expected conversion proportion of 0·05 per year and 0·10 per year, respectively, in the absence of the intervention. With these parameters, and assuming a coefficient of variation of 0·10 (because the villages were well matched), there was 80% power to detect a 53% reduction in annual DAT conversion rate in children, and a 56% reduction in dogs.[21]

To confirm any significant effects detected by these regressions, we also applied a more robust *t* test, comparing the average observed incidence to expected incidence ratios for treated versus control villages. All analyses were done with STATA version 7.

Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, writing of the report, or the decision to submit the paper for publication.

Results

After the trial began, some errors were noted in the preintervention prevalence database, such that the ordering of pairs was slightly inaccurate. Nevertheless, preintervention seroprevalence in children in the nine control villages did not differ significantly from that recorded in the nine treated villages (table 1); nor did preintervention LST prevalence differ between control and treated villages. In dogs, preintervention seroprevalence was also indistinguishable between the two groups (table 1). Hence, the treated and control villages were successfully matched by preintervention transmission rate.

43 DAT conversions were identified in the 2219 DAT-negative children who were retested (table 2). Conversion rate was significantly associated with age (figure 2; p<0·005), presumably because of age-related variability in susceptibility to infection, not exposure, as shown by previous prospective surveys in this endemic zone.[4] Again as expected, there was also some indication that seroconversion rates were higher in LST-negative children than in LST-positive children, even after controlling for age (odds ratio 4·2, 95% CI 1·0–17·6, p=0·05). After controlling for age, LST status, and preintervention village seroprevalence, a significant effect of treatment on risk of DAT conversion was shown by logistic regression, clustering the data on village (odds ratio for DAT conversion in treated *vs* control villages 0·57, 0·36–0·90, p=0·017). We confirmed this result with a more stringent test. In this case, we first generated expected DAT conversion rates in each village on the basis of logistic regressions incorporating age, LST status, and preintervention village seroprevalence (table 2); we then compared the mean ratios of observed to expected DAT conversion rate for the treated and untreated villages by *t* test—ie, giving the results of each village equal weight. The mean observed to expected ratio was significantly lower in the treated villages (p=0·023).

20 LST conversions were recorded in the 1285 LST-negative children who were retested (table 2). The risk of LST conversion during the prospective investigation (0·016) was less than the risk of DAT conversion (0·027) in the same group of initially LST-negative children, as expected from our previous finding that the cell-mediated response develops later than the humoral response following leishmania infection.[4] With these few data, there was no significant difference in LST conversion rate between the intervention and control villages. After controlling for preintervention village seroprevalence, the odds ratio for LST conversion in treated villages was 0·66 (0·41–1·08, p=0·096). Nor was any significant difference detected by the *t* test comparison of mean ratios of observed to expected LST conversion rates (p=0·45, data not shown).

48 of 465 dogs lost collars during the survey; 24 were replaced within 5–15 days. The remaining 24 were not replaced, since the collars were lost after the transmission season had ended (ie, between October and December, 2000). 102 dogs were not retested, since they were lost during the survey (11% of control dogs and 10% of collared dogs), and 92 dogs arrived after the first survey. 28 of these 92 dogs arrived before August, and are included in the conversion rate analysis, since they had a reasonable risk of infection during the transmission season, and were all DAT negative on arrival. None of the dogs that arrived after the season converted.

42 DAT conversions were recorded in the 820 DAT-negative dogs who were retested (table 3). As seen in children, there was a significant association between age and seroconversion rate (p=0·006), and we therefore controlled for age in the models. Village variability in preintervention transmission rate was standardised by controlling for preintervention seroprevalence in children. This is a better indicator of current rates than dog seroprevalence, which is cumulative because no dog serorecoveries were noted during the trial (of 88 positives retested), compared with 23 serorecoveries amongst 183 positive children retested.

A significant effect of treatment on the risk of DAT conversion was shown by logistic regression clustering the data on village (odds ratio for DAT conversion in treated

