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Original Article

Evaluation of the antifeeding and insecticidal effects of a deltamethrin-impregnated collar on *Lutzomyia longipalpis*

Avaliação dos efeitos repelência e inseticida de uma coleira impregnada com deltametrina sobre *Lutzomyia longipalpis*

Fernanda Morcatti Coura[1], Fabiola de Oliveira Paes Leme[2], Fernanda dos Santos Alves[3], Roberto Baract de Araujo[4], Adriane Pimenta da Costa Val Bicalho[5*]

[1] Departamento de Ciências Agrárias, Instituto Federal de Minas Gerais - IFMG, Campus Bambuí, , MG, Brasil
[2]Departamento de Clinica e Cirurgia Veterinárias,Escola de Veterinária da Universidade Federal de Minas Gerais
[3] Departamento de Clinica e Cirurgia Veterinárias da Escola de Veterinária da UFMG
[4] Departamento de Clinica e Cirurgia Veterinárias da Escola de Veterinária da UFMG
[5] Departamento de Clinica e Cirurgia Veterinárias da Escola de Veterinária da UFMG

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A B S T R A C T

Canine leishmaniasis (CanL) is a vector-transmitted zoonotic disease that can be prevented using topical insecticides. Deltamethrin-impregnated collars (DMC) are an efficient method applied to dogs to prevent CanL; however, few reports have analyzed the efficiency of these collars, especially in dogs that are frequently bathed. The purpose of this study was to evaluate the antifeeding and insecticidal outcomes of a DMC used in dogs. DMCs were used on 12 mongrel dogs. Dogs were exposed to phlebotomine sand flies before the use of DMC and at 7, 21, 60, and 120 days after donning a DMC. Six dogs were bathed biweekly, and six were not bathed during the experiment. After exposure, sand flies were captured and classified as dead, alive, male, and engorged or non-engorged females to calculate the antifeeding and insecticidal effects. The use of a DMC showed insecticidal effects on male and female sand flies, with minimal effects in engorged females. The prevention of blood-feeding by sand flies were also observed. The insecticidal and antifeeding effects were better in bathed than in non-bathed dogs, showing that baths did not influence the effects and can be continued during control of CanL. Our results indicate that DMC can be used to control CanL but should be used combined with other control measures.

Palavras-chave:
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R E S U M O

A leishmaniose visceral canina (LVC) é uma doença zoonótica transmitida por vetor e que pode ser prevenida através da utilização de inseticidas tópicos. Coleiras impregnadas com deltametrina (CID) são um método eficiente e aplicável em cães para prevenir a LVC; no entanto, poucos estudos analisaram a eficácia dessas coleiras, especialmente em cães que são frequentemente banhados. O objetivo desse estudo foi avaliar os efeitos repelência e inseticida de uma CID usada em cães. Foram utilizadas coleiras impregnadas em 12 cães sem raça definida. Os cães foram expostos a flebótomos antes do uso da CID e aos 7, 21, 60 e 120 dias após a colocação das coleiras. Seis dos 12 cães receberam banhos a cada 15 dias e seis cães não foram banhados durante o período do experimento. Após a exposição, flebótomos eram capturados e classificados em mortos, vivos, machos, fêmeas ingurgitadas e fêmeas não ingurgitadas, para calcular os efeitos repelência e inseticida. A utilização da CID demonstrou efeito inseticida em febótomos machos e fêmeas, com mínimos efeitos em fêmeas ingurgitadas A prevenção do ingurgitamento por sangue dos flebótomos foi observado. Os efeitos inseticida e repelência foram melhores em cães submetidos a banho que naqueles que não foram banhados, demonstrando que banhos não influencia os efeitos da coleira e podem ser dados durante o controle da LVC. Nossos resultados indicam que a CID pode ser utilizada no controle da LVC mas deve utilização em conjunto com outras medidas de controle.

