

## ORIGINAL ARTICLE

# Evaluation of 65% Permethrin Spot-on and Deltamethrin-impregnated Collars for Canine *Leishmania infantum* Infection Prevention

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## Impacts

- Treatment of dogs during sand flies season with synthetic pyrethroids significantly reduce the risk of *Leishmania infantum* infection.

## Key words:

*Leishmania infantum*; dogs; protection; permethrin; deltamethrin

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## Summary

During the 2004 and 2005 sand fly seasons, we evaluated the efficacy of a 65% spot-on solution of permethrin (Exspot, Schering & Plough) and deltamethrin-impregnated collar (Scalibor, Intervet) in reducing *Leishmania infantum* infection, in a canine leishmaniasis (CanL) endemic region (Liguria) in Italy. Immunofluorescent assay (IFA) revealed that three of 120 dogs (2.5%) treated with a 65% spot-on solution of permethrin, as three of 119 dogs (2.5%) treated with deltamethrin-impregnated collar have shown seroconversion after sand fly season. On the contrary, seroconversion was 15% in 188 untreated control dogs. Treatment reduced the risk of infection by 84%. The difference in treated dogs and control ones is highly significant ( $\chi^2 = 12.4$ ;  $P = 0.0004$ ). Our results show that treatment with 65% spot-on solution of permethrin and the deltamethrin-impregnated collar are effective in reducing the risk of acquiring *L. infantum* infection.

## Introduction

Canine leishmaniasis (CanL), caused by *Leishmania infantum*, is a widespread disease in South America and in the Mediterranean area (Dantas-Torres, 2006a), even if new foci have been recently reported in continental area of Europe (Ferroglio et al., 2005). This diphasic parasite is transmitted by the sand fly of the genus *Lutzomyia* in the New World and *Phlebotomus* in the Old World. The dog is the main reservoir. *L. infantum* can also affect humans, causing human visceral leishmaniasis (HVL). CanL control is mainly achieved by treatment of dogs, but it has a limited effect on the infectivity of dogs to sand fly (Gradoni et al., 1987; Alvar et al., 1994). Even if a vaccine is commercially available in Brazil (Dantas-Torres, 2006b), no vaccine is actually registered in Europe for prophylaxis, and the only preventive measure to protect dogs from *L. infantum* infection is the treatment with repellent against the sand flies.

Reithinger et al. (2001) have compared different insecticides and have confirmed that treatment of dogs can significantly reduce the risk of infection. A topical solution of 65% permethrin has been proven to be effective in reducing the number of sand flies that take blood meal on treated dogs in experimental trials (Molina et al., 2001; Reithinger et al., 2001). A field trial, carried out in Brazil using 65% permethrin spot-on, showed that even in field conditions this solution can significantly reduce the risk of *L. infantum* infection in treated dogs (Giffoni et al., 2002).

Different papers have proven deltamethrin-impregnated collar to be effective for protecting dogs against a sand fly bite (Killick-Kendrick et al., 1997; Halbig et al., 2000; David et al., 2001; Maroli et al., 2001; Reithinger et al., 2001, 2004; Foglia Manzillo et al., 2006), but only one citation proved the efficacy of 65% spot-on solution of permethrin in a field trial (Giffoni et al., 2002). In the present work, we compared a 65% spot-on solution of

permethrin and deltamethrin-impregnated collar in a CanL endemic region (Liguria) in Italy.

## Materials and Methods

Test animals were selected in kennel dogs and client-owned dogs in the province of Imperia (Liguria Region, Italy), recorded as endemic area with an old stable focus of CanL (Zaffaroni et al., 1999; Poggi et al., 2002). Selected dogs for pharmacological treatment were chosen according to their life style (permanently outdoor), age (>12 months), absence of clinical signs and negative immunofluorescent assay (IFA) ( $\leq 1 : 20$ ) carried out according to Mancianti and Meciani (1988).

From a kennel with a 21% prevalence infection (E. Ferroglia, unpublished data) a total of 120 dogs, 60 in 2004 and 60 in 2005, were enrolled in the trial. In January–February (2004–2005) all dogs tested negative to the IFA test, and then animals were treated with a 65% spot-on solution of permethrin (Exspot; Schering & Plough, Baton Rouge, LA, USA) every 30 days during sand fly seasons from May to October. Treated dogs were tested in January–February of the following year to evaluate seroconversions.

Client-owned dogs (60 in 2004 and 60 in 2005) protected with a deltamethrin-impregnated collar (Scalibor; Intervet, Boxmeer, the Netherlands) from the mid of May to the end of October, were recruited in veterinary clinics in the same areas following standard procedures for *L. infantum* infection status evaluation. Seven dogs lost collars during the summer, but collars were replaced within 48 h by owners. One dog died in a car accident in summer 2005. Only data from 59 dogs were available for 2004.

Finally, a negative control group was identified in the same area in January–February 2005 and 2006 by enrolment of 188 client-owned dogs (95 in 2005 and 93 in 2006) referred to a veterinary clinic. These dogs did not receive any treatment against sand flies during the summer of 2004 and 2005 and were negative at IFA, respectively, in January–February 2004 and 2005. This option derived from ethical reasons, as CanL is present in the study area.

