Comparison of an amitraz-impregnated collar with topical administration of fipronil for prevention of experimental and natural infestations by the brown dog tick (*Rhipicephalus sanguineus*)

Agustin Estrada-Peña, DVM, and Frederic Ascher, DVM

Objective—To compare the efficacies of amitraz and fipronil for prevention of experimental and natural infestations of *Rhipicephalus sanguineus*.

Design—Prospective study.

Animals—30 dogs.

Procedure—In 3 trials, dogs were allocated to 3 groups of 10 each. In trial 1, dogs were experimentally infested on day –1, and on day 0 were fitted with an amitraz-impregnated collar, treated topically with fipronil, or not treated. Ticks were counted daily until day 7, when viability of ticks and their progeny was determined. In trial 2, dogs were treated on day 0 and experimentally infested on days 7, 8, 10, and 13. Ticks were counted on days 8, 10, 13, and 18, and viability of ticks and their progeny was determined on day 18. In trial 3, dogs were exposed weekly to a tick-infested environment from day –3 to day 70. Dogs were treated on day 0, and ticks were counted and removed weekly from day 3 to day 77.

Results—Fipronil and amitraz were acaricidal and inhibited attachment and feeding. Amitraz had a significantly greater effect than fipronil on numbers of live, feeding ticks, egg hatchability, and larval viability, indicating partial ability to interrupt the tick life cycle. In field conditions, amitraz remained effective over the entire observation period.

Clinical Implications—Amitraz had stronger and more sustained effects against tick infestation than fipronil. (*J Am Vet Med Assoc* 1999;214:1799–1803)

The brown dog tick (*Rhipicephalus sanguineus*) is one of the most widespread tick species in the world.[1-3] Common in dogs,[4] it can be a vector for various pathogens such as *Babesia canis*, *Ehrlichia canis*, *Haemobartonella canis*, and *Hepatozoon canis*.[5] Disease transmission is dependent on the number of attached ticks and blood-feeding activity,[6] because most pathogens are present in tick salivary glands and transmitted during engorgement via salivary secretion and regurgitation. Attributes of an effective acaricide, therefore, include the ability to kill ticks and inhibit their attachment and feeding. Extended duration of action and the ability to decrease or eliminate the environmental reservoir of immature tick stages (interrupt the life cycle) are also desirable, particularly for control of *R sanguineus*.

Various acaricides, including amitraz and, more recently, fipronil, are currently available. Amitraz is a neurotoxic compound of the formamidine family. Its potent acaricidal properties were first reported by Allan and Palmer,[7] although its mechanism of action has yet to be clearly established. In vitro and in vivo studies, using various formulations, including impregnated collars,[8] have indicated its detachment,[9] repellent,[10] and acaricidal effects. Comparisons with other acaricides have also been published.[11,12] Fipronil is a phenylpyrazole compound that has powerful blocking effects against γ-**aminobutyric acid (GABA)**-dependent chloride ions. There are few reports of its effects on ticks.[13,14]

The purpose of the study reported here was to compare the efficacy of an amitraz-impregnated collar with topical application of fipronil for protection against infestation with *R sanguineus* in laboratory and field conditions and determine the effects of the 2 drugs on tick reproduction and viability of offspring.

Materials and Methods

Animals—Thirty sexually intact mixed-breed dogs (16 male and 14 female), weighing 15 to 20 kg (33 to 44 lb), were included in this study. Dogs were housed outdoors in individual pens, received water and dry food ad libitum, and were managed in accordance with guidelines of the European Committee for Care of Animals with Scientific Purposes.

Ticks—*Rhipicephalus sanguineus* ticks used for experimental infestation were laboratory-reared adults of a colony that originated from ticks collected in the region of Zaragoza, Spain, in 1991. Parasitic stages were maintained by feeding on New Zealand White rabbits, and molting stages were maintained at 22 C (71.6 F) and 85% relative humidity, with 14 hours of light/day.

