Efficacy of a collar impregnated with amitraz and pyriproxyfen for prevention of experimental tick infestations by *Rhipicephalus sanguineus*, *Ixodes ricinus*, and *Ixodes scapularis* in dogs

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**Objective**—To compare the efficacy of collars impregnated with 9% amitraz or 9% amitraz and 0.5% pyriproxyfen (PPF) for control of newly established tick infestations by *Rhipicephalus sanguineus*, *Ixodes ricinus*, and *Ixodes scapularis* in dogs and determine whether egg production by surviving female ticks was decreased.

**Design**—Prospective study.

**Animals**—72 dogs.

**Procedure**—Dogs were fitted with 1 of 3 test collars impregnated with amitraz, amitraz and PPF, or only excipients (untreated controls). In 3 trials corresponding to each of the 3 tick species, dogs were infested with 150 unfed adult ticks on days 8, 10, 13, and 18. The number of feeding female ticks was recorded on days 10, 13, 18, and 28. Surviving females were weighed and permitted to oviposit under controlled conditions.

**Results**—Collars impregnated with amitraz and PPF decreased tick loads as efficiently as collars containing amitraz alone. Inclusion of PPF into the collar did not significantly decrease the efficacy of amitraz. The few female ticks that survived after feeding on dogs treated with collars containing PPF were unable to oviposit.

**Conclusions and Clinical Relevance**—Collars impregnated with amitraz were efficient in preventing tick infestations in dogs but did not inhibit oviposition in the few surviving female ticks. Incorporation of PPF into the amitraz-impregnated collar resulted in impairment of the reproductive ability of ticks. (J Am Vet Med Assoc 2005;226:221–224)

In dogs, the importance of ticks relates to their blood-depriving activity and their role as vectors for various pathogens. *Rhipicephalus sanguineus* is a peridomestic tick species of considerable concern because of its ability to colonize or be prevalent in the close vicinity of dog habitats (eg, kennel, house, and garden). Possibly resulting in massive infestations.

*Rhipicephalus sanguineus* is a vector for *Babesia canis*, *Ehrlichia canis*, *Haemobartonella canis*, and *Hepatozoon canis*. The probability of disease transmission is dependent on the number of ticks attached to the dog. *Ixodes ricinus* and *Ixodes scapularis* are commonly found in gardens, entering the yard from surrounding wooded areas, and may transmit *Borrelia burgdorferi* to dogs and, incidentally, to humans. Acaricides applied to dogs focus on killing attached ticks or repelling ticks that have not yet attached; however, their ability to prevent laying of viable eggs by surviving female ticks that manage to feed may be limited. Impairing the reproductive ability of ticks that feed on dogs despite acaricidal treatment may provide an additional protective feature for dogs in suburban areas that have been treated.

Pyriproxyfen (PPF) is an arthropod growth regulator that has been evaluated as an oviposition and molt inhibitor in ticks in experimental laboratory conditions. Previous studies were performed with the raw chemical, and to the authors' knowledge, comparative analyses describing the efficacy of formulated PPF for the control of tick infestations in dogs have not been performed. The purpose of the study reported here was to compare the efficacy of collars impregnated with 9% amitraz or 9% amitraz and 0.5% PPF for control of newly established tick infestations by *R sanguineus*, *I ricinus*, and *I scapularis* in dogs and determine whether egg production by surviving female ticks was decreased.

**Materials and Methods**

**Dogs**—Seventy-two sexually intact (40 males and 32 females) mixed-breed dogs from 1 to 3 years old and weighing from 15 to 20 kg (33 to 44 lbs) were included in the study. Dogs had no previous contact with ticks and were obtained from a local dog breeder. Dogs were housed outdoors in individual pens and received water and dry food ad libitum. Dogs were managed in accordance with the guidelines of the European Committee for Care of Animals for Scientific Purposes.

**Ticks**—The strain of *R sanguineus* used in this study was collected outside of Zaragoza, Spain, in 1993; the strain of *I ricinus* was collected from a garden in Czeske Budejovice (Czech Republic) in 1999, and the strain of *I scapularis* was collected in York, Pa, in 1999. All 3 strains were maintained under controlled laboratory conditions and fed on New Zealand white rabbits. Feeding of ticks on rabbits eliminates transovarial transmission of pathogens in ticks, therefore, ticks were determined to be free of known pathogens. Nonparasitic stages of *R sanguineus* were maintained in flasks at 25°C (77°F) and 90% relative humidity, whereas those of *I ricinus* and *I scapularis* were maintained in flasks at 20°C (68°F) and 95% relative humidity in total darkness. Freshly molted nymphs of *I ricinus* and *I scapularis* were forced to enter diapause by exposure to a temperature of 5°C (41°F) for 3 months. This procedure ensures the continuity of the colonies. The same conditions were used for engorged females and developing eggs.