* Corresponding author: adriane@vet.ufmg.br

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INTRODUCTION

Canine leishmaniasis (CanL) is caused by *Leishmania (Leishmania) infantum* (Kinetoplastida: Trypanosomatidae), which is highly prevalent in many Mediterranean subregions, Europe, and South America (KILLICK-KENDRICK *et al.*, 1997; REGUERA *et al.*, 2016). Moreover, canine visceral leishmaniasis is a public health problem since dogs are reservoirs of the microorganism. Thus, efforts to control the disease in dogs have focused on humans and dogs themselves (KILLICK-KENDRICK *et al.*, 1997; SEVÁ *et al.*, 2016). This vector-transmitted disease is an emerging condition; CanL is adapting to the environmental changes occurring in the world and is rapidly spreading into new regions or countries that were not previously affected by the disease (MIRÓ *et al.*, 2008; REGUERA *et al.*, 2016). The vectors of the CanL agent are insects of the Psychodidae family, and *Lutzomyia longipalpis* sand flies (family: Psychodidae, subfamily: Phlabotominae) are the main urban vector of leishmaniasis in Brazil (KILLICK-KENDRICK, 1999; SHARMA; SINGH, 2008; FREITAS *et al*., 2012; MIRÓ *et al*., 2014).

Restriction of contact of the vector with its host is the best method to control any vector-borne disease (SHARMA; SINGH, 2008). Therefore, effective prevention against bites of sand flies in dogs can be achieved using a combination of measures, including the use of topical insecticides in these animals (SOLANO-GALLEGO *et al.*, 2011). Dog collars made of chlorinated polyvinyl that are saturated with the synthetic pyrethroid deltamethrin at a concentration of 4% are among the options of potentially viable alternatives for the defense of dogs against CanL (ALEXANDER; MAROLI, 2003).

The antifeeding effect is essential to secure dogs against sand flies via these products, and it is, therefore, essential to prevent bites. Reducing bites and feeding reduces the rate of infected vectors and the subsequent transmission of the disease (MIRÓ *et al.*, 2008; SEVÁ *et al.*, 2016). The antifeeding effect also depends on its insecticidal effect, since there is a reduction of the number of newly infected vectors (DAVID *et al*., 2001; COURTENAY *et al*., 2009; MOLINA *et al*., 2012; SEVÁ *et al*., 2016).

Individual protective measures of dogs obtained from the use of deltamethrin-impregnated collars (DMC) has been examined by several authors (DAVID *et al*., 2001; HALBIG *et al*., 2000; KILLICK-KENDRICK *et al*., 1997; REITHINGER *et al*., 2001; COURTENAY *et al*., 2009; SEVÁ *et al*., 2016), but the results were contrasting. No report was found in the literature on the efficiency of these collars used in dogs that are frequently bathed.

Therefore, the purpose of this study was to evaluate the insecticidal and antifeeding effects of DMC on *L. longipalpis* in the presence and absence of baths in collar-wearing dogs. The antifeeding effect was defined as the inhibition of sand flies blood-feeding, and the insecticidal effect was defined as the ability of the product to cause death in both male and female sand flies.

MATERIAL AND METHODS

Animals

Twelve mongrel dogs of both sexes and who weighed10-25 kg were used in the study. The dogs were kept in individual kennels, with an open area and shelter at the Experimental Kennel of Small Animals, located in the Veterinary School of XXXX. The dogs were fed with dog food *ad libitum*. Environmental enrichment was performed, such as walks and pet bottles.

All dogs were submitted to a complete physical examination and were negative for CanL in the immunofluorescence antibody tests (IFATs) and enzyme-linked immunosorbent assay (ELISA) before admission to the experimental groups. The clinical laboratory parameters were within normal for the species.

The project of this experiment was accredited by the Ethics Committee on Animal Experimentation, XXXX (CEUA Protocol number: 315/2012).

Phlebotomines

Male and female *Lutzomyia longipalpis* from a closed colony were used in this study and maintained at the Department of Parasitology, Institute of Biological Sciences, XXX. Dogs were exposed to phebotomines at an approximate proportion of 30 females to 5 males (MAZLOUMI GAVGANI *et al.*, 2002).

Cage for exposure of dogs to sand flies

To feed on the dogs, the sand flies were released in a cage optimized for this experiment according to (KILLICK-KENDRICK *et al.*, 1997). The cage consisted of a rectangular metal frame (height: 70 cm; width: 70 cm; length: 180 cm). A camera covered with traditional plain tulle fabric was attached to the frame at both ends, and the metal frames were of the same size. However, the right end was closed with a zipper and had a 50-cm fabric that enabled closure with a string. The left end also had 50-cm tissue, which was closed with a string after the dogs were positioned. Two sleeves (diameter: 7 cm) were constructed at the sides of the exterior portion of the cage so that the inside could be accessed without risk of the sand flies escaping. These sleeves were also used to release sand flies inside the cage after the dogs were positioned, and their ends were closed.