Experimental design—The 30 dogs were allocated by weight and sex into 3 groups of 10 and remained within their group for 3 separate trials. Dogs were shampooed with a commercial product[a] before each trial. Amitraz[b] collars and fipronil[c] were applied according to the manufacturer's directions. Each dog in 1 group (fipronil) was treated with 1 tube of fipronil (1.34 ml) that was applied to the skin between the shoulder blades; each dog in another group (amitraz) was treated with an amitraz-impregnated collar that was fitted around the neck; and each dog in the third group (control) was not treated.

From the Department of Parasitology, Faculty of Veterinary Medicine, University of Zaragoza, 50013-Zaragoza, Spain (Estrada-Peña); and Virbac SA, 1 06510-Carros, Cedex, France (Ascher).
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Trial 1—The first trial compared acaricidal effects of the products. All dogs were experimentally infested with 100 female and 100 male ticks on day –1 by distributing the ticks evenly along the dorsal midline from the top of the head to the base of the tail. Dogs were restrained for a period of 10 to 15 minutes to allow the ticks to move freely into the coat and spread over the body. Dogs in the amitraz and fipronil groups were treated on day 0. From day 0 to day 7, female ticks were counted daily by careful visual inspection and classified as alive-fed (alive and engorged with blood), alive-unfed (moving in the coat or attached to the skin, but not engorged), dead-fed (dead, engorged), or dead-unfed (dead, not engorged). On day 7, viability of surviving female ticks and their progeny was determined as follows. Ticks were forcibly detached, weighed, and allowed to oviposit. Weight of egg clusters and percentages of egg hatchability, larvae surviving for 10 days, and feeding larvae at 30 days were determined. Larvae were permitted to feed on New Zealand White rabbits under laboratory conditions as stated.

Trial 2—The second trial was performed 14 days after completion of the first trial and compared inhibitory effects of the products on attachment and feeding behavior. Dogs were treated on day 0 and experimentally infested with 100 male ticks and 100 female ticks on days 7, 8, 10, and 13. On days 8, 10, 13 and 18, female ticks were counted and classified as described for the first trial and removed by picking after each count, before each reinfestation. On day 18, viability of surviving female ticks and their progeny was determined as for the first trial.

Trial 3—The third trial was performed 22 days after completion of the second trial, during a period of activity of *R sanguineus* in the region, and compared the duration of effect of the products. Dogs were naturally infested during a 2-hour walk in a *R sanguineus*-infested suburban region on day –3 and weekly thereafter from day 7 to day 70. Dogs in the amitraz and fipronil groups were treated on day 0, and female ticks were classified and counted on days –3, –2, 2, 3, and weekly thereafter from day 14 to day 77 (ie, 7 days after each reinfestation). Ticks were removed from dogs on day 3 and weekly thereafter from day 14 to day 77.

Statistical analyses—Tick counts were analyzed by use of ANOVA for repeated measures, followed by Newman-Keuls pairwise test to compare counts among groups. Counts of alive-fed, dead-fed, and dead-unfed female ticks were analyzed. For viability measurements, variance homogeneity was checked by the Barlett test. When variances were homogeneous, comparisons were carried out by ANOVA followed by pairwise tests using the contrast method. When variances were not homogeneous, comparisons were evaluated with the nonparametric Kruskal-Wallis test. In all instances, statistical significance was set at $P \leq 0.05$.

Results

Trial 1—In the control group, mean number of alive-fed ticks increased from 0.4 on day 0 to 44.6 on day 7, confirming the feeding ability of the ticks used for experimental infestation. Mean number of dead-unfed and dead-fed ticks increased only slightly; thus, most alive-unfed ticks had become alive-fed ticks (Fig 1). In the fipronil and amitraz groups, the number of alive-fed ticks was significantly lower than in the control group throughout the trial, whereas the number of dead-fed and dead-unfed ticks increased, indicating that live ticks had died. The number of dead-unfed ticks was significantly greater in the amitraz group (mean on day 7, 47.0) than in the fipronil group (mean on day 7, 30.4), and the numbers of alive-fed and dead-fed ticks were significantly lower in the amitraz group than in the fipronil group (Fig 1) throughout the trial. This indicated that ticks in the amitraz group died without having had a blood meal, whereas approximately 27% of ticks in the fipronil group that died did so after feeding. Treatment effects on the viability of

