Available online at www.sciencedirect.com
SCIENCE DIRECT®
Veterinary Parasitology 130 (2005) 293–304
veterinary parasitology
www.elsevier.com/locate/vetpar

Efficacy of a combination of imidacloprid 10%/permethrin 50% versus fipronil 10%/(*S*)-methoprene 12%, against ticks in naturally infected dogs

Domenico Otranto [a,*], Riccardo Paolo Lia [a], Cinzia Cantacessi [a], Gianluca Galli [b], Paola Paradies [a], Egidio Mallia [a], Gioia Capelli [c]

[a] *Department of Animal Health and Welfare, Faculty of Veterinary Medicine, University of Bari, Str. Prov. per Casamassima Km 3, 70010 Valenzano (Bari), Italy*
[b] *Bayer Italia s.p.a., Divisione Sanità Animale, Milan, Italy*
[c] *Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (Padua), Italy*

Received 24 January 2005

Abstract

Preventing tick bites is a fundamental step towards reducing the impact of tick-borne protozoal, bacterial and viral diseases (TBDs) in humans and animals. The aim of this study was to evaluate the efficacy of a combination of imidacloprid 10%/permethrin 50% and of fipronil 10%/*S*-methoprene 12% against ticks in naturally infected dogs and to assess methodological parameters to calculate drug efficacy on tick immature stages.

From July to August 2004, 45 privately owned dogs of various sexes, ages, breeds, coat length and habits were enrolled in a trial carried out in an area (radius approximately 50 km) in Southern Italy. Three homogeneous groups (both for dog population and tick population) were formed: 15 dogs treated with imidacloprid 10% and permethrin 50% spot-on (group A), 15 dogs treated with fipronil 10% and methoprene 12% spot-on (group B) and 15 untreated dogs (group C). The dogs in each group were then sub-grouped according to their age and weight. Two different treatments were administered (time 0 and +28 days) to groups A and B, and the dogs were checked weekly for tick infestation until day +56 post-treatment (p.t.). Twenty-four areas distributed on the whole body surface were examined for ticks at each follow-up, while only at time 0 and at day +56 p.t., ticks were collected from the dogs and identified.

For the immature stages a semi-quantitative method was adopted and the load of immature stages was evaluated and grouped into four classes up to day +56 p.t. when the mean number of immature ticks (MIT) for each infection class was evaluated.

All the adult ticks collected were identified as brown dog ticks (*Rhipicephalus sanguineus*). Immature stages were first compared at day +28 p.t.. The efficacy of both products used in groups A and B on adult ticks was high and generally very similar. Conversely, the efficacy of imidacloprid 10% and permethrin 50% against immatures was higher than that of fipronil 10% and methoprene 12% throughout the observation period with statistically significant differences ($p < 0.05$) at day +28 p.t. (i.e. group A = 98.52%, group B = 72.40%).

* Corresponding author. Tel.: +39 080 4679839; fax: +39 080 4679839.
E-mail address: d.otranto@veterinaria.uniba.it (D. Otranto).

0304-4017/$ – see front matter
doi:10.1016/j.vetpar.2005.04.014

On the whole, in analysing the efficacy of both products against adult plus immature ticks, it was found that the combination of imidacloprid 10% and permethrin 50% was more effective than fipronil 10% and methoprene 12%, with the differences being statistically significant at day +28 p.t. (group A = 98.43%, group B = 77.56%).

Keywords: Ticks; Dogs; *Rhipicephalus sanguineus*; Control; Imidacloprid; Permethrin; Fipronil; Efficacy; Field trial; Tick-borne diseases

1. Introduction

Throughout the world, ticks, together with fleas, are the most widespread ectoparasites affecting pets. Tick infections are important because ticks are vectors of several protozoal, bacterial and viral pathogens causing tick-borne diseases (TBDs) in humans and animals (e.g. borreliosis, ehrlichiosis, rickettsiosis, babesiosis and viral encephalitis) (Sonenshine, 1991, 1993; Lane and Crosskey, 1995). In animals, the transmission of TBDs occurs both transovarial (i.e. all tick developmental stages are infective) and transstadial (i.e. only nymphs (N) and adults (A)).

In particular, *Rhipicephalus sanguineus*, commonly known as ''kennel tick'' or ''brown dog tick'', is the species most commonly retrieved in regions with warm climates, feeding on dogs, both in urban and rural habitats. *Rhipicephalus* groups include a number of species that act as vectors of a wide range of pathogens, such as *Rickettsia conorii*, *Ehrlichia canis, Hepatozoon canis* and *Babesia canis* (reviewed in Sonenshine, 1991, 1993).

It is well known that to prevent the transmission of TBDs, prophylactic protection against ticks is needed. In the past, several drugs have been employed against ticks: first arsenic and chlorinated hydrocarbons and later organophosphates and amidines. However, disadvantages included high toxicity and harmful side effects for both animals and humans, resistance and environmental pollution (de Castro, 1997). Pet owners and environmentalists steered pharmaceutical research toward the development of drugs which were both effective against a wide range of ectoparasites affecting pets, and safe and less-toxic for animals and humans.

Synthetic pyrethroids have been employed for tick control not only for their acaricidal activity but also for their repellent properties.