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Experimental design—Dogs were allocated by weight and sex into 9 groups of 8 dogs each. Three separate trials were performed with the 3 species of ticks. Two groups of dogs in each trial were used to test the 2 collars, and the remaining group served as the untreated control group. Dogs were bathed with a nonacaricidal commercial product before the beginning of the trial. Treatments were applied according to the manufacturer’s recommendations and included a collar impregnated with 9% amitraz (AMZ group) and a collar impregnated with 9% amitraz plus 0.5% PPF (AMZ plus PPF group).

Trials—Challenge infestation trials were performed to determine the ability of the collars to prevent new tick infestations in dogs and disrupt the life cycle of ticks. The ability of the treatments to repel ticks on dogs under experimental conditions is difficult to control; therefore, the protective effect of the collar was checked by comparing the number of ticks feeding on treated and untreated dogs. The mortality rate of larval ticks recorded in the different treatment groups was calculated.

Repeated infestations were performed to determine the ability of the treatment to protect dogs with ticks. Each dog in every group was infested with 100 female and 50 male ticks. Infestations were confined to the ears, and ears were covered with a muslin sleeve to keep the area of the ticks restricted. The sleeve was fixed to the ear with adhesive tape, and an Elizabethan collar was placed on the dog to keep it from dislodging or tearing the sleeve. Infestations were performed outside the pens to avoid contamination of the pens. The floor and walls of every pen were washed daily to remove ticks that may have escaped from the dog. A single treatment was applied on day 0. Untreated control dogs were fitted with the same type of collar without active ingredients (placebo) on day 0.

The first tick infestation was performed in every group on day 8. Two days later (day 10), feeding ticks were forcibly detached, counted, and classified as alive-fed (se, alive and engorged with blood) or dead. Dogs were reinfested as previously described on day 10. Feeding ticks were detached on day 13, counted, and classified as alive-fed or dead. After ticks were counted and classified on day 13, the third infestation was performed and the number of ticks feeding on dogs subsequently counted and classified on day 18. The fourth and last infestation was performed on day 18, and ticks were counted and classified on day 28. Surviving ticks from this fourth challenge test were weighed and permitted to oviposit under controlled conditions. The weight of egg clusters, percentage of eggs hatching, percentage of larvae surviving at 10 days, and percentage of larvae surviving at 30 days were determined. The egg output of each female tick was weighed by use of a precision balance. A cluster of 100 eggs was counted and classified on day 28. Surviving ticks from this last infestation was performed on day 18, and ticks were counted, and classified as alive-fed or dead. After ticks previously described on day 10. Feeding ticks were detached on day 0. Untreated control dogs were fitted with the same type of collar without active ingredients (placebo) on day 0.

Results

Infestations performed on day 8 were unsuccessful as determined by low tick counts on day 10, even in untreated control groups. This may be explained by the short time interval between infestation and tick counts. Therefore, data from the first infestation were not included in the statistical analyses. However, infestations performed on days 10, 13, and 18 resulted in an adequate number of ticks feeding on dogs in untreated control groups. The most prominent treatment effect was observed for R. sanguineus. Treatment with amitraz decreased the number of ticks feeding on dogs to 6.1%, 2.9%, and 2.7% on days 13, 18, and 28, respectively, compared with the number of ticks feeding on dogs in the untreated control group. Percentages of feeding female ticks recorded in the AMZ plus PPF groups were 3.5%, 5.7%, and 7.8% at the same respective time intervals. For I. scapularis and I. ricinus species, tick count reductions in treated groups were slightly lower, with percentages of feeding female ticks ranging from 6% to 9.8% for I. scapularis and from 7.9% to 15.7% for I. ricinus, from day 13 to day 28. A significant decrease in the number of female ticks feeding on treated dogs was detected, compared with values recorded in the untreated control groups on days 13, 18, and 28 (Table 1).

Table 1—Mean ± SD number of feeding female ticks on dogs after treatment with collars impregnated with 0.9% amitraz (AMZ), 0.9% amitraz and pyriproxyfen (AMZ + PPF), or a placebo (untreated control) at various intervals after repeated infestations on days 8, 10, 13, and 18.