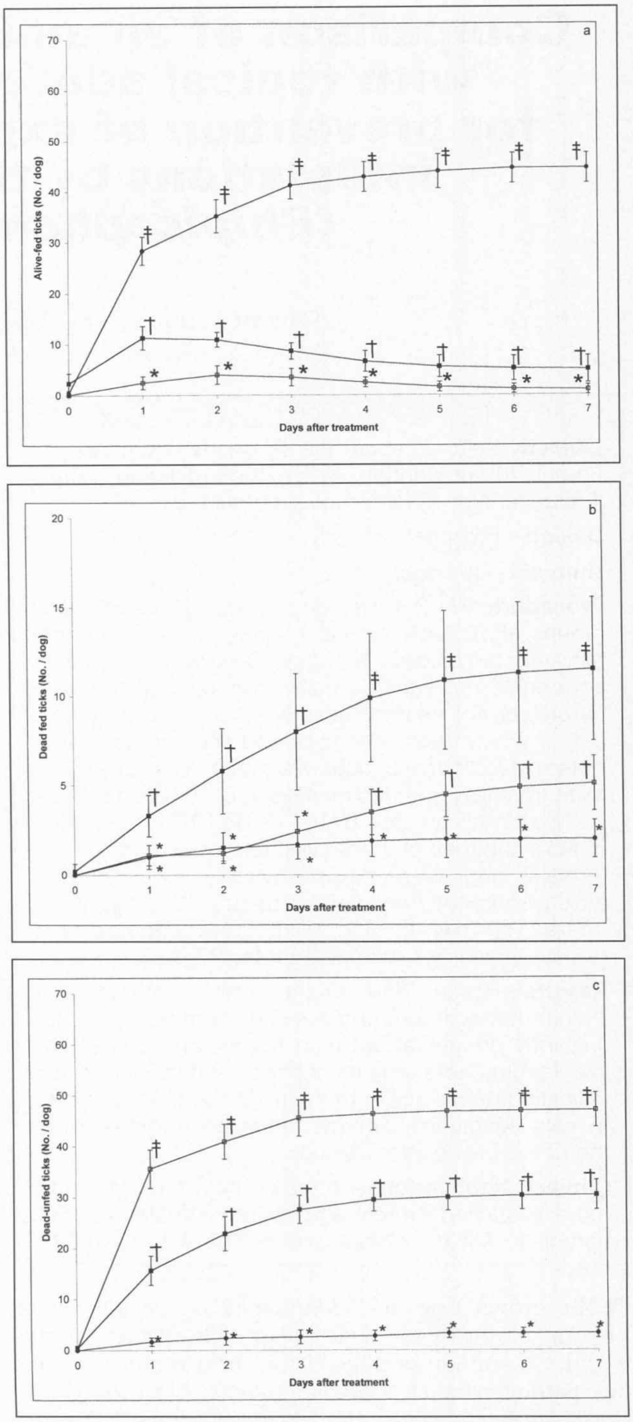

Figure 1—Mean number of female *Rhipicephalus sanguineus* ticks that were alive-fed (a), dead-fed (b), or dead-unfed (c) on dogs that were experimentally infested with 100 ticks on day –1. Dogs were treated with fipronil (■) or amitraz (□) on day 0, or not treated (●). Bars represent SD. Groups with unlike symbols differ significantly ($P < 0.05$).

Table 1—Mean (SD) measurements of viability for adult female *Rhipicephalus sanguineus* and their larvae after removal from dogs that were untreated (control) or treated with amitraz or fipronil 7 days prior to tick removal

Variable	Control	Fipronil	Amitraz*
Female weight (mg)	277 (30)	259 (41)	224 (19)
Egg cluster weight (mg)	142 (15)	135 (21)	116 (10)
Egg hatchability (%)	99 (4)	97 (9)	42 (19)
Larval survival (%)†	99 (2)	98 (6)	40 (20)
Larval feeding (%)‡	97 (5)	97 (6)	38 (19)

*All values were significantly ($P < 0.05$) different from fipronil and control values. †Percentage of larvae alive at 10 days of age. ‡Percentage of larvae actively feeding at 30 days of age.