In particular, a combination of imidacloprid 10% and permethrin 50% has been recently developed (Advantix®; Bayer HealthCare AG, Animal Health Division, Germany) in a spot-on formulation with the aim of providing treatment for, and prophylaxis against, ticks, fleas, mosquitoes and phlebotomine sand flies (Mencke et al., 2003).

The efficacy of this association against ticks has been experimentally demonstrated by using different approaches such as hair taken from treated dogs or by attaching adult female ticks to the shaved lateral thorax of treated dogs (Mehlhorn et al., 2003) or by leaving treated dogs in contact with adult ticks (Epe et al., 2003; Young et al., 2003).

Another product containing fipronil 10% and (*S*)-methoprene 12% (Front Line Combo®; Merial S.A.S., France) is available in a spot-on formulation to treat infection by fleas, ticks and louses.

The efficacy of imidacloprid 10% and permethrin 50% versus fipronil 10% and methoprene 12% has been assessed only in laboratory conditions against *R. sanguineus* (Young et al., 2003) and in a multi-centre field trial on dogs from 23 veterinary clinics in Germany, France and Italy (Hellmann et al., 2003).

The aim of this study was to evaluate the efficacy of imidacloprid 10%/permethrin 50% and fipronil 10%/methoprene 12% combinations against ticks in dogs under natural conditions and to assess methodological criteria for calculating the efficacy of the products on immature ticks affecting dogs in the field.

2. Materials and methods

2.1. Study area

The trial was conducted from July to August 2004 on dogs living in the same area (within a radius of 50 km) in the municipalities of Oliveto Lucano and Accettura (Potenza province, Basilicata region, Southern Italy, latitude 40° and 41°N, longitude 15° and

Fig. 1. Survey area: schematic representation of the province of Potenza (Basilicata region, Southern Italy) (latitude 40° and 41°N, longitude 15° and 17°E).

17°E, between 548 and 1367 m above sea level (a.s.l.)) (Fig. 1). The mean temperature and relative humidity values were daily recorded by a meteorological station.

2.2. Study design

Forty-five owned dogs of various sexes, ages, breeds and coat length were enrolled in the trial (Table 1). The dogs were kept as hunting or guard dogs, or pets. Some were fenced in, others were allowed to move freely within a radius of about 200 m from their home, others still spent all day close to grazing cows, sheep or goats. Since all the dogs lived in the same area (see below) it was assumed they were exposed to the same tick load.

Dogs were excluded from the trial, if they were under seven weeks of age, had a history of apparent reactions to a component of the test or control products, presented with skin lesions at the application site or had pre-existing medical conditions, had been treated in the previous three months with products active against ticks or lived in a tick-treated environment.

Three different groups were formed as follows:

Group A: 15 dogs treated with imidacloprid 10%/ permethrin 50% spot-on.
Group B: 15 dogs treated with fipronil 10%/ methoprene 12% spot-on.
Group C: 15 untreated dogs.

In particular, within each group there were also three sub-groups of five animals divided according to age (under 1.5 years of age; between 1.5 and 3.5 years; over 3.5 years) and weight (between 4 and 10 kg; between 10 and 22 kg and more than 22 kg). Age and weight were considered the two most important factors, along with coat length, that influence tick infections in dogs. The animals were thus divided according to weight, age and coat length and randomly assigned to one of the three groups.

At day 0, the dogs were identified and photographed, and data on the owner and their dogs (breed, sex, age, weight, lifestyle and attitude, coat length) were recorded on a separate file for each dog.

Two different treatments were administered to groups A and B at baseline (time 0) and +28 days (±1

Table 1
Characteristics of the 45 dogs enrolled in the trial

	n	%
Breed		
Mixed breed	24	53.3
Pure breeds	21	46.7
Sex		
Female	22	48.9
Male	23	51.1
Age (months)		
<18	12	26.7
18–36	19	42.2
>36	14	31.1
Weight		
<10	15	33.3
10–22	15	33.3
>22	15	33.3
Coat		
Short	16	35.6
Medium	14	31.1
Long	15	33.3
Attitude		
Hunting	8	17.8
Companion/pet	11	24.4
Guard	12	26.7
Sheepdog	14	31.1
Presence of fleas		
No	8	17.8
Yes	37	82.2

day), and the dogs were checked weekly for ticks (days 0, +7, +14, +21, +28, +35, +42, +49 and +56 (±1 day) p.t.). Twenty-four areas distributed on the whole body surface were examined for ticks at each follow-up (Fig. 2). The number and developmental stages of the ticks found in the 24 body sites were recorded weekly and reported in a separate file for each dog (Fig. 2).

Any adverse effects of the drugs were evaluated by clinical examination of the animals.

The drugs were administered according to the manufacturer's instructions on the basis of the animal body weight ranges. The batch number and expiry date were recorded.

2.3. Ticks collection and identification

At time 0, before the first treatment, and at day +56 p.t. (last examination), ticks were collected from the dogs in the three groups to record the parasitic load for each anatomical site, and to identify the species of ticks.

The ticks were stored in 70% ethanol, using a different vial for each animal, and were identified in the lab according to their stage [adult (A) – male and female (M and F), nymph (N) and larva (L)]. Tick species were identified using the morphological keys of Manilla (1998).

