http://dx.doi.org/10.4322/rbcv.2015.353

Antifeeding and short-term insecticidal effects against *Lutzomya longipalpis* in dogs treated with permethrin or deltamethrin*

Efeitos não alimentação e inseticida em curto prazo sobre *Lutzomyia longipalpis* em cães tratados com permetrina ou deltametrina

Fernanda dos Santos Alves,** Renata Duarte de Oliveira,*** Guilherme Ribas de Aguiar,*** Ana Carolina Junqueira de Moura,** Fabiola de Oliveira Paes Leme,**** Roberto Baracat de Araujo,**** Adriane Pimenta da Costa Val*****

Abstract

Canine visceral leishmaniasis is a zoonotic disease caused by parasites of the genus *Leishmania* and transmitted by phlebotomine sandflies of the genus *Lutzomyia* in the New World and *Phlebotomus* in the Old World. The individual protection of dogs by treatment with permethrin 65% or by a 4% deltamethrin-impregnated collar is evaluated in this study based on the antifeeding of the products and the short term insecticidal antifeeding effects in the following sites on the dog's body: the inner side of the ear and the inguinal region. Both products produced a decrease in blood feeding and an increase in the antifeeding of the sandflies and the effects were not homogeneous, as there were significant differences between the effects on the pinna and on the inguinal region. The treatments of dogs with synthetic pyrethroids are important strategies to reduce the infection rates in endemic regions however the antifeeding and antifeeding effects were low and may not be sufficient to ensure a dog's individual protection.

Keywords: insecticides, *Leishmania infantum,* psychodidae, zoonosis.

Resumo

Leishmaniose visceral canina é uma zoonose causada por parasitos do gênero *Leishmania* e transmitida por flebótomos do gênero *Lutzomyia* no Novo Mundo e *Phlebotomus* no Velho Mundo. A proteção individual do cão através do tratamento com produtos contendo permetrina 65% ou com a coleira impregnada com deltametrina 4% é avaliada no presente estudo, baseado nos efeitos de não alimetação e inseticida imediatos no pavilhão auditivo direito e região inguinal direita. Ambos os produtos demonstraram redução no ingurgitamento das fêmeas e aumento da mortalidade, mas o efeito não foi homogêneo, uma vez que foram verificadas diferenças estatisticamente significativas nos efeitos nas diferentes regiões. O tratamento dos cães com piretroides é uma importante estratégia para redução das taxas de infecção em regiões endêmicas, no entanto, os efeitos mortalidade e repelência foram baixos e podem não ser suficientes para a proteção individual do cão.

Palavras-chave: inseticidas, *Leishmania infantum*, Psychodidae, zoonose.

Introduction

Canine visceral leishmaniasis (CVL) is a disease caused by parasites of the genus *Leishmania*, class Kinetoplasta and family Trypanosomatidae (GRAMICCIA; GRADONI, 2005). Among the 15 known species that infect human beings, 13 have a zoonotic nature (GRAMICCIA, 2011). Dog infections are of major importance in public health because they are the main reservoir of CVL caused by *Leishmania infantum* (syn. *Leishmania chagasi*) (BANETH, 2006; REITHINGER et al, 2001; SHARMA; SINGH, 2008), and the vectors are the phlebotomines of the genus *Lutzomyia* in the New World and *Phlebotomus* in the Old World (GRAMICCIA; GRADONI, 2005; SHARMA; SINGH, 2008).

Phlebotomine sandflies are holometabolous insects, i.e., they have different stages as follows: egg, four larval stages (instars), pupa and adult. The length of each stage depends on the climatic conditions and food availability. These insects can be found in almost every faunal region but are abundant in neotropical regions (SHERLOCK, 2003). Phlebotomine sandflies are small flying insects, and their hairy body length ranges from 2 to 3 mm; they can be found around human habitations; they are silent; and they are highly active at dawn and at night (BRAZIL; GOMES BRAZIL, 2003) [8]. According to Brazil and Gomes Brazil (2003), they are more active in the afternoon and at night. These insects have a gonadotrophic cycle, i.e., oviposition is preceded by a blood meal (ELNAIEN et al, 1994). According to field and laboratory studies, *Lutzomyia longipalpis* may have more than one blood meal before the oviposition (Giffoni et al., 2002). The need for more than one blood meal may be because of climatic factors such as high temperature and low humidity