Table 2—Mean (SD) measurements of viability for adult female *R sanguineus* and their larvae after removal from dogs that were untreated (control) or treated with amitraz or fipronil 13 days prior to tick infestation. Ticks were removed 5 days later

Variable	Control	Fipronil	Amitraz*
Female weight (mg)	278 (26)	271 (26)	213 (34)
Egg cluster weight (mg)	143 (13)	140 (14)	110 (16)
Egg hatchability (%)	99 (2)	99 (2)	41 (27)
Larval survival (%)†	98 (3)	97 (4)	34 (23)
Larval feeding (%)‡	93 (4)	93 (4)	33 (25)

See Table 1 for key.

surviving ticks and their progeny was estimated by measurements performed on 25 ticks removed at random from control dogs, 49 ticks removed from fipronil-treated dogs, and 10 ticks removed from amitraz-treated dogs. Mean values for female tick weight, egg cluster weight, percentages of egg hatchability, surviving larvae at 10 days, and larvae feeding at 30 days were significantly lower in the amitraz group; differences between the fipronil and control groups were not detected **(Table 1)**.

Trial 2—In the amitraz group, the number of alive-fed ticks was consistently lower than in the other groups and remained constant throughout the trial **(Fig 2)**. Numbers of dead-unfed ticks were consistently higher in the amitraz group than in the fipronil group, and numbers of dead-fed and alive-fed ticks were consistently lower. This indicated that amitraz killed ticks before feeding, whereas ticks in the fipronil group died either before or after having a blood meal. Treatment effects on the viability of surviving ticks and their progeny were estimated by measurements performed on 25 ticks removed at random from control dogs, 25 ticks removed from fipronil-treated dogs, and 10 ticks removed from amitraz-treated dogs. Results of all measurements were significantly lower in the amitraz group; differences between the fipronil and control groups were not detected **(Table 2)**.

Trial 3—After natural infestation, most ticks in the control group were alive-fed (range, 4.9 to 8.5 ticks/dog). In the treated groups, ticks were alive-fed on days –3 and –2 (before treatment), and dead-fed on days 2 and 3. In the fipronil group, the number of alive-fed ticks increased from day 14 to day 77, whereas the numbers of dead-fed and dead-unfed ticks decreased from day 35 to day 77 **(Fig 3)**. In the amitraz group, ticks of all classifications were scarce throughout the trial. In the control group, none of the dogs were free of ticks throughout the trial. In the fipronil group, the percentage of dogs that were free of ticks on specific days after treatment were: day 2, 30%; day 3, 60%; day 14, 70%; day 21, 70%; day 28, 20%; and day 35, 20%. In the amitraz group, the percentage of dogs that were free of ticks on specific days after treatment were: day 2, 100%; day 3, 100%, day 14, 90%; day 21, 70%; day 28, 90%, and day 35, 80%.