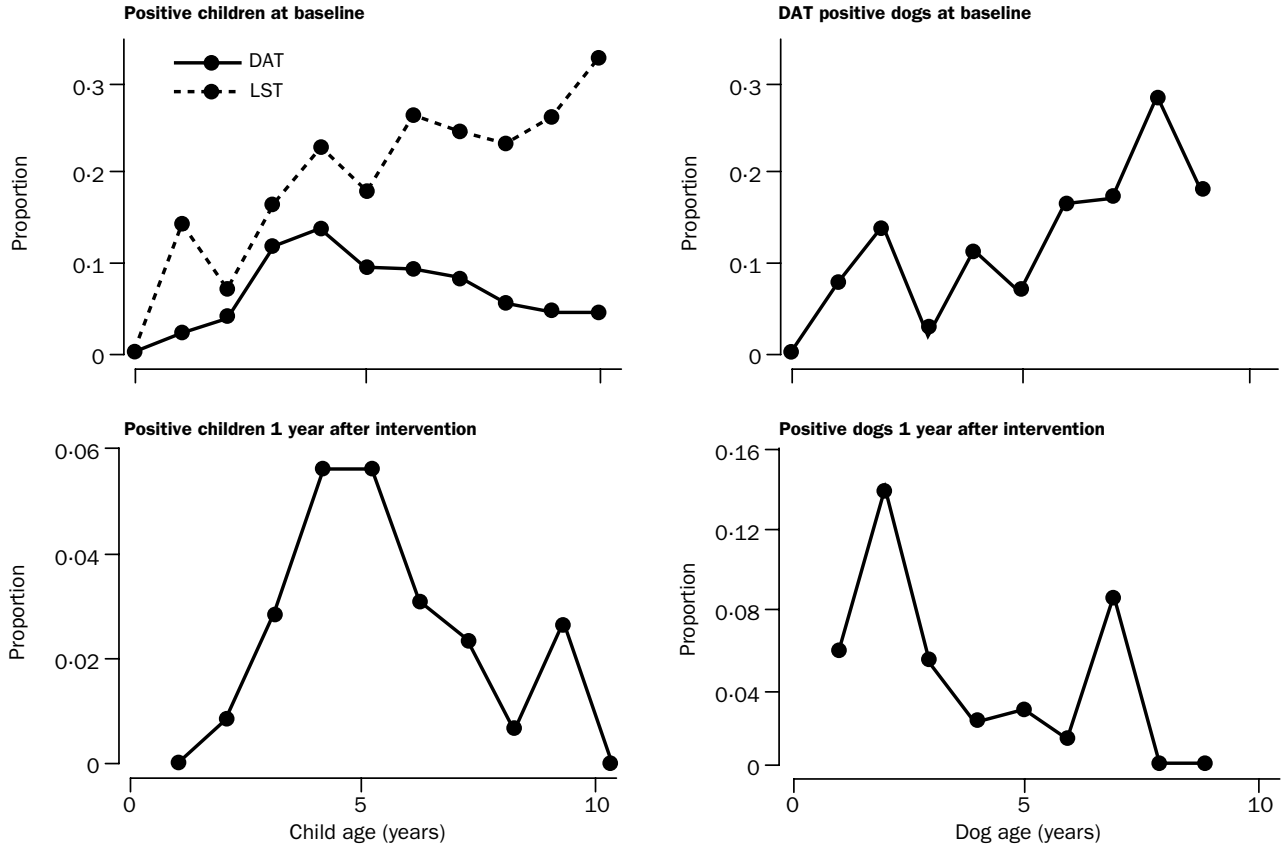

Figure 2: **Proportion of seropositive children and dogs at baseline, and 1 year after intervention**
DAT=direct agglutination test, LST=leishmanin skin test.

	Frequency (number positives/number tested)		
	Observed	Expected	Ratio O/E
Control villages			
1	7·14% (2/28)	4·53%	1·58
2	7·89% (3/38)	5·78%	1·36
3	8·00% (2/25)	4·31%	1·86
4	6·98% (3/43)	3·73%	1·87
5	0 (0/23)	5·22%	0
6	6·61% (8/121)	5·34%	1·24
7	6·85% (5/73)	5·56%	1·23
8	7·50% (6/80)	5·90%	1·27
9	5·71% (2/35)	5·75%	1·00
Average	6·65% (31/466)	5·1%	1·27
Treated villages			
10	0 (0/25)	4·55%	0
11	3·70% (2/54)	4·52%	0·82
12	2·27% (1/44)	4·76%	0·48
13	6·25% (2/32)	4·85%	1·29
14	0 (0/17)	5·22%	0
15	3·39% (2/59)	4·66%	0·74
16	0 (0/28)	5·16%	0
17	4·76% (3/63)	5·49%	0·87
18	3·13% (1/32)	5·21%	0·60
Average	3·11% (11/354)	4·9%	0·53

O/E=observed/expected ratio. For village names, see table 1.