*Recebido em 9 de outubro de 2014 e aceito em 13 de maio de 2015.
** Mestrado, Escola de Veterinária, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brasil.
***Graduando em Medicina Veterinária, Escola de Veterinária, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brasil.
****Professor, Escola de Veterinária, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brasil.
*****Professor, Escola de Veterinária, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brasil.
Autor para correspondência: adriane@ufmg.br

that reduce the oviposition. It is not known if this characteristic is common to other phlebotomine species, but the epidemiological phenomenon is of major importance because it may amplify the transmission of the *Leishmania infantum* parasites by these vectors (ELNAIEN et al., 1994).

Conventional control strategies against CVL include insecticide spraying around houses (DDT, cypermethrin or deltamethrin), culling of *Leishmania* – specific seropositive dogs, diagnosis and treatment of human cases and public education (Giffoni et al., 2002; Antoniou et al., 2004, BRASIL, 2006). Although the compulsory culling of seropositive dogs in Brazil has occurred since 1963 (Decree n. 51.838, the Ministry of Health of Brazil, March 14, 1963), the efficacy and ethics of this method have been frequently questioned (Moreira; Souza, 2004). The Manual for the Surveillance and Control of Visceral Leishmaniasis cites as preventive measures the control of stray dogs and environmental sanitation (Brasil, 2006).

Topical formulations with long residual effects are desirable for sustainable control by public health campaigns. The interval between the applications may depend on the renovation rate of the canine population (births/immigration and deaths/emigration) and, in the case of the collars, it may depend on how often the collars are lost or damaged (Courtenay et al., 2009). The choice of the method for topical insecticide application depends on the relative strength and persistence of their antifeeding and antifeeding effects, the cost of the intervention and the practical applicability of these methods within the community (Mazloumi Gavgani et al., 2002). The potential insecticide protection against sandflies will rely on its antifeeding effect (or anti-feeding) that is essential for avoiding the bites and consequently reducing disease transmission (Miró et al., 2007) and on its insecticidal effect (or antifeeding), also essential in reducing disease transmission (David et al., 2001; Molina et al., 2001; Mazloumi Gavgani et al., 2002; Courtenay et al., 2009; Franc; Boushira, 2009).

Classically, the xenodiagnosis for leishmaniasis is performed in the inner side of the dog's ear (Costa Val et al., 2007). In a study, *Leishmania* were more readily identified in an ear skin biopsy than in biopsies from the nose and abdominal areas. There was no significant difference in the parasite load determined by optical microscopy in the biopsies from the ear, nose and abdominal skin. According to these authors, the anatomical region of the skin that is best for diagnosis cannot be inferred (Xavier et al., 2006).

Although the literature regarding the epidemiological aspects of the use of insecticides in dogs is extensive (David et al., 2001; Molina et al., 2001; Mazloumi Gavgani et al., 2002; Courtenay et al., 2009; Franc; Boushira, 2009), little is known regarding the individual protection of dogs. This study was designed to compare the effectiveness of permethrin 65% and a 4% deltamethrin-impregnated collar on their antifeeding and insecticidal effects against *L. longipalpis* and to define whether there is a difference between the pinna and the inguinal region of the dog for xenodiagnosis.

Materials and methods

This study was approved by the Ethics Committee on Animal Experimentation of the Universidade Federal de Minas Gerais (protocol n. 084/2010).

Animals

Ten male and 12 female dogs were divided into 3 groups as follows: one control group (GC) with 6 dogs, one group of 8 dogs treated with permethrin 65% (GP) and one group of 8 dogs treated with deltamethrin 4% (GD).

Treatments

The control group (GC) received no treatment prior to the exposure to the phlebotomine sandflies. The second group (GP) was treated with a *spot-on* permethrin 65% product applied between the scapulae, at a dosage based on the weight of the dog. Total efficacy was considered after three applications, each separated by 30 days according to the product's label. The third group was treated with deltamethrin-impregnated collars, and the efficacy was considered from 21 to 120 days, according to the product's label. The exposure to sand flies occurred during the product´s efficacy, according to its label.