Deltamethrin-impregnated collar (DMC)

The collars used in the experiment consisted of a white PVC band (length: 65 cm). The collars were saturated

with deltamethrin 4% and placed around the dogs' neck after the first exposure to sand flies (weight: 25 g).

Experimental groups

The dogs were randomly divided into two groups. The first group (Group 1; G1) consisted of six dogs (four males and two females), which were bathed with neutral shampoo every 15 days throughout the experiment. The second group (Group 2; G2) consisted of six dogs (three males and three females), which were not bathed throughout the experimental study.

Treatments

Dogs were exposed to sand flies to determine the insecticidal and antifeeding effects at specific times: T_0 (prior to placement of the DMC), T_7 (seven days after placement, corresponding to the onset of action of the collar), T_{21} (21 days after application, corresponding to the peak distribution of the insecticide on the animal's skin), T_{60} (60 days after application), T_{90} (90 days after application), and T_{120} (120 days after application).

Animals were sedated using a combination of acepromazine (0.2%; 0.05 mg/kg) and pethidine hydrochloride (50 mg/ml; 5 mg/kg) via intramuscular administration. After sedation, each dog was placed in a lateral recumbency in the exposure cage, and the ends of the cage were closed and checked. The sand flies were then released into the interior of the cage using the sleeves. The environment was kept partially dark, and the sand flies remained in contact with the dog for 40 min (GUARGA *et al.*, 2000). After this period, the sand flies were aspirated using a low-power vacuum drive, which was fitted with a PVC pipe (length: 30 cm). The insects were captured into a miniature Center for Disease Control and Prevention (CDC)-style trap and subsequently frozen. After aspiration of all living sand flies and their capture with the trap, the dog's fur and the floor of the cage were vacuum-aspirated to obtain the dead sand flies. After recovery from sedation, each dog was placed back in its individual kennel. T_0 (prior to placement of the DMC) was used as a control group to analyze the effect with and without DMC use.

Classification of the sand flies

The insects were classified as living or dead and male or female. The female sand flies were classified as engorged or non-engorged using a stereoscope. Engorgement was determined according to the presence of any amount of blood in the female's abdomen.

Statistical analysis

All data analysis was carried out using GraphPad Prism version 6.04 for Windows, (GraphPad Software, La Jolla California USA, www.graphpad.com). Each of the effects listed below were compared using the Chii-Square Test with an α error of 5% in the same group (G1 or G2) or between groups (G1xG2).

The insecticidal effect proportion was calculated comparing the number of total males and females sand flies with dead male and female sand flies.

The antifeeding effect proportion was calculated comparing the total number of female sand flies with the number of non-engorged female sand flies.

The insecticidal effect proportion on engorged females was calculated comparing the total number of female sand flies with the total number of dead engorged sand flies.

The absence of effect proportion was calculated comparing the total number of female sand flies with the number of alive engorged sand flies.

RESULTS

Insecticidal and antifeeding effects of DMC over time in the same group and between groups

Statistical analysis showed an insecticidal effect on sand flies after the placement of DMCs on dogs in G1 ($p<0.05$) (Table 1). In G2, there was a statistical change in mortality percentages of the sand flies exposed to DMC at T21 and T60; however, there was a decrease of the insecticidal effect of the DMC on sand flies at T120 ($p<0.05$) (Table 1).

When comparing G1 and G2, there was a difference at T0 and T60, but overall, both groups showed similar results on the insecticidal effects, with G2 showing less effect at T120.

For the antifeeding effect, the number of non-fed females increased over time in G1 after the placement of DMCs, which meant that the females were not blood-feeding. However, the statistical analysis showed that there was a difference only at T120 ($p<0.05$) (Table 2).

Table 1. Mean values of dead insects in Groups 1 and 2.

Group	T_0	T_7	T_{21}	T_{60}	T_{90}	T_{120}
G1	1.08 ^{aA}	14.70 ^{bA}	10.22 ^{bA}	3.90 ^{bA}	9.91 ^{bA}	9.49 ^{bA}
G2	11.31 ^{aB}	15.38 ^{aA}	17.97 ^{bA}	16.97 ^{bB}	9.9 ^{aA}	6.59 ^{bA}

Values followed by the same small letters (in columns) or capital letters (in rows) do not statistically differ from others as compared by the Chi-Square Test ($p<0.05$). Small letters indicate differences between the same group (G1 or G2), and capital letters indicate differences between the two groups (G1 x G2).