2.4. Estimation of tick load

At days +7, +14, +21, +28, +35, +42, +49 p.t., no ticks were actually collected but their number, stage (adult, immature), sex (M/F), location on the animal's body were recorded on file (Fig. 2).

For the immature stages, a semi-quantitative method was adopted, involving visual inspections and the recording on file of the estimated number of ticks found in an area of 1 cm^2 for each of the 24 body sites.

In particular, the examination area was defined using a plastic template over the flat sites (e.g. neck, thorax, abdomen), and when this was not possible (e.g. interdigital and periocular areas, ears), the sites were considered as independent units of calculation.

The load of immature stages was evaluated by the same inspector throughout the observation period and grouped into the following four classes:

Class 1: low density (<10 immatures).
Class 2: medium density ($10 < x < 50$ immatures).
Class 3: high density ($50 < x < 100$ immatures).
Class 4: very high density (>100 immatures).

The load of immature stages was then calculated a posteriori at day +56 p.t. by collecting all the ticks from 10 sites per infection class. In order to calculate the efficacy of the products on the immature stages, the mean number of immature ticks (MIT) for each infection class was evaluated using the following formula:

$$\mathrm{MIT} = \left(\frac{\mathrm{S1} + \mathrm{S2} + \cdots + \mathrm{S10}}{10}\right) \pm 2\mathrm{S.D.}$$

where MIT represents the mean number of immature ticks in each load class and $\mathrm{S1} + \mathrm{S2} + \cdots + \mathrm{S10}$ is

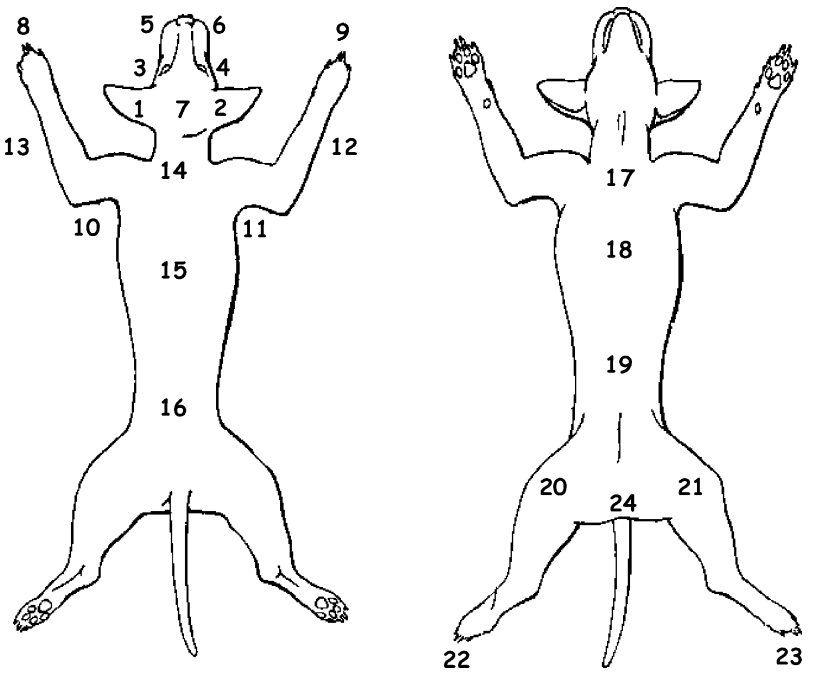

Fig. 2. File reporting the 24 body areas examined for ticks.

the number of immature stages collected at day +56 p.t. in each of the 10 sites.

The MIT for each of the four infection classes was then multiplied by the number of body sites in which that parasitic load was estimated.

2.5. Calculation of the efficacy and epidemiological analysis

The efficacy of each treatment was calculated at each inspection (at day +7, +14, +21, and +28 after the first treatment and at days +35, +42, +49, and +56 after the second treatment) by comparing the number of live or dead ticks found in the treated dogs with the number of ticks found in the control group, according the following formula (modified from Abbott, 1987): Percentage of efficacy = (mean number of ticks on control animals − mean number of ticks on treated animals)/mean number of ticks on control animals) × 100.

The homogeneity of the dog population and the three study groups was evaluated using the chi-square test in relation to dog epidemiological data at time t_0.

The homogeneity of the tick population collected at time t_0 in each group was evaluated comparing the mean tick numbers by variance analysis (ANOVA) and the Kruskal–Wallis test for independent samples.

The mean number of ticks counted on each day post-treatment in the three groups was compared using parametric (ANOVA) and non-parametric tests (Mann–Whitney *U*-test), as suggested by Duncan et al. (2002). If both tests were either significant or non-significant, efficacy or non-efficacy was proved beyond reasonable doubt. Statistical significance was set at $p < 0.05$ for both tests. The software used was SPSS for Windows, version 12.0.

3. Results

The three groups proved to be homogenous from the epidemiological point of view for both individual dog characteristics (sex, age, breed, weight, hair length, attitude) and parasitic load ($p < 0.05$) (Table 2).