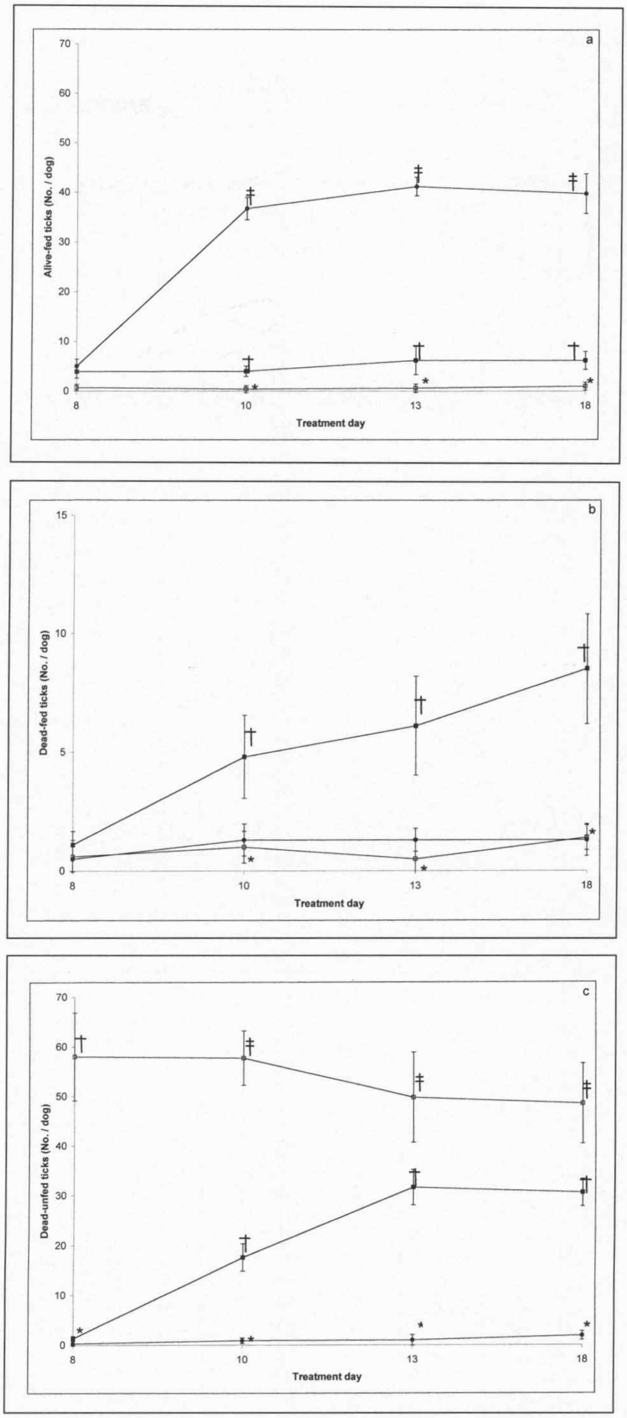

Figure 2—Mean number of female *R sanguineus* ticks that were alive-fed (a), dead-fed (b), or dead-unfed (c) on dogs that were experimentally infested with 100 ticks on days 7, 8, 10, and 13. *See* Figure 1 for key.