<table>
<thead>
<tr>
<th>Species</th>
<th>Treatment</th>
<th>10</th>
<th>13</th>
<th>18</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. sanguineus</td>
<td>AMZ</td>
<td>1 ± 0.1</td>
<td>2 ± 0.1</td>
<td>1 ± 0.2</td>
<td>1 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>AMZ + PPF</td>
<td>2 ± 0.2</td>
<td>1 ± 0.1</td>
<td>2 ± 0.2</td>
<td>3 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>7 ± 1.1</td>
<td>33 ± 4.1</td>
<td>35 ± 9.5</td>
<td>27 ± 10.4</td>
</tr>
<tr>
<td>I. scapularis</td>
<td>AMZ</td>
<td>1 ± 0.3</td>
<td>2 ± 0.1</td>
<td>2 ± 0.2</td>
<td>2 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>AMZ + PPF</td>
<td>2 ± 0.2</td>
<td>1 ± 0.3</td>
<td>2 ± 0.5</td>
<td>2 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>4 ± 0.8</td>
<td>27 ± 3.9</td>
<td>23 ± 6.1</td>
<td>27 ± 6.8</td>
</tr>
<tr>
<td>I. ricinus</td>
<td>AMZ</td>
<td>1 ± 0.1</td>
<td>2 ± 0.2</td>
<td>3 ± 1.1</td>
<td>2 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>AMZ + PPF</td>
<td>2 ± 0.3</td>
<td>2 ± 0.3</td>
<td>3 ± 0.9</td>
<td>3 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>4 ± 0.5</td>
<td>27 ± 7.1</td>
<td>22 ± 4.9</td>
<td>26 ± 8.4</td>
</tr>
</tbody>
</table>

Values for day 10 were not included in the statistical analyses because of low numbers of ticks feeding on dogs in the placebo (untreated control) group.

Within a species, values with different superscript letters are significantly (P < 0.05) different.
The number of female ticks killed by treatment before feeding (engorgement) is an important consideration. Efficacy of both impregnated collars was best for prevention of *R. sanguineus*. In the AMZ group, 92% to 97% of ticks were killed before the beginning of feeding. In the AMZ plus PPF group, the corresponding values ranged from 95% to 98%. Mortality rates were slightly lower for *I. scapularis* (88% to 93% for the AMZ group and 86% to 95% for the AMZ plus PPF group) and *I. ricinus* (91% to 96% for the AMZ group and 93% to 98% for the AMZ plus PPF group; Figure 1).

Surviving female ticks were collected and maintained under controlled conditions to evaluate the effects of treatment on oviposition (Table 2). Weight of female ticks was consistently lower in ticks that had fed on treated dogs; however, this finding was not significant. Consequently, those female ticks oviposited a slightly smaller amount of eggs, compared with female ticks that had fed on untreated control dogs; however, this finding was not significant. Female ticks that had fed on dogs treated with amitraz plus PPF did not lay any viable eggs. Larval survival and feeding was not affected by amitraz treatment. Results were consistent across tick species and treatment groups.

**Table 2**—Mean values for reproductive ability of surviving female ticks on dogs after treatment with collars impregnated with 0.9% AMZ, 0.9% AMZ + PPF, or a placebo at various intervals after repeated infestations on days 8, 10, 13, and 18.

<table>
<thead>
<tr>
<th>Species</th>
<th>Treatment</th>
<th>Tick weight (mg)</th>
<th>Egg cluster weight (mg)</th>
<th>Eggs hatching (%)</th>
<th>Larvae surviving at 10 days (%)</th>
<th>Larvae surviving at 30 days (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>R. sanguineus</em></td>
<td>AMZ</td>
<td>159.0</td>
<td>70.3</td>
<td>84.3</td>
<td>81.4</td>
<td>79.4</td>
</tr>
<tr>
<td></td>
<td>AMZ + PPF</td>
<td>174.8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>216.7</td>
<td>91.2</td>
<td>96.9</td>
<td>96.9</td>
<td>92.9</td>
</tr>
<tr>
<td><em>I. scapularis</em></td>
<td>AMZ</td>
<td>190.3</td>
<td>80.3</td>
<td>97.2</td>
<td>96.5</td>
<td>92.7</td>
</tr>
<tr>
<td></td>
<td>AMZ + PPF</td>
<td>170.8</td>
<td>0.6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>249.6</td>
<td>196.6</td>
<td>98.6</td>
<td>96.9</td>
<td>93.8</td>
</tr>
<tr>
<td><em>I. ricinus</em></td>
<td>AMZ</td>
<td>180.8</td>
<td>71.8</td>
<td>96.2</td>
<td>93.3</td>
<td>89.5</td>
</tr>
<tr>
<td></td>
<td>AMZ + PPF</td>
<td>176.8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>249.6</td>
<td>196.5</td>
<td>99.6</td>
<td>96.9</td>
<td>93.4</td>
</tr>
</tbody>
</table>