On the whole, 1772 adult ticks were collected at day 0 with a mean load of 39 ticks per dog and a

Table 2
Composition and homogeneity of animals in the three groups enrolled in the trial, divided according to variables related to the population sample

Factor	Group A		Group B		Group C		Homogeneity	
	n	%	*n*	%	*n*	%	Test	*p*
Breed								
Mixed breed	8	33.3	7	29.2	9	37.5	$\chi^2 = 0.536$	0.765
Pure breeds	7	33.3	8	38.1	6	28.6		
Sex								
Female	8	36.4	5	22.7	9	40.9	$\chi^2 = 2.31$	0.315
Male	7	30.4	10	43.5	6	26.1		
Age								
<18	5	41.7	3	25.0	4	33.3		
18-36	5	26.3	7	36.8	7	36.8	$\chi^2 = 1.064$	0.900
>36	5	35.7	5	35.7	4	28.6		
Weight								
<10	5	33.3	5	33.3	5	33.3		
10–22	5	33.3	6	40.0	4	26.7	$\chi^2 = 0.80$	0.938
>22	5	33.3	4	26.7	6	40.0		
Coat								
Short	5	31.3	6	37.5	5	31.3		
Medium	6	40.0	4	26.7	5	33.3	$\chi^2 = 0.668$	0.955
Long	4	28.6	5	35.7	5	35.7		
Attitude								
Hunting	0	–	5	62.5	3	37.5		
Companion/pet	6	54.5	3	27.3	2	18.2	$\chi^2 = 7.756$	0.256
Guard	4	33.3	3	25.0	5	41.7		
Sheepdog	5	35.7	4	28.6	5	35.7		
Treatments in the previous year								
No	4	30.8	4	30.8	5	38.5	$\chi^2 = 0.216$	0.897
Yes	11	34.4	11	34.4	10	31.3		
Presence of fleas								
no	2	25.0	3	37.5	3	37.5	$\chi^2 = 0.304$	0.859
yes	13	35.1	12	32.4	12	32.4		

Group A (treated with imidacloprid 10%/permethrin 50%), group B (treated with fipronil 10%/methoprene 12%), group C (untreated controls).

maximum of 166 ticks (Table 3); no statistical differences were detected in the parasitic load in the three groups (Table 4).

All the ticks collected were identified as *R. sanguineus*. Immature stages were first appeared at day +28.

The MIT values for each of the four infection classes calculated at $t + 56$ resulted as follows:

Class 1: low density (3.8).
Class 2: medium density (23.5).
Class 3: high density (75.1).
Class 4: very high density (158.5).

The mean tick load of adult and immature ticks in groups A, B and C from day 0 to day +56 is reported in Table 5.

Table 3
Mean and maximum number of adult ticks collected at day 0 on all 45 dogs

Ticks (adults)	Mean–maximum (S.D.)	Male	Mean–maximum (S.D.)	Female	Mean–maximum (standard deviation)
1772	39–166 (46)	1057	23–112 (29)	714	16–93 (20)

Table 4
Tick load and homogeneity tests for tick population in the three groups at day t_0

Groups	Ticks	Mean (S.D.)	Maximum	Homogeneity-test	
				Kruskall–Wallis	ANOVA $\ln(x+1)$
A	552	37 (42)	166	$\chi^2 = 0.314$	$F = 0.035$
B	590	39 (44)	149	$p = 0.855$	$p = 0.969$
C	630	42 (53)	153		

Group A (treated with imidacloprid 10%/permethrin 50%), group B (treated with fipronil 10%/methoprene 12%), group C (untreated controls).

Table 5
Mean tick load (± S.D.) of adult and immature ticks in group A (treated with imidacloprid 10%/permethrin 50%), group B (treated with fipronil 10%/methoprene 12%), group C (untreated controls) from day 0 to day 56

Days	Group A		Group B		Group C	
	Adults	Immatures	Adults	Immatures	Adults	Immatures
0	36.80 (41.95)	–	39.27 (39.27)	–	42 (52.58)	–
7	0.80 (0.94)	–	0.73 (1.33)	–	6.4 (5.50)	–
14	0.33 (0.49)	–	0.93 (1.22)	–	8.13 (6.51)	–
21	0.87 (1.13)	–	1.53 (1.92)		17.33 (39.70)	–
28	1.20 (2.04)	15.11 (30.06)	1.60 (1.45	230.80 (291.98)	14.40 (29.38)	1021.43 (1492.26)
35	0.00 (0.0)	16.68 (33.74)	0.20 (0.56)	87.53 (183.72)	27.93 (56.84)	929.17 (1266.37)
42	0.13 (0.35)	1.52 (3.46)	0.33 (0.90)	20.32 (38.23)	23.00 (55.64)	987.5 (1219.23)
49	0.07 (0.27)	3.64 (12.07)	0.07 (0.26)	9.65 (19.35)	59.20 (61.50)	892.82 (1522.27)
56	0.20 (0.41)	0.76 (2.13)	0.13 (0.52)	105.01 (366.40)	71.27 (77.30)	560.29 (1332.56)

The efficacy of the products (i.e. the percentage-wise parasitic load reduction) was calculated for adults, males, females, immatures and total population (adults + immatures), as reported in Table 6 and Figs. 3–6.

The efficacy of the combination of imidacloprid 10% and permethrin 50% against adult ticks was slightly higher than that of fipronil 10% and methoprene 12% throughout the observation period, except for $t+7$ and $t+56$ (−1.04% and −0.09% respectively) (Table 6). None of these differences was statistically significant.

