Antifeeding and short-term insecticidal effects against *Lutzomyia 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

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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 alimentação 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.


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.

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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 cm² 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)

<table>
<thead>
<tr>
<th></th>
<th>Fed</th>
<th>Unfed</th>
<th>Males</th>
<th>Dead (total)</th>
<th>Alive (total)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>RP</td>
<td>261</td>
<td>81</td>
<td>119</td>
<td>14</td>
<td>447</td>
<td>461</td>
</tr>
<tr>
<td>RIR</td>
<td>201</td>
<td>85</td>
<td>127</td>
<td>13</td>
<td>400</td>
<td>413</td>
</tr>
<tr>
<td>TOTAL</td>
<td>462</td>
<td>166</td>
<td>246</td>
<td>27</td>
<td>847</td>
<td>874</td>
</tr>
</tbody>
</table>
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).

<table>
<thead>
<tr>
<th></th>
<th>Females</th>
<th></th>
<th>Males</th>
<th>Dead (total)</th>
<th>Alive (total)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fed</td>
<td>Unfed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RP</td>
<td>169</td>
<td>289</td>
<td>325</td>
<td>39</td>
<td>744</td>
<td>783</td>
</tr>
<tr>
<td>RIR</td>
<td>79</td>
<td>295</td>
<td>339</td>
<td>17</td>
<td>696</td>
<td>713</td>
</tr>
<tr>
<td>TOTAL</td>
<td>248</td>
<td>584</td>
<td>664</td>
<td>56</td>
<td>1440</td>
<td>1496</td>
</tr>
</tbody>
</table>

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).

<table>
<thead>
<tr>
<th></th>
<th>Females</th>
<th></th>
<th>Males</th>
<th>Dead (total)</th>
<th>Alive (total)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fed</td>
<td>Unfed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RP</td>
<td>47</td>
<td>285</td>
<td>255</td>
<td>11</td>
<td>576</td>
<td>587</td>
</tr>
<tr>
<td>RIR</td>
<td>100</td>
<td>207</td>
<td>217</td>
<td>7</td>
<td>517</td>
<td>524</td>
</tr>
<tr>
<td>TOTAL</td>
<td>147</td>
<td>492</td>
<td>472</td>
<td>18</td>
<td>1093</td>
<td>1111</td>
</tr>
</tbody>
</table>

**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% on the GP. A significant small number of blood fed insects was found when the RP of both groups was compared as follows: 31% (GC) to 12% (GP), a statistically significant (p<0.0001) result.

Analyzing the data from Table 3, there was a 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 insects (1.62%) and live (98.8%) insects at the end of the exposure.

**Comparison between the GP and GD groups**

When comparing the results 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 statistically significant result (p<0.005).

**Discussion**

Several studies have evaluated the efficacy of the 4% deltamethrin-impregnated collar (Killick-Kendrick et al., 1997; Halbiddavid et al., 2001; Reithinger et al., 2001; Mazloumi; Gavagni, 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 Gavagni, 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 Gavagni, 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 an 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 phlebotomines 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 an 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 70.1% 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.

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