All dogs included in the trial did not leave the area during sand fly season. Treated and control dogs sera were retested by IFA in January–February (2005 for 2004 sand fly season and 2006 for 2005 sand fly season) of the following year to evaluate seroconversions and considered positive when IFA titre was  $\geq 1 : 160$ . The EPI INFO ver.6 statistical package (Dean et al., 1995) was used to compare prevalence data between dog groups and between seasons with a  $\chi^2$  test. Differences in prevalence were considered as significant when  $P \leq 0.05$ .

## Results

Results of the trial are shown in Table 1. Data reported are cumulative for 2004 and 2005 seasons because there was not significant difference in the two seasons. Data obtained showed that there was no difference between permethrin and deltamethrin-treated groups, while a difference between these two groups and the control group was statistically significant ( $\chi^2 = 12.4$ ;  $P = 0.0004$ ). In both permethrin and deltamethrin-treated groups, a 84% reduction of incidence, by serology, was observed compared with the control group.

## Conclusions

Our results demonstrate that treatment with a 65% spot-on solution of permethrin, as well a deltamethrin-impregnated collar, are effective in reducing the risk of acquiring *L. infantum* infection. Both treatments showed reduction of infection (84%) determined by serology, in treated dogs compared with the control group. In treated dogs, the incidence was 2.5% for both treated groups, while incidence in untreated control group (15%) was significantly higher ( $\chi^2 = 12.4$ ;  $P = 0.0004$ ). In the kennel, seroprevalence to *L. Infantum* seems to be slightly increased than in the rest of the area (15%) (Poggi et al., 2002). However, this data is not significant, so we can assume that exposure risk is the same in both groups. This difference in exposure of the permethrin-treated group underlines the protective effect of the 65%

**Table 1.** Evaluation of a 65% permethrin spot-on (Exspot, Schering & Plough) and of deltamethrin-impregnated collar (Scalibor, Intervet) in preventing *Leishmania infantum* infection determined by Immunofluorescent assay (IFA) in dogs in an endemic area of canine leishmaniasis in Italy during two (2004–2005) sand fly seasons. Dogs tested negative at an IFA ( $< 1 : 20$ ) carried out in January/February were enrolled and retested in January/February the following year. IFA was considered as positive when  $\geq 1 : 160$

Treatment	Sand fly season 2004 positive/total (incidence; 95% confidence interval)	Sand fly season 2005 positive/total (incidence; 95% confidence interval)	Total (2004–2005 sand fly seasons) positive/total (incidence; 95% confidence interval)
Permethrin	1/60 (1.7%; 0.1–10.1%)	2/60 (3.3%; 0.6–12.5%)	3/120 (2.5%; 0.65–7.7%)
Deltamethrin	2/59 (3.3%; 0.6–12.5%)	1/60 (1.7%; 0.1–10.1%)	3/119 (2.5%; 0.65–7.7%)
No treatment	14/95 (14.7%; 8.6–23.8%)	16/93 (17.2%; 10.5–26.7%)	30/188 (15%; 11.1–22.2%)

spot-on permethrin treatment. The incidence in the control group (15%) did not differ significantly compared with the one (12%) reported in the same area in 2001 (Poggi et al., 2002).

A 65% spot-on solution of permethrin has been shown to be efficacious in reducing the risk of a sand fly bite in the laboratory (Molina et al., 2001; Reithinger et al., 2001) and in a field trial (Giffoni et al., 2002). Our results also confirm previous reports on the efficacy of deltamethrin-impregnated collars against sand flies (Killick-Kendrick et al., 1997; Halbig et al., 2000; David et al., 2001; Maroli et al., 2001; Reithinger et al., 2001, 2004; Foglia Manzillo et al., 2006). Reithinger et al. (2004) suggested that treatment of dogs with insecticide could be a feasible alternative to dog culling for controlling CanL in Brazil. This approach clearly represents a new control strategy for CanL, even if Moreno and Alvar (2002) suggested that more studies are needed to evaluate the impact of such treatment on humans. The treatment of dogs with synthetic pyrethroids not only protects them from sand fly bites, but also, due to the 'knock-down' effect of these fast acting insecticides, will kill sand flies taking a blood meal on infected dogs before the vector can infect a second host (dog or human). Even if dog owners treat dogs to protect them from a sand fly bite and *L. infantum* infection, a public health perspective would encourage the synthetic pyrethroids 'knock-down' effect and the prevention of a blood meal. A field trial carried out in Iran, where a mass treatment of dogs with a synthetic pyrethroid was done, has proven to be effective in reduction of HVL cases in children (Mazloumi Gavvani et al., 2002). So, the use of insecticides against sand fly must be considered not only for the protection against sand fly bite of treated dogs, but also it must be proposed, at least in endemic areas with a high incidence of human cases, in a wider public health perspective. Topical application of permethrin can have a relatively immediate effect, while, to be fully protective, collars need to be placed on dogs 10 days before exposure to the sand fly (Reithinger et al., 2001). Collars are effective for 34 weeks (Reithinger et al., 2001), while application of 65% spot-on solution of permethrin needs to be repeated every 30 days (Molina et al., 2001; Giffoni et al., 2002). Collars need also to be routinely checked, as up to 6.9% of collars had to be replaced in a field trial because of failure or because of their loss (Maroli et al., 2001) and 5.9% needed to be replaced in the current trial.

Considering the importance of protecting dogs against sand fly bites and as a public health issue, it would be interesting to evaluate the potential role of mass use of 65% spot-on solution of permethrin to reduce CanL incidence in dogs and humans in CanL endemic areas.

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