The highest efficacy against adult ticks in both groups (i.e. 100% group A and 99.89% group B) was reported after the second treatment and remained high (i.e. 99.72% group A and 99.81% group B) till day +56 p.t. (i.e. four weeks after the second treatment).

Conversely, the efficacy of imidacloprid 10%/permethrin 50% against immatures was higher than that of fipronil 10%/methoprene 12% with statistically

Table 6
Efficacy of the association of imidacloprid 10%/permethrin 50% (group A), fipronil 10%/methoprene 12% (group B) against ticks in different stages (adult and immature) and sex (male and female)

Days	Adult		Male		Female		Immature		Adult and immature	
	Group A	Group B	Group A	Group B	Group A	Group B	Group A	Group B	Group A	Group B
7	87.50	88.54	92.59	88.89	85.51	88.41	–	–	87.50	88.54
14	95.90	88.52	94.64	94.64	96.97	83.33	–	–	95.90	88.52
21	95.00	91.15	100.00	97.92	92.07	87.20	–	–	95.01	91.15
28[a]	91.67	88.89	97.83	89.13	87.10	88.71	98.52	77.40	98.43	77.56
35	100.00	99.28	100.00	99.40	100.00	99.20	98.20	90.58	98.20	90.58
42	99.42	98.55	99.25	97.76	99.53	99.05	99.85	97.94	99.85	97.94
49	99.89	99.89	100.00	99.72	99.81	100.00	99.59	98.92	99.59	98.92
56	99.72	99.81	100.00	100.00	99.52	99.68	99.86	81.26	99.85	83.35

[a] Second treatment.

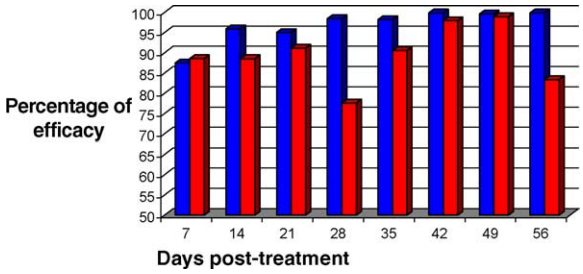

Fig. 3. Efficacy of the association of imidacloprid 10%/permethrin 50% (group A–in blue), fipronil 10%/methoprene 12% (group B–in red) against ticks (adult and immature). Treatments were administered to groups A and B at baseline (time 0) and +28 days (±1 day).

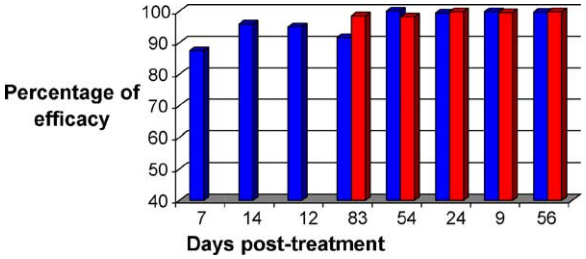

Fig. 4. Efficacy of the association of imidacloprid 10%/permethrin 50% (group A) against adult (blue) and immature (red) ticks. Treatments were administered at baseline (time 0) and +28 days (±1 day).

significant differences ($p < 0.05$) at day +28 (i.e. group A = 98.52%, group B = 72.40%) (Table 6). On the whole, the efficacy of imidacloprid 10%/permethrin 50% against immatures was higher than that of fipronil 10%/methoprene 12% throughout the observation period and remained higher (99.86%) than that recorded for group B (81.26%) at day +56 (Table 6 and Fig. 6).

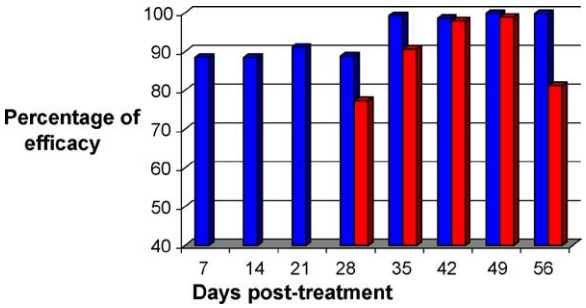

Fig. 5. Efficacy of the association of fipronil 10%/methoprene 12% (group B) against adult (blue) and immature (red) ticks. Treatments were administered at baseline (time 0) and +28 days (±1 day).

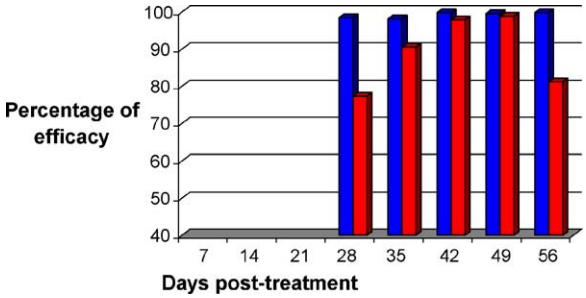

Fig. 6. Efficacy of the association of imidacloprid 10% and permethrin 50% (group A–blue), fipronil 10% and methoprene 12% (group B–red) against immature ticks. Treatments were administered at baseline (time 0) and +28 days (±1 day).