Table 3: **Post intervention incidence of leishmania infection in domestic dogs**

vs control villages 0·46, 0·30–0·70, p=0·0003). We also confirmed this result by the more robust *t* test. In this case, we generated expected DAT conversion rates in each village on the basis of logistic regressions incorporating age and preintervention seroprevalence by village (table 2). The mean ratio of observed to expected DAT conversion rate for the treated villages was significantly less than that for the untreated villages by *t* test (p=0·008).

Discussion

Our results show an epidemiological effect on *L infantum* transmission from the community-wide implementation of insecticide-impregnated collars on domestic dogs. We noted not only that collars protect dogs, as suggested by a field trial in Italy,[23] but also that there was a reduction in the incidence rate of leishmania transmission in children. In view of the few conversions seen in children during the trial, that the results were significant is surprising, and should be interpreted with caution. Nevertheless our best estimate is that the odds of seroconversion in dogs during one transmission season was reduced by 54%, and in children by 43%. We assume that all infected dogs and children had seroconverted by the time of the second survey. This assumption is reasonable since the prepatent period in naturally infected dogs, and probably children, is about 3 months.[24] However, the prepatent period for skin-test conversion in children is substantially longer than this time,[4] and many more infected children will probably skin test convert after the second survey (accounting for why the analysis of the LST data was less informative than the DAT data). Protection against leishmania will probably increase over time if new collars are provided in subsequent years, because the proportion of infected dogs (and hence the transmission rate) will decline steadily as seropositive dogs die and are replaced by seronegative dogs. Although we have no direct evidence, there is no obvious reason why the rate of clinical symptoms of zoonotic visceral leishmaniasis would not decrease with the rate of leishmania infection.

The choice of method for topical insecticide application as a public health means of zoonotic visceral leishmaniasis control (ie, insecticide-impregnated dog collars or topical lotions) will ultimately depend on: first, the relative strength and persistence of their effects on sandfly bloodfeeding and survival; second, the cost of the intervention, and third, the practical applicability of these methods in the community (eg, the willingness to apply topical formulation to dogs, or the efficiency with which lost collars are replaced). At-risk populations are more likely to comply with a topical insecticide treatment programme than the highly unpopular dog culling policy in place in some countries. As well as providing protection for much longer than topical lotions,[17] collars also show that insecticide has been applied, which is an advantage for investigators when following up treated dogs during a control campaign. Balancing these advantages, pour-on lotions are probably easier to use than collars, and wear and tear is not a concern. In our survey, 5% of all collars needed to be replaced before the end of the transmission season (ie, within 5 months). This is a relevant proportion, and might be a drawback in regions with longer transmission seasons than Iran. Collar design could be improved to reduce this rate. High collar coverage rates need to be maintained to have a meaningful effect on leishmania transmission rates; which would require not only rapid replacement of lost collars, but also rapid collaring of new dogs recruited into the population. In our study site, the dog population turnover rate was about 10% per year; Where the turnover rate is much higher, for example, in Brazil,[24] maintaining high collar coverage rates will be a much greater logistic challenge.

In countries with endemic disease and large stray dog populations, the strategy of targeting domestic dogs only may not be thorough enough. In Iran, this strategy is sound because all stray dogs are killed as a generic disease control measure. Because all stray dogs are put down, killing them does not have the same logistic difficulties as killing domestic dogs, which are only slain if they have a positive leishmania diagnosis. Furthermore, stray dogs can be killed immediately after detection, whereas domestic dogs are culled only annually after serosurvey, and there are no compliance issues associated with stray dogs. However, even if stray dogs are culled, the effectiveness of either collars or lotions on domestic dogs will be restricted where wild canids or other mammals have an important reservoir role. In Iran, there is some evidence for *L infantum* infection in jackals and foxes,[19] but their role in maintaining the domestic transmission cycle is not known. The significant reduction in transmission that resulted from collaring of all domestic dogs in this trial, suggests that wild canids are not a major concern in northwest Iran. The protective effect of dog collars against leishmania transmission was as good, or better, than that shown in dog culling trials.[10,11] With respect to cost, the extent to which the collars should be subsidised by government will be determined, not only by the national budget, but also by a comparative evaluation of the public versus private good. If dog owners are willing to pay to protect their dog, then the demand for government resources could be reduced.

Contributors

A S Mazloumi Gavgani coordinated the study with the assistance of M H Hodjati and was responsible for managing all aspects of the fieldwork, data management and laboratory diagnosis. H Mohite was responsible for the dog sampling programme. C R Davies was responsible for the study concept, securing funding, the study design, and the data analysis. All the investigators contributed to the writing of the paper.

Conflict of interest statement
None declared.

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