Exposure of the dogs to the sandflies

Approximately 30 female and 30 male *L. longipalpis* sandflies bred in a closed colony from the Parasitology Department of the Biological Science Institute of the Universidade Federal de Minas Gerais were used. For the exposure and evaluation of the antifeeding and insecticidal effect of the products and the possible feeding of the sandflies, a Flebocontainer was used, as in the model described by Costa-Val [20]. The Flebocontainer is a semitransparent white PVC container 10 cm high by 8.7 cm in diameter. At the top of the lid there is a 6-cm diameter gap covered with nylon mesh of 80 openings per cm^2 secured with a silicone adhesive. At a height of 7.5 cm, there is a 15-mm diameter opening for the sandflies. The Flebocontainer was placed through the nylon portion of the screen directly in contact with the skin of the animals. The dogs were sedated with acepromazine (0.22 mg/kg, IM) before the exposure. Two Flebocontainers were used per dog as follows: one was placed on the right pinna (RP), and the other was placed on the right inguinal region (RIR). After 40 minutes, the dead sandflies were counted and recorded. The live insects were killed by freezing, and all the female insects were classified as fed or unfed using a stereoscope (Metrimpex/PZO Labimex.,Hungary)

Statistical analysis

The data were analyzed using the Chi-square test because of the dichotomous nature of the data.

Results

The results of GC, GP and GD are presented in Tables 1, 2 and 3, respectively.

Table 1: The number of fed females, unfed females and males in the right pinna (RP) and in the right inguinal region (RIR) of the control group (GC)

	Females		Males	Dead (total)	Alive (total)	Total
	Fed	Unfed				
RP	261	81	119	14	447	461
RIR	201	85	127	13	400	413
TOTAL	462	166	246	27	847	874

Table 2: The number of fed females, unfed females and males in the right pinna (RP) and in the right inguinal region (RIR) of the dogs treated with 65% permethrin (GP).

	Females		Males	Dead (total)	Alive (total)	Total
	Fed	Unfed				
RP	169	289	325	39	744	783
RIR	79	295	339	17	696	713
TOTAL	248	584	664	56	1440	1496

Table 3: The number of fed females, unfed females and males in the right pinna (RP) and in the right inguinal region (RIR) of the dogs treated with deltamethrin 4% (GD).

	Females		Males	Dead (total)	Alive (total)	Total
	Fed	Unfed				
RP	47	285	255	11	576	587
RIR	100	207	217	7	517	524
TOTAL	147	492	472	18	1093	1111

Antifeeding effect

In the GC, no statistically significant difference was found in the dead insects that were fed and exposed to RP compared to those exposed to RIR.

When comparing the results of the GC to those of the GP, considering the total number of sandflies, the antifeeding effect is statistically significant (p<0, 0001) in the GP. For all the female sandflies exposed to the dogs, 34% fed on the GC and 17% fed on the GP. A significant small number of blood fed insects was found when the RP of both groups was compared as follows: on the GC dogs, 33%, and on GP, 21%. Similar results were obtained with the sandflies exposed to the RIR as follows: 31% (GC) to 12% (GP), a statistically significant (p<0.0001) result.

Analyzing the total number of sandflies from Table 2, 70,19% did not feed on a blood meal, whereas 29,81% fed on the dogs, resulting in a statistically significant antifeeding effect (p<0.005). Comparing the results obtained from the exposure to RP and RIR, there was a statistically significant antifeeding effect for the RIR, where fewer insects fed.

There was a statistically significant effect (p<0.0001) when comparing the number of fed phlebotomines on the GC dogs (36%) with those that fed on the GD dogs (13%). The identical effect was observed when exclusively comparing the insects exposed to the RP as follows: 39% (GC) to 7% (GD). There was a reduction of 50% in the total number of sandflies that fed on the RIR as follows: 34% fed on the GC dogs and 17% on the GD dogs, a statistically significant result (p<0.0001).

Analyzing the data from Table 3, deltamethrin 4% had a significant antifeeding effect (p<0.05), and 23% of all the female insects exposed had a blood meal, and 74% did have a blood meal. Comparing the regions of exposure, the highest number of fed sandflies were found on those exposed to RIR, with a statistically significant effect (p<0.01).