The efficacy of imidacloprid 10%/permethrin 50% (group A) against both adults and immatures was higher than that recorded in group B except for day +7 (group A = 87.50%; group B = 88.54%) with a statistically significant difference ($p < 0.009$) at day +28 (group A = 98.43%; group B = 77.56%) (Table 6 and Fig. 3).

From day +14, till the end of the observation period, imidacloprid 10%/permethrin 50% showed higher than 95% efficacy against both adults and immatures. In particular, the efficacy increased after day +28 and remained higher than 99% from day +49 till the end of the observation period (day +56 group A = 99.85% and group B = 83.35%).

The mean percentage values regarding the distribution of immatures on the animal body showed that immature stages were homogeneously distributed on all the body sites in group C (head 31.8%, body 29.02% and legs 39.18%), while in groups A and B, most immatures were present on the legs (group A = 82.42%; group B = 70.84%), with fewer on the head (group A = 13.08%; group B = 20.34%) and the body (group A = 4.48%; group B = 8.82%).

The mean number of ticks detected between day 0 and day +56 on groups A and B are reported in Table 7. No adverse systemic or topic reactions were registered in all the treated dogs (groups A and B).

4. Discussion

Up to now, to our knowledge, no field work has ever been conducted to evaluate the efficacy of tick repellents in dogs over a period of two months. This

Table 7
Differences in mean tick load and statistical significance (p) in group A (treated with imidacloprid 10%/permethrin 50%) and group B (treated with fipronil 10%/methoprene 12%), from day 0 to day 56, according to stages and sex

Days	Adult ticks				Male				Female			
	Mean group A	Mean group B	ANOVA ln(x + 1) (p)	Mann–Whitney U-test (p)	Mean group A	Mean group B	ANOVA ln(x + 1) (p)	Mann–Whitney U-test (p)	Mean group A	Mean group B	ANOVA ln(x + 1) (p)	Mann–Whitney U-test (p)
0	36.80	39.27	0.851	0.724	22.53	24.20	0.994	0.917	14.27	15.07	0.839	0.917
7	0.80	0.73	0.678	0.547	0.13	0.20	0.796	0.944	0.67	0.53	0.629	0.592
14	0.33	0.93	0.114	0.159	0.20	0.20	1.000	1.000	0.13	0.73	0.058	0.078
21	0.87	1.53	0.339	0.381	0.00	0.13	0.153	0.150	0.87	1.40	0.388	0.405
28	1.20	1.60	0.214	0.137	0.13	0.67	0.053	**0.041**	1.07	0.93	0.940	0.771
35	0.00	0.20	0.165	0.150	0.00	0.07	0.326	0.317	0.00	0.13	0.153	0.150
42	0.13	0.33	0.574	0.888	0.07	0.20	0.449	0.524	0.07	0.13	0.559	0.550
49	0.07	0.07	1.000	1.000	0.00	0.07	0.326	0.317	0.20	0.13	0.326	0.317
56	0.20	0.13	0.539	0.343	0.00	0.00	–	–	14.27	15.07	0.539	0.343

	Immature				Total (adult and immature)			
	Mean group A	Mean group B	ANOVA ln(x + 1)	Mann–Whitney U-test	Mean group A	Mean group B	ANOVA ln(x + 1)	Mann–Whitney U-test
0	–	–	–	–	36.80	39.27	0.804	0.724
7	–	–	–	–	0.80	0.73	0.000	0.547
14	–	–	–	–	0.33	0.93	0.000	0.159
21	–	–	–	–	0.87	1.53	0.339	0.381
28	15.11	230.80	**0.004**	**0.046**	16.31	232.40	**0.009**	**0.026**
35	16.68	87.53	0.000	0.556	16.68	87.73	0.000	0.556
42	1.52	20.32	0.000	0.107	1.65	20.65	0.000	0.164
49	3.64	9.65	0.000	0.575	3.71	9.72	0.000	0.590
56	0.76	105.01	0.457	0.272	0.96	105.14	0.000	0.299

In bold the significant mean differences are reported for both parametric (ANOVA) and non-parametric (U-test) statistical tests.

is probably due to several factors which make this type of field investigation and data analysis difficult. In fact, the main difficulties in the field are associated with:

- constantly monitoring a homogenous and significant sample of dogs living in the same environment thus under the same parasitic load;
- locating a well defined area with a high parasitic pressure/load to evaluate drug efficacy;
- selecting animals that have not been treated with other acaricides;
- finding cooperative owners willing to arrange weekly examinations;
- finding owners who agree to leaving their dogs untreated (control group).

In the present study these difficulties have been overcome by choosing animals living in the same area and thus assuming that dogs were exposed to the same tick load through the observation period. Furthermore, in this study the authors proposed a semi-quantitative method for evaluating immature stages in order to calculate the efficacy of the test products.

To our knowledge, immature stages of ticks have neither been used to asses the efficacy of any acaricidal product in the field and/or in laboratory trials, nor for fipronil (e.g. Cruthers et al., 2001) or the imidacloprid/permethrin combination (Cruthers et al., 2003; Mehlhorn et al., 2003; Young et al., 2003). However, immature ticks play an important role in evaluating the repellent efficacy of a drug, since they are usually present in high numbers at the same time, in a small area. In fact, each female *R. sanguineus* lays from 4000 to 6000 eggs at a time for a total of about 15,000 eggs throughout its life depending on environmental factors. Nevertheless, only 5% of the larvae will survive before blood feeding, while this percentage is higher (20%) in nymphs (Sonenshine, 1991, 1993).