In G2, the antifeeding effect was not observed with the use of DMCs, and there was no difference when comparing G1 and G2 (Table 2).

Table 2. Mean values of non-fed females in Groups 1 and 2.

Group	T_0	T_7	T_{21}	T_{60}	T_{90}	T_{120}
G1	71.01 aA	86.27aA	87.72aA	91.78aA	77.67aA	94.36bA
G2	91.03 aA	74.01aA	72.00aA	90.91aA	84.68aA	94.24aA

Values followed by the same small letters (in columns) or capital letters (in rows) do not statistically differ from others as compared by the Chi-Square Test ($p<0.05$). Small letters indicate differences between the same group (G1 or G2), and capital letters indicate differences between the two groups (G1 x G2).

Insecticidal effect in engorged and dead females

The numbers of dead and engorged females were compared between time points and groups. The use of DMC showed insecticidal effect in engorged females in G1 only at T7 and T21 ($p<0.05$). In G2, there was a decrease in the insecticidal effect in engorged females, specifically at T7, T60, T90, and T120 ($p<0.05$) (Table 3).

Table 3. Mean values of engorged and dead females in Groups 1 and 2.

Group	T_0	T_7	T_{21}	T_{60}	T_{90}	T_{120}
G1	6.25 aA	29.17 bA	50.00 bA	2.00 aA	2.00 aA	5.26 aA
G2	74.00 aA	12.90 bA	56.10 aA	7.69 bA	3.57 bA	5.56 bA

Values followed by the same small letters (in columns) or capital letters (in rows) do not statistically differ from others as compared by the Chi-Square Test ($p<0.05$). Small letters indicate differences between the same group (G1 or G2), and capital letters indicate differences between the two groups (G1 x G2).

Effect in live engorged females

No statistical difference was observed between the time points in G1 or between groups G1 and G2 (Table 4). In G2, the number of alive-engorged female sand flies increased at T7, T60, T90, and T120.

Table 4. Mean values of engorged and alive females in Groups 1 and 2.

Group	T_0	T_7	T_{21}	T_{60}	T_{90}	T_{120}
G1	93.75 aA	70.83 aA	93.75 aA	50.00 aA	98.00 aA	100.00 aA
G2	26.00 aA	100.00 bA	43.90 aA	100.00 bA	96.42 bA	100.00 bA

Values followed by the same small letters (in columns) or capital letters (in rows) do not statistically differ from others as compared by the Chi-Square Test ($p<0.05$). Small letters indicate differences between the same group (G1 or G2), and capital letters indicate differences between the two groups (G1 x G2).

DISCUSSION

The results of our study showed that the effects of DMCs on *L. longipalpis* in the presence of baths in collar-wearing dogs are slightly higher than in non-bathed dogs. An insecticidal outcome in sand flies was observed after the placement of DMCs on dogs, and some antifeeding effect on non-fed females was also observed, which showed that DMCs help preventing blood-feeding of sand flies on dogs using a DMC. However, it was also noticed that for engorged females, the insecticidal effect was observed only until 21 days of use, and if dogs were not bathed, the effect was more negatively affected. Also, when the absence of effect was evaluated, the number of engorged females alive did not change over time. In summary, these results indicate that some sand flies blood-feed from dogs in the presence of a DMC and might die only during a particular phase of use. However, DMCs have an insecticidal effect in sand flies and can prevent blood-feeding, which showed that they can be used as a control measure of CanL and that dogs can be bathed during the use of a DMC.

The insecticidal effect of deltamethrin in G1 and G2 was observed in the statistical analysis. This shows that DMCs have an insecticidal effect, which is not affected by frequent baths in dogs. Other studies confirmed the insecticidal effect of DMCs. Sandfly mortality reached 96% (in the 4th week) and decreased to 35% in the 35th week, with a gradual decrease in the effect after the 22nd week following application (DAVID *et al.*, 2001). According to other studies, the use of DMCs showed a 30% mortality in sand flies (REITHINGER; TEODORO; DAVIES, 2001).