Short term insecticidal effect

There was not a statistically significant difference between the number of dead insects on the GC dogs (1%) and on the GP dogs (2%). Comparing the data on the phlebotomines exposed to the RP, there was no significant difference between the number of dead sandflies on the GP dogs (3%) and on the GC dogs (1%). Similar results were found when comparing the antifeeding of the sandflies exposed to the RIR as follows: there was a death of 1% of insects on the GC dogs and 2% on the GP dogs.

An analysis of the data from Table 2 shows no statistically significant difference (p<0.0001) for the antifeeding effect, and 96,26% of the insects was alive, and 3,74% was dead by the end of the exposure. There was a statistical difference (p<0.05) between the antifeeding effect on the RP and on the RIR, and the effect was more pronounced on the RP.

In the GD group, the deaths of 1% of the phlebotomines were observed when they were exposed to the RP or to the RIR, and the same result was obtained in the GC. Similar results were found between the number of dead insects from the GC (1%) and from the GD (1%) in the RP and in the RIR.

Analyzing the data from Table 3, there was a statistically significant difference (p<0.0001) between the number of dead (1,62%) and live (98,8%) insects at the end of the exposure.

Comparison between the GP and GD groups

When the antifeeding effects of those groups are compared (Table 4), the statistical analyses showed a significant difference (p<0.05) as follows: 2% of dead insects on the GP dogs and 1% on the GD dogs. The antifeeding effect was better on the GP dogs (40%) than on the GD dogs (33%), with a statically significant result (p<0.005).

Table 4: GP and GD exposures

	Females		Males	Dead (total)	Alive (total)	Total
	Fed	Unfed				
Permethrin 65%	248	584	664	56	1440	1496
Deltamethrin 4%	147	492	472	18	1093	1111

Discussion

Several studies have evaluated the efficacy of the 4% deltamethrin-impregnated collar (Killick-Kendrick et al., 1997; Halbiddavid et al., 2001; Reithinget et al., 2001; Mazloumi; Gavgani, 2002; Ferroglio et al., 2008; Franc; Boushira, 2009), and some have evaluated the efficacy of 65% permethrin (Molina et al., 2001; Reithinger et al., 2001; Ferroglio et al., 2008; Molina et al., 2012); some studies have concluded that a deltamethrin-impregnated collar (Mazloumi Gavgani, 2001; Ferroglio et al, 2008) or 65% permethrin (Giffoni et al., 2002; Ferroglio et al., 2008) are valuable resources for the prevention of infection in dogs (Giffoni et al., 2002; Mazloumi Gavgani, 2002; Ferroglio et al., 2008).

Most studies demonstrate high antifeeding and antifeeding effects of the deltamethrin impregnated collars (Killick-Kendrick et al., 1997; David et al., 2001; Reithinger et al, 2001 Ferroglio et al., 2008), but different phlebotomine species were tested. One of the studies demonstrated that the use of a 4% deltamethrin-impregnated collar reduced the number of fed insects on the treated dogs over a period of 2 to 34 weeks after the attachment, with less than 13% of the sandflies fed. The species tested was laboratory reared *Phlebotomus perniciosus* (Killick-Kendrick et al., 1997). Other authors reported that the highest antifeeding effect was achieved on the second month after the attachment, with 68% of unfed wild-caught *Lutzomyia intermedia* (Reithinger et al., 2001). In another study that used *Lutzomyia longipalpis* females, the authors observed a antifeeding effect of 99,3% after 4 weeks of attachment, 100% from 8 to 12 weeks after attachment, and up to 96% after 16 to 20 weeks after attachment (David et al., 2001). There was a reduction from 51% to 11% in the feeding of *Phlebotomus papatasi* caught on the wild 1 week after the attachment (Halbig et al., 2000). The present study observed a antifeeding effect of 74% using *Lutzomyia longipalpis* sandflies reared in a laboratory colony. Many other differences among these studies may explain the different results. Two of the studies exposed the dogs to sandflies for 2 hours (Killick Kendrick et al., 1997; David et al., 2001), and another 2 exposed the dogs for 7 hours (David et al, 2001; Reithinger et al., 2001). The present study exposed the dogs to the sandflies for 40 minutes and used a different methodology, the Flebocontainer, instead of placing the dogs inside cages in which the sandflies were released (Killick-Kendrick et al., 1997; Halbig et al., 2000; David et al., 2001; Reithinger et al., 2001). Some studies tested the collars for consecutive weeks (Killick-Kendrick et al., 1997; David et al, 2001), whereas others tested the collars after 8 days of attachment (Halbig et al., 2000) and after 0, 5 and 12 days of attachment (Reithinger et al., 2001). This study exposed the dogs to the sandflies after varying days of attachment, but within the validity period of the product, as recommended by the manufacturer.