The semi-quantitative method presented here for the evaluation of immature stages can be considered a useful tool for calculating the efficacy of a drug in the field. The importance of immature ticks for animal and human health is due to the fact that they transmit a number of bacterial and protozoal TBDs, and they are important for maintaining tick infestations in the environment. Therefore, effective control of immature ticks is advisable to reduce the damage caused by ticks in animals, humans and the environment.

Up to now the efficacy of imidacloprid 10%/permethrin 50% versus fipronil 10%/methoprene 12% has been assessed only in laboratory conditions, by leaving treated dogs in contact with adult ticks for 2 h at days +7, +14, +21, +28 and +35 (Young et al., 2003).

Furthermore, a European multi-centre field trial was conducted to evaluate the efficacy of both the products on dogs from 23 veterinary clinics in Germany, France and Italy by weekly collection of ticks on treated dogs till day +28 (Hellmann et al., 2003). At day +28, that trial reported an efficacy against *Rhipicephalus* spp. of 98.5% and 89.4% on animals treated with a combination of imidacloprid 10%/permethrin 50% and fipronil 10%/methoprene 12%, respectively, while the efficacy registered in the present study on the same day p.t. (+28) on adult *R. sanguineus* was 91.67% and 88.89%, respectively. The study by Hellmann and colleagues (2003) was probably influenced by the fact that the immature stages were not detected and that the animals came from very different environments with very different parasitic load conditions.

In the present study, the efficacy of both products in groups A and B on adult ticks is high and very similar. In particular, the highest percentage (100%) was reported in group A at day +35 (1 week after the second treatment). From day +14 to day +28, group A reported higher efficacy values than group B. Conversely, these differences were not detectable after the second treatment when both products displayed efficacy values ranging from 99.42% to 100% (group A) and from 98.55% to 99.81% (group B).

As regards the immature stages, the high number at day +28 (1021.43 in group C) is related to the biology of *R. sanguineus* in the study area. The highest load of immature stages ($t + 28$), in fact, occurred about one week after the day of maximum humidity (r.u. = 92%) recorded at day +21 (Fig. 7).

As regards the efficacy of both products against immature ticks, a higher efficacy was reported in group A than group B; this difference was statistically significant at day +28 (Table 7). After the second treatment, the efficacy of the products on immature

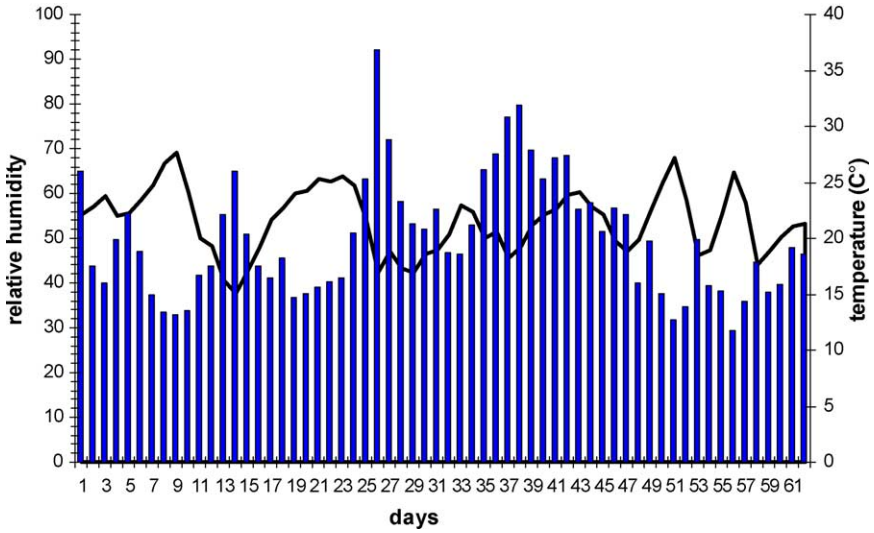

Fig. 7. Mean temperature (black line) and relative humidity (blue bars) recorded from July to the end of August 2004, including the observation period (i.e. 5 July (day 0), and 31 August (day +56)). Treatments were administered at baseline (corresponding to days 5–6) and +28 days (corresponding to days 33–34) (±1 day).

ticks in group A was consistently above 99%, except for day +35 (98.20%).

In groups A and B, the immature ticks were mainly concentrated on the legs and interdigital spaces (group A = 82.42%, group B = 70.84%), followed by the head (group A = 13.08%, group B = 20.34%) and trunk (group A = 4.48%, group B = 8.82%). Conversely, the distribution of immature stages in group C was homogeneous (i.e. head = 31.8%, trunk = 29.02% and legs = 39.18%). The higher concentration of immature ticks on the legs in groups A and B could be explained by the fact that less product ''reaches'' the extremities, which are the body regions most exposed to immature tick infection. Although no experimental trials have so far measured the amount of active ingredient in trunk and legs of treated dogs, it is reasonable to assume that when a product is applied topically and remains dermal, more active ingredient will be removed from the legs than from more proximal body regions.

