

THE ANTI-ECTOPARASITE EFFECTS OF DIFFERENT TOPICAL SPOTS-ON FORMULATION AGAINST TICKS AND FLEAS IN NATURALLY INFESTED STRAY AND OWNED DOGS

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Abstract: Ectoparasites poses potential hazards and are a constant challenge for veterinarians and pet owners worldwide. This clinical field study was conducted to assess the efficacy of different topical spot-on formulations against ticks and fleas in naturally infected dogs admitted for clinical examination at the Veterinary Hospital, Faculty of Veterinary Medicine, Sharkia Governorate, Egypt. Herein, forty dogs showed ectoparasites infestation were enrolled. These dogs were divided into four groups (ten each). Group1 (G1) considered as untreated control. The other three groups (G2, G3, and G4) were treated with fipronil 9.8% (Fipron spot-on solution), a combination of 9.8% fipronil/(S)-9.8% methoprene (Frontline Plus®), and imidacloprid/permethrin (K9-Advantix®), respectively. Clinical (all the dogs were clinically monitored throughout the experiment), parasitological (ticks and fleas (adult and eggs) were counted and identified on days 0, 1, 2, 7, 15, 21, 30, and 45 after treatment, and zootechnical (live-weight gain) analyses were conducted. Throughout the experiment, no adverse symptoms were observed in any of the treated animals. The synergistic effect of each topical spot-on was compared for eliminating ticks and fleas, aiming to obtain the ideal treatment model. Our results suggest that fipronil/(S)-methoprene outperformed fipronil alone in controlling ticks and fleas in naturally infested dogs. Also, it may be productive in the struggle against different tick and flea species infesting dogs as it gives protection for at least four weeks. In comparison to the other two topical spot-on formulations used in this study, imidacloprid/permethrin was the least effective, particularly against fleas. Overall, because these ectoparasites concern animal welfare and human health, this study underlines the importance of continued ectoparasites monitoring and educating pet owners about the impacts of flea and tick infestations as well as pathogenic hazards.

Key words: dogs; ectoparasites; Fipronil(S)-methoprene; Imidacloprid/permethrin; spot-on

Introduction

Dogs are the oldest domesticated and friendly pets as it's common for owners to treat them like a member of the family (1). Hence, they have activities to support owners' physical, social, and companionship and improve their mood, particularly for older people and children (2, 3). However,

the close interactive relationship between dogs and humans could be associated with many human infectious diseases, including bacterial, fungal, viral, and parasitic infections (4-6). Tick infestation is a common veterinary health concern in dogs and often seen in poorly nourished dogs in shelters and kennels but is diagnosed in well cared dogs too, primarily associated with under-

lying health issues (7). However, various tick species infest domestic dogs, *Rhipicephalus sanguineus* (*R. sanguineus*) is one of the most commonly distributed (8). Fleas and ticks display hematophagous activities, and some species consider as intermediate hosts and/or pathogen vectors, such as *Ehrlichia* spp., *Anaplasma* spp., *Rickettsia* spp., *Babesia* spp., and *Dipylidium caninum* (9). Furthermore, many pathogens are transmitted by dog fleas and have zoonotic potential (10). *Ctenocephalides felis* (*C. felis*) and *Ctenocephalides canis* (*C. canis*) are the most common flea-infested dogs, even though *Pulex irritans* and *Pulex simulans* can often be considered (11). Adverse health effects of ectoparasites, particularly life-threatening anemia, a wide range of dermatological problems, and vector-borne pathogens, may require long-term protection coverage for ticks and fleas (12, 13). Notwithstanding this, many dog owners still record tick infestations on their dogs, even though they use acaricidal agents (14).

Furthermore, in low-income communities, interventions to treat such parasites are frequently performed throughout the advanced stages of the disease, causing discomfort to pets and their owners (15). Several topical spots, such as fipronil, permethrin, and methoprene, have been used to treat pet animals infected with ectoparasites, which spread through the skin or direct contact with the parasites, or absorb and spread systemically (16). In contrast, minor compounds were used orally, such as comforthis and nitenpyram (17). Several combinations of acaricidal properties, which are considered effective and safe for treatment have been used to protect dogs against fleas or ticks or both, as fipronil/methoprene, imidacloprid/permethrin, or fipronil/permethrin (18, 19). Unfortunately, in many African countries, including Egypt, appropriate policies regarding pet ownership and their effects on individual and community health do not exist. So its control is crucial to maintaining the health and welfare of pets and protecting people from their infestations and vectorial capacity for transmission of zoonotic infections. Thus, the current study was conducted to recruit and monitor the anti-ectoparasite effects of different topical spot-on formulations against ticks and fleas in naturally infested stray and owned dogs admitted for clinical examination at the Veterinary Hospital, Faculty of Veterinary Medicine, Sharkia Governorate, Egypt.