**Discussion**

Both collars evaluated in this study were effective in preventing tick infestations in dogs. Although *R. sanguineus* was most susceptible to the acaricidal effects of amitraz, good levels of control were achieved for *I. ricinus* and *I. scapularis*. The combination of amitraz and PPF in the same plastic matrix of the collar did not impair the efficacy of amitraz and efficiently disrupted the life cycle of the ticks by stopping oviposition in exposed female ticks.

The efficacy of amitraz treatment in the control of *R. sanguineus* ticks has been previously described. Amitraz has an interesting tick detachment activity on partially engorged females of *R. sanguineus*. To the authors’ knowledge, the present study is the first to report the efficacy of an acaricide treatment against high levels of infestation by *I. scapularis* and *I. ricinus*. The combination of amitraz and PPF in the same plastic matrix of the collar did not impair the efficacy of amitraz and efficiently disrupted the life cycle of the ticks by stopping oviposition in exposed female ticks.

Environmental application of ivermectides on a large scale for the control of *I. scapularis* in widespread areas has been considered. The efficacy of collars impregnated with 9% amitraz and pyriproxyfen (AMZ + PPF) on days 13 (black bar), 18 (white bar), and 28 (striped bar) after repeated infestations on days 10, 13, and 18.

![Figure 1](image-url)

*Figure 1—Percentage of female ticks killed before feeding (engorgement) on dogs after treatment with collars impregnated with 0.9% amitraz (AMZ) or 0.9% amitraz and pyriproxyfen (AMZ + PPF) on days 13 (black bar), 18 (white bar), and 28 (striped bar) after repeated infestations on days 10, 13, and 18.*
traz to prevent transmission of *B burgdorferi* to tick-naive dogs has been reported.\(^2\) *Ixodes ricinus* is also an important parasite of dogs and humans in humid and cold areas of Europe. Involvement of *I ricinus* in the transmission of several pathogens to domestic animals and humans has been documented.\(^4\)

Amitraz prevents ticks from biting by affecting the CNS of the parasite and paralyzing its mouth parts.\(^9\) Collars containing amitraz are rapidly effective against several tick species.\(^9\) Pyriproxyfen is a new compound that impairs the life cycle of arthropods\(^11\) working apparently as a juvenile hormone analogue. Pyriproxyfen modulates vitellogenin synthesis in fat body cells of insects\(^12\) and may also be involved in the inhibition of insect ecdysteroids,\(^13\) which are important triggers for the metamorphosis process.\(^14\) Thus, PPF inhibits insect molting. The same may apply for ticks also. A marked activity of PPF on ticks was recorded under laboratory conditions\(^4\) using rabbits treated topically with the raw chemical and then infested with R sanguineus ticks. All female ticks that had fed on rabbits treated with PPF at a concentration of 1.8 mg/cm\(^2\) of skin did not oviposit subsequently. The same PPF concentration inhibited molting of immature ticks after feeding on treated rabbits.

To the authors' knowledge, the study reported here is the first to evaluate the efficacy of an application of PPF formulated together with an insecticide. The combination of an acaricide and tick-repellent compound, amitraz, with an arthropod development inhibitor, PPF, enlarges the scope of treatment by disrupting the life cycle of the tick. Although established permanent populations of *R sanguineus* in kennels and various other dog habitats are frequent findings, the combined use of amitraz and PPF may enhance the efficacy of control measures against this species of tick, especially in high-infested areas. Use of PPF in dog collars may help as a juvenile hormone analogue, pyriproxyfen. The same may apply for ticks also. A marked activity of PPF on ticks was recorded under laboratory conditions\(^4\) using rabbits treated topically with the raw chemical and then infested with R sanguineus ticks. All female ticks that had fed on rabbits treated with PPF at a concentration of 1.8 mg/cm\(^2\) of skin did not oviposit subsequently. The same PPF concentration inhibited molting of immature ticks after feeding on treated rabbits.

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### References