The antifeeding effect found in this study was 1.62%, reflecting the small number of insects that died after being exposed to the dogs wearing a 4% deltamethrin-impregnated collar. Similar data were obtained in another study in which the antifeeding after 7 hours of contact was 13% in the treated animals and 9% in the untreated animals, without statistically significant results (Halbig et al., 2000). Another study showed a antifeeding of 96% of the sandflies exposed after 4 weeks of attachment and of 35% after 35 weeks, which is a statistically significant result when compared to the control group (David et al., 2001). Other studies showed a 30% antifeeding of fed females after 1 month of use and no significant antifeeding effect after 2 months (Reithinger et al., 2001) and a antifeeding effect ranging from 25 to 64% during the tests from the second to the 34th weeks, statistically significant results when compared to dogs that were not wearing the collar (Killick-Kendrick et al., 1997). In the present study, the antifeeding and the antifeeding effects caused by the 4% deltamethrin-impregnated collar observed were small, although statistically significant when compared to the number of fed and alive sandflies, respectively. Such different results may have occurred for the following reasons: in two studies, the authors tested the products against phlebotomonines from the genus *Phlebotomus* (Killick-Kendrick et al, 1997; Halbig et al., 2000); in the present study, the dogs were exposed to sandflies for 40 minutes whereas in other studies, they were exposed for 2 hours (Killick-Kendrick et al., 1997; David et al., 2001) or for 7 hours (Halbig et al., 2000; Reithinger et al., 2001); and the methodology of exposure, as discussed in the previous paragraph.

Few studies tested the individual protection of dogs after the application of 65% permethrin (Molina et al., 2001; Reithinger et al, 2001; Molina et al., 2012). In one of the studies, the 65% permethrin provided a antifeeding effect of 78,5% on the 7th day, with a range of 0 to 42,1% from days 14 to 91 and the antifeeding effect was 100% on the 7th day and 91,4% on the 28th day (Molina et al., 2001). In another study, the blood feeding rates only dropped significantly by 2 months on the permethrin-treated dogs, according to the authors (Reithinger et al., 2001). Some authors demonstrated that the antifeeding effect gradually declined after the topical treatment, but it was acceptable on day 22 (67.6%) (Molina et al., 2012). The topical 65% permethrin was able to protect dogs for 22 days according to one study (Molina et al., 2001), but in the present study, the antifeeding effect was 70,19% and the antifeeding was 3.74%. The different results may be because of different species of phlebotomines studied because 2 of the studies used laboratory-reared *Phlebotomus perniciosus* (Molina et al., 2001; Molina et al., 2012), another used wild-caught *Lutzomyia intermedia* and the present study used *Lutzomyia longipalpis* from a laboratory colony. The time elapsed since the application of the product was not known in the present study, and it was 0, 5 and 12 days after treatment (Reithinger et al, 2001), -8 to 91 days after treatment (Molina et al., 2001), and 1 to 43 days after application (Molina et al., 2012). As to the different climates and the methodologies of exposure of the insects to the dogs, the present study used the Flebocontainer and the other studies exposed the dogs to the sandflies in net cages (Molina et al., 2001; Reithinger et al., 2001; Molina et al., 2012).

Synthetic pyrethroids disperse through a dog's skin by dermal secretions (David et al., 2001), and 65% permethrin impregnates the stratum corneum (Molina et al., 2001). This study suggests that deltamethrin and permethrin do not distribute equally through a dog's body because there were significant differences between the RP and RIR. Permethrin showed a higher antifeeding effect on the RIR and a higher antifeeding effect on RP, and the deltamethrin showed less antifeeding on the RIR and no significant difference in the antifeeding effect on the RP and RIR.