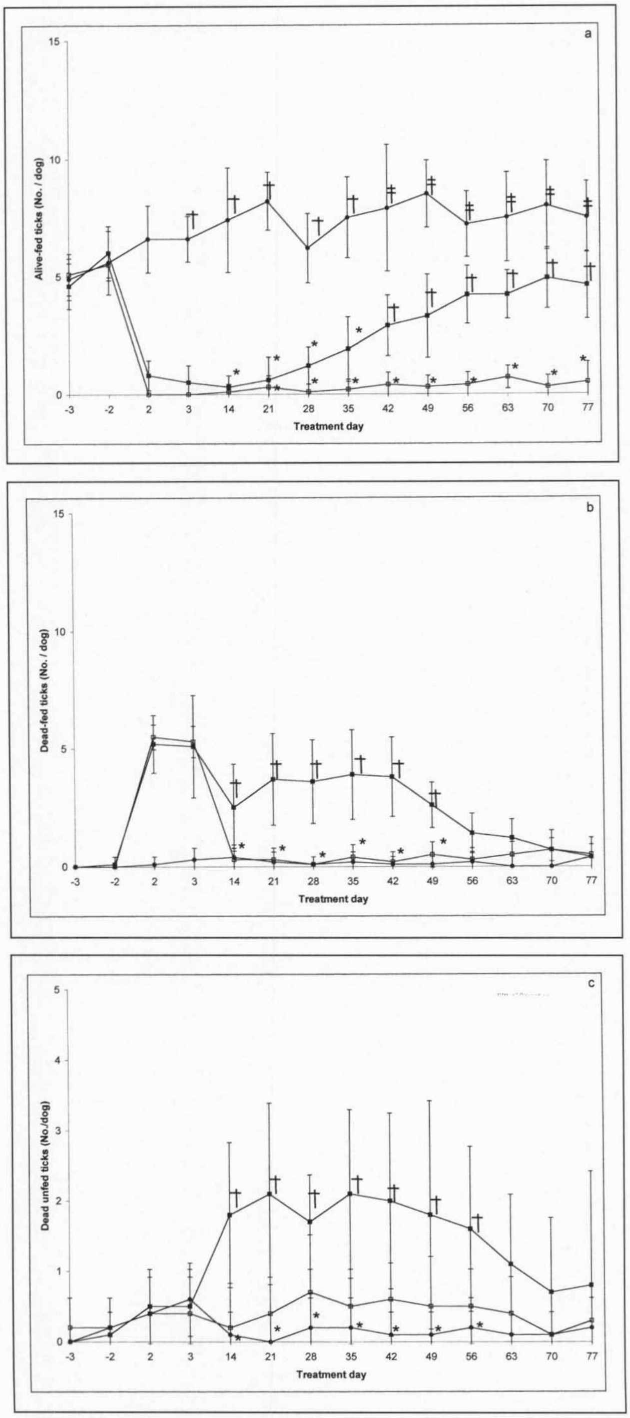

Figure 3—Mean number of female *R sanguineus* ticks that were alive-fed (a), dead-fed (b), or dead-unfed (c) on dogs that were naturally infested on day –3 and weekly from day 7 to day 70. *See* Figure 1 for key.

Discussion

Amitraz and fipronil were effective in treating and preventing tick infestation. The amitraz-impregnated collar appeared to be more efficacious, had a longer duration of effect, and affected the tick life cycle, reducing egg hatchability and percentages of surviving and feeding larvae.

In other studies, investigators have counted ticks and compared their numbers with a control group or counted immature and adult ticks separately.[9] In our study, we counted only female ticks, because another study[10] indicated that there is no sex difference for detachability. Furthermore, the classification of female ticks into 4 groups allowed better estimation of the effects of each treatment.

Although fipronil proved effective, amitraz had significantly greater effects in all 3 trials. Amitraz killed ticks more quickly than fipronil and had tick-repellent effects. Folz et al[11] examined the effects of amitraz on partially or fully engorged adult *R sanguineus* ticks and concluded that amitraz was an effective repellent and detachment acaricide. The results of our study confirmed its ability to kill ticks quickly, before blood was ingested. Fipronil also killed ticks before feeding, but approximately 27% of dead ticks in the first trial had ingested a blood meal before dying. In the third trial, under field-type conditions, the amitraz group had few ticks, and many dogs had none; in the fipronil group, fed-dead and unfed-dead ticks were detected. These results indicated that amitraz was efficacious as a tick repellent. In trial 3, in which dogs were subjected to repeated tick infestation for 70 days after treatment, amitraz was more effective than fipronil throughout the trial.

Differences in formulation of the 2 acaricides may explain differences in their effects. Amitraz is impregnated into a plastic matrix that allows controlled, prolonged release of the active ingredient, whereas fipronil is deposited in a single dose on the skin. Folz et al[11] studied a liquid concentrate formulation of amitraz and detected a rapid decrease in effectiveness against attached feeding adult *R sanguineus*.

Rhipicephalus sanguineus has adapted to its host and its host's environment. To control this species, it is important to treat the host and to decrease or eliminate the environmental reservoir of mature and immature stages.[15] In an in vitro study of formamidine and related compounds, Knowles and Roulston[15] detected a dose-dependent effect on production and viability of eggs of the Yeerongpilly strain of *Boophilus microplus*. Results of the in vivo study reported here confirmed that amitraz affected the production of eggs (mean egg cluster weight), egg hatchability, and viability of larvae (percentage of surviving and feeding larvae). Fipronil did not have similar effects.

[a]Seboderm, Virbac, Carros, France.
[b]Preventic, Virbac, Carros, France.
[c]Frontline, Rhone Merieux, Lyon, France.

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