Several studies in different countries have demonstrated the use of the DMC in defending CanL (HALBIG *et al.*, 2000; REITHINGER *et al.*, 2001; FERROGLIO *et al.*, 2008; SEVÁ *et al.*, 2016). The use of a DMC may be an alternative to the extermination of dogs in Brazil, although the effect depends on the coverage and loss rates (REITHINGER *et al.*, 2004). A significant reduction was observed in the seroconversion of children and dogs in Iran, but the effectiveness depends on large-scale use by the community (GAVGANI *et al.*, 2002). This observation was corroborated by a study conducted by Sevá *et al.* (SEVÁ *et al.*, 2016) in Brazil, that showed a

strong reduction of seroprevalence among dogs and humans when collars were used in 90% of the canine population. These results were obtained using a model formulated to study the efficacy of control measures of CanL and considered that the collar insecticide effect is instantaneous.

The safeguard achieved with the use of collars requires great effort, since application of the collars should cover a high number of the dog population, which represents both infected and not infected dogs;the collar is not so effective when used individually (ALEXANDER; MAROLI, 2003; SEVÁ *et al.*, 2016). Moreover, the collars must be replaced every six months or immediately in the case of misplacement or damage (SEVÁ *et al.*, 2016).

Some antifeeding effect was observed in G1. Moreover, the antifeeding result continued until T120, which prevented more than 90% of females feeding. The results found in the literature are changeable because the studies differ in techniques, and any comparison should be made carefully. An 80% reduction was observed in blood-feeding of sand flies (HALBIG *et al.*, 2000) in contrast to the results obtained by other authors who observed a reduction as high as 100% (after 8 and 12 weeks of collar use) (DAVID *et al.*, 2001). The greatest antifeeding effect was demonstrated after 8 weeks of use, although at a less significant (69%) proportion (REITHINGER; TEODORO; DAVIES, 2001). Our study revealed a slightly better antifeeding effect in G1 than in G2. The best antifeeding effect in G1 may be due to either a product in the shampoo composition or absence of odor and secretion in the dogs that would have been removed after each bath, thereby reducing dog attractiveness to sand flies, and increasing the antifeeding effect in G1.

Studies evaluating the performance of the DMC after baths are rare in the literature. Dogs in G1 were bathed with a mild shampoo every other week, while the other six dogs (G2) were not bathed. The conditions to which the dogs in G1 were subjected to may be considered more usual in the current Brazilian scenario, in which the owners bathe their pets every 1-2 weeks. This practice can be used as a control measure of CanL. Overall, our results demonstrate that DMCs have insecticidal and antifeeding effects, and baths do not interfere in its efficacy to control the vector.

Several studies have evaluated the ability of the DMC to stop blood-feeding of female sand flies, resulting in death (KILLICK-KENDRICK *et al*., 1997; DAVID *et al*., 2001; REITHINGER *et al*., 2004). The effect on the number of dead and engorged females was observed at T7 and T21 when dogs were bathed but was not demonstrated in non-bathed dogs. However, the engorged and alive sand flies were not affected. Other studies demonstrated that 100% of the engorged females died in the first 16 weeks. This rate decreased to 35% after 35 weeks (DAVID *et al.*, 2001), and it is most likely that the few females that survived had no contact with the insecticide after feeding (KILLICK-KENDRICK *et al.*, 1997).

Cages that enabled exposure of the animals' entire body to sand flies were used in other studies (KILLICK-KENDRICK *et al*., 1997; HALBIG *et al*., 2000; DAVID *et al*., 2001; REITHINGER *et al*., 2004), as well as in the present study. Some differences between the times of exposure and species used in the studies may explain some of the diverse results between the studies. Only one study used *L. longipalpis* from a closed colony (REITHINGER *et al.*, 2004), whereas vectors found in the Old World were used in other studies (KILLICK-KENDRICK *et al*., 1997; DAVID *et al*., 2001; REITHINGER *et al*., 2001). Moreover, some differences can be attributed to the number of dogs used in the studies, the time groups were exposed to sand flies, and the variation of the humidity, temperature conditions, and different generations of sand flies.

CONCLUSIONS

DMCs effect on *L. longipalpis* in the presence of baths in collar-wearing dogs are not affected, and its use can be continued in collar-wearing dogs. DMCs have insecticidal effects in sand flies and can prevent blood-feeding. Indeed, there are also effects in engorged sand flies. The use of combined procedures to control CanL should be considered in endemic areas such as Brazil.

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