When comparing the antifeeding and antifeeding effects between 65% permethrin and 4% deltamethrin-impregnated collars, only one study was found (Reithinger et al., 2001), and 65% permethrin showed better results in the present study. The other study showed no significant difference between the group of dogs treated with the 4% deltamethrin-impregnated collar and the group treated with permethrin 65% one week after the initial treatment; there was no significant difference between the treated and the control groups (Reithinger et al., 2001). Analyzing the effects of the 1st and 2nd months, the authors observed a significant decrease in the number of fed sandflies, but the best effect was obtained from the deltamethrin group. After 2 months, the authors observed a more effective antifeeding effect with deltamethrin with a reduction of 69% of the sandflies that were ingurgitated and no antifeeding effect in both groups (Reithinger et al., 2001). The different results of the present study may have been caused by the lack of information concerning the time of application of the products instead of by a difference in the methodology and of the phlebotomine species.

Conclusions

The treatment of dogs with synthetic pyrethroids for their individual protection is an important strategy to reduce the infection rates in endemic areas. As observed in this study, the antifeeding and antifeeding effects were small, and the synthetic pyrethroids might not provide individual protection to dogs.

Between the products tested, permethrin exhibited better results on both effects when applied following the manufacturer's instructions and respecting the treatment period. The antifeeding effect of permethrin was small, and it may not provide ideal protection.

The antifeeding effect of the 4% deltamethrin-impregnated collar is small, and few insects died after direct contact with dogs wearing it. The antifeeding of sandflies between the regions studied exhibited no statistically significant difference.

The differences between the vector species, the climate conditions and the resistance to pyrethroids may have caused the less efficient effects. Improvement in the efficacy of the products tested is required to provide better individual protection to the dogs in the endemic areas.

Acknowlegments

The authors would like to thank FAPEMIG and Pró-Reitoria de Pesquisa da UFMG for the support on this research.

References

ANTONIOU M, Messaritakis I, Christodoulou V, Ascoksilaki I, Kanavakis N, Sutton AJ, Carson C, Courtenay O. Increasing incidence of zoonotic visceral leishmaniasis on Crete, Greece. *Emerg Infect Dis* 2001 v. 15, p. 932-934.

BANETH G: Leishmaniasis. In *Infectious diseases of the dog and the cat*. 3rd edition. Edited by Greene CE, St Louis: Elsevier; 2006, p. 685-695.

BRASIL. Ministério da Saúde. Departamento de Vigilância Epidemiológica. *Manual de vigilância e controle da leishmaniose visceral*, Brasília: Ministério da Saúde, 2006.

BRAZIL, RP, Gomes Brazil B: Biologia dos flebotomíneos neotropicais. In: *Flebotomíneos do Brasil.* Edited by Rangel EF and Laison R. Rio de Janeiro: Editora Fiocruz, 2003, p. 257-274.

COSTA VAL AP, Cavalcanti RR, Gontijo NF, Michalick MSM, Alexander B, Williams P, Melo MN. Canine visceral leishmaniasis: Relationships between clinical status, humoral immune response, haematology and *Lutzomyia (Lutzomyia) longipalpis* infectivity. *The Veterinary Journal* 200. v. 174, n. 3, p. 636-643.

COURTENAY O, Kovacic V, Gomes AF, Garcez LM, Quinnel RJ. A long lasting topical deltamethrin treatment to protect dogs against visceral leishmaniasis. *Med Vet Entomol* 2009. v. 23, p. 245-256.

DAVID JR, Stamm LM, Bezerra HS, Souza RN, Killick-Kendrick R, Lima JW. Deltamethrin-impregnated dog collars have a potent anti-feeding and insecticidal effect on *Lutzomyia longipalpis* and *Lutzomyia migonei*. *Mem Inst Oswaldo Cruz* 2001, v. 96, p. 839-847.

ELNAIEM DA, Ward RD, Young PE. Development of Leishmania chagasi (Kinetoplastida: Trypanosomatidae) in the second blood meal of its vector Lutzomyia longipalpis (Diptera:Psychodidae). *Parasitol Res* 1994 v. 80, p. 414-419.

FRANC M, Boushira E. Efficacy of a combination of a fipronil-(S)-methoprene spot on formulation and a deltamethrine-impregnated collar in controlling fleas and sandflies on dogs. *Vet Ther* 2009. v. 10, n. 1-2, p.71-77.