On the whole, in analysing the efficacy of both products against adult plus immature ticks, it was found that the combination of imidacloprid 10%/permethrin 50% was more effective than that of fipronil 10%/methoprene 12%, with the difference being statistically significant at day +28 (group A = 98.43%, group B = 77.56%).

5. Conclusion

The significant difference between groups A and B against immature ticks at +28 days p.t. may be due to the fact that the concentration of the active ingredient in group B had fallen below an effective level faster than in group A. Alternatively, the fipronil/*S*-methoprene combination (group B) may be less effective against immature ticks compared to the imidacloprid/permethrin combination (group A). Furthermore, the higher humidity reported between days 21 and 28 p.t., might have significantly increased the amount of immature ticks. The differences in efficacy reported between group A and B on day 28 p.t. may be due to the additional repellent activity of the permethrin in the product combination applied to the dogs in group A. This hypothesis is also confirmed by the higher efficacy reported in group A versus group B on the following days until the end of the study (Table 7).

In addition, the field trial on the efficacy of imidacloprid 10%/permethrin 50% versus fipronil 10% (w/v) and methoprene 12% is the first longitudinal study aimed at evaluating repellent efficacy using two treatments (at days 0 and +28), which the manufacturers advise for both products.

Finally, permethrin is not only well known for its acaricidal activity but it has also been proven to have

repellence activity when combined with imidacloprid (Mehlhorn et al., 2003; Stanneck et al., 2004). On the basis of our results in the field, it seems that the product's repellence and acaricidal activity should be able to prevent the transmission of TBDs, particularly given the important role played by immatures.

Acknowledgments

The authors wish to thank Norbert Mencke Bayer Health Care Animal Health Division Veterinary Services, Germany for his suggestions.

References

Abbott, W.S., 1987. A method of computing the effectiveness of an insecticide. J. Am. Mosq. Control Assoc. 3, 302–303.

Cruthers, L., Slone, R.L., Guerrero, J., Robertson-Plough, C., 2001. Evaluation of the speed of kill of fleas and ticks with Frontline Top Spot in dogs. Vet. Ther. 2 (2), 170–174.

Cruthers, L., Slone, B.S., Arther, R.G., Davis, W., Settje, T., 2003. K9 Advantix (Imidacloprid plus Permethrin) for controlling ticks and mosquitoes on dogs. Suppl. Compend. Contin. Educ. Pract. Vet. 25, 15–18.

de Castro, J.J., 1997. Sustainable tick and tick borne disease control in livestock improvement in the developing countries. Vet. Parasitol. 71, 77–97.

Duncan, J.L., Abbott, E.M., Arundel, J.H., Eysker, M., Klei, T.R., Krecek, R.C., Lyons, E.T., Reinemeyer, C., Slocombe, J.O., World Association for the Advancement of Veterinary Parasitology (WAAVP), 2002. World Association for the Advancement of Veterinary Parasitology (WAAVP): second edition of guidelines for evaluating the efficacy of equine anthelmintics. Vet. Parasitol. 103, 1–18.

Epe, C., Coati, N., Stanneck, D., 2003. Efficacy of the compound preparation imidacloprid 10%/permethrin 50% spot-on against ticks (*I. ricinus, R. sanguineus*) and fleas (*Ct. felis*) on dogs. Parasitol. Res. 90, 122–124.

Hellmann, K., Knoppe, T., Krieger, K., Stanneck, D., 2003. European multicenter field trial on the efficacy and safety of a topical formulation of imidacloprid and permethrin (Advantix) in dogs naturally infested with ticks and/or fleas. Parasitol. Res. 90, 125–126.

Lane, R.P., Crosskey, R.W. (Eds.), 1995. Medical Insects and Arachnids. Chapman & Hall, London.

Manilla, G., 1998. Fauna d'Italia, vol. XXXVI. Acari-Ixodida. Edizioni Calderini, Bologna, pp. 210–236.

Mehlhorn, H., Schmahl, G., Mencke, N., Bach, T., 2003. In vitro and in vivo studies on the effect of a combination containing 10% imidacloprid and 50% permethrin against *Ixodes ricinus* ticks. Parasitol. Res. 89, 323–325.

Mencke, N., Volf, P., Volfova, V., Stanneck, D., 2003. Repellent efficacy of a combination containing imidacloprid and permethrin against sand flies (*Phlebotomus papatasi*) on dogs. Parasitol. Res. 90, 108–111.

Sonenshine, D.E., 1991. Biology of Ticks, vol. I. Oxford University Press, Oxford.

Sonenshine, D.E., 1993. Biology of Ticks, vol. II. Oxford University Press, Oxford, 465 pp.

Stanneck, D., Bach, T., Krieger, K., 2004. Repellent and acaricidal efficacy of imidacloprid 10%/permethrin 50% spot-on against ticks (*Ixodes ricinus*) on dogs in comparison to fipronil 10% spot-on. In: BSAVA Scientific Proceedings, p. 102.

Young, D.R., Arther, R.G., Davis, W.L., 2003. Evaluation of K9 Advantix vs. Frontline Plus topical treatments to repel brown dog ticks (*Rhipicephalus sanguineus*) on dogs. Parasitol. Res. 90, 116–118.