FERROGLIO E, Poggi M, Trisciuoglio A. Evaluation of 65% permethrin spot-on and deltamethrin-impregnated collars for canine *Leishmania infantum* infection prevention. *Zoonoses Public Health* 2008 v. 55, p. 145-148.

GRAMICCIA M, 2011. Recent advances in leishmaniosis in pet animals: epidemiology, diagnostics and anti-vectorial prophylaxis. *Vet Parasitol* 2011. v. 181, p. 23-30.

GRAMICCIA M, Gradoni L. The current status of zoonotic leishmaniases and approaches to disease control. *International Journal for Parasitology* 2005. v. 35, p. 1169-1180.

GIFFONI J, Almeida C, Santos S, Ortega V, Barros A. Evaluation of 65% permethrin spot-on for prevention of canine visceral leishmaniasis effect on disease prevalence and the vectors (Diptera Psychodidae) in a hyperendemic area. *Vet Ther* 2002. v. 3, n. 4.

HALBIG P, Hodjati MH, Mazloumi-Gavgani AS, Mohite H, Davies CR. Further evidence that deltamethrin-impregnated collars protects domestic dogs from sandflies bites. *Med Vet Entomol* 2000. v. 14, p. 223-226.

KILLICK-KENDRICK R, Killick-Kendrick M, Focheux C, Dereure J, Puech MP, Cadiergues MC. Protection of dogs from bites of phlebotomine sandflies by deltamethrin collars for control of canine leishmaniasis. *Med Vet Entomol* 1997. v. 11, p. 105-111.

MAURICIO IL, Gaunt MW, Stothard JR, Miles MA. Genetic typing and phylogeny of the Leishmania donovani complex by restriction analysis of PCR amplified gp63 intergenic regions. *Parasitology* 2001. v. 122, p. 393-403.

MAZLOUMI GAVGANI AS, Hodjati MH, Mohite H, Davies CR. Effect of insecticide-impregnated dog collars on incidence of zoonotic visceral leishmaniasis in Iranian children: a matched-cluster randomized trial. *The Lancet* 2002. v. 360, p. 374-379.

MIRÓ G, Gálvez R, Mateo M, Montoya A, Descalzo MA, Molina R. Evaluation of efficacy of a topically administered combination of imidaclopRIR and permethrin against *Phlebotomus perniciosus* in dog. *Vet Parasitol* 2007. v. 143, p. 375-379.

MOLINA R, Espinosa-Góngora C, Gávez R, Montoya A, Descalzos MA, Jiménez D, Dado D, Miró G. Efficacy of 65% permethrin applied to dogs as a spot-on against *Phlebotomus perniciosus. Vet Parasitol* 2012. V. 187, p. 529-533.

MOLINA R, Lohse JM, Nieto J. Evaluation of a topical solution containing 65% permethrin against the sandfly (*Phlebotomus perniciosus*) in dogs. *Vet Ther* 2001. v. 2, p. 261-267.

MOREIRA FD, Souza VMM, Sreenivasan M, Nascimento EG, Pontes de Carvalho L. Assessment of an optimized dog-culling program in the dynamics of canine *Leishmania* transmission. *Vet Parasitol* 2004. v. 122, p. 245-252.

REITHINGER R, Teodoro U, Davies CR. Topical insecticide treatments to protect dogs from sand fly vectors of leishmaniasis. *Emerg Infec Dis* 2001. v. 7, n. 5, p. 872-876.

SHARMA U, Singh S. Insect vectors of *Leishmania*: distribution, physiology and their control. *Journal Vector Borne Diseases* 2008. v. 45, p. 255-272.

SHERLOCK IA: Importância médico-veterinária. In: *Flebotomíneos do Brasil.* Edited by Rangel EF and Laison R, Rio de Janeiro: Editora Fiocruz, 2003, p. 15-21.

XAVIER SC, de Andrade HM, Monte SJ, Chiarelli IM, Lima WG, Michalick MS, Tafuri WL, Tafuri WL. Comparison of parafin-embedded skin biopsies from diferente anatomical regions as sampling methods for detection of *Leishmania* infection in dogs using histological, immunohistochemical and PCR methods. *BMC Vet Res*. 2006. v. 2, n. 17.