Material and methods

Ethical approval

This study was issued by the Institutional Animal Care and Use Committee (IACUC) of the Zagazig University (Approval No.: ZU-IACUC/2/ F/122/2021).

Animals

This study was conducted throughout March–April 2021, in a separate building room, at the Veterinary Hospital, Faculty of Veterinary Medicine, Sharkia Governorate, Egypt. Forty mixed breeds, including stray and owned dogs (20 of each) were randomly selected based on the criteria of possessing ectoparasites, any breed, sex, and were not subjected to ectoparasiticides six weeks before enrolling for inclusion in this study. The selected dogs weighted 6.5 to 37 kg (avg. 16 kg), 28 males and 12 females, and their age ranged from above six months to 3 years old. Pregnant and lactating dogs with ectoparasites in clinical consultation or those treated with ectoparasiticides (within one month of first treatment) were excluded from this study. Dogs were handled in compliance; in addition, the dogs' owners approved and signed informed consent about the study procedures before enrolment.

Clinical examination

On the first day of therapy, all enrolled dogs were examined and screened for ticks and fleas by a clinical body examination. Next, an ID number was assigned to each dog. Much attention was given to the ear pinnae, and inside the external ear canal. The dogs were then checked on the neck, chest area, legs, armpits, and interdigital spaces. All dogs were housed in separate cages in the same place. They were given commercial food and drinking water ad libitum and were under one week of observation. The enrolled dogs exhibited alopecia, pruritus, scales and were restless and continuously rubbed their bodies against the walls and scratched their legs.

Ticks and fleas collection and identification

Ticks were manually recovered from dogs using blunt medium-sized steel forceps without injuring mouthparts (20), while fleas were collected with a moistened flea comb (21).

For further examinations, the samples were put in clean, labeled plastic tubes and transferred to the Parasitology Department, Faculty of Veterinary Medicine, Zagazig University, Egypt. Furthermore, ticks and fleas pooling samples from examined dogs were cleared in lactophenol media, then mounted permanently on polyvol, and left in a hot air oven at 50°C for 24 h (22, 23). After that, tick and flea species were morphologically identified using light (Leica, USA) and stereomicroscopes (Optika, Italy) according to standard keys (24, 25, 26). The infected dogs with ticks were examined by thumb counts on days 0, 1, 2, 7, 15, 30 and 45, while both adult and eggs of fleas were examined by comb-counting with the use of fine flea comb on the same previous days as previously described (27).

Dog grouping and treatment protocols

Dogs were divided into four groups of ten dogs each, and were caged individually in an indoor animal unit. One week before the experiment, all dogs were managed equally to be in similar condition. After evaluating the percentage of infestations with ticks and fleas, G1 was considered as an untreated control and remained without treatment. All the other three groups were subjected to therapy on the first day of the experiment with a spot-on formulation. Ten dogs in G2 were treated with fipronil 9.8% (Fipron spot-on solution by Bioveta, Pet & Animal Supplies, Turkey) at a dosage of 0.67 mL for a dog weighing from 2 to 10 kg and 1.34 mL for a dog weighing from 10.1 to 20 kg and 2.68 mL for those weighing from 20.1 to 40 kg. Dogs in G3 were treated with a combination of 9.8% fipronil/ (S)-9.8% methoprene (Frontline Plus®, Merial Limited, Duluth, Ga., Georgia) at a dose of 0.67 mL for those weighing 2 to 10 kg, 1.34 mL for those weighing 10.1 to 20 kg, and 2.68 mL for those weighing more than 20.1 kg. Finally, G4 received the prescribed dose of imidacloprid/permethrin (K9-Advantix®, Bayer Animal Health, Shawnee Mission, Ks., Missouri, USA). Small dogs, less than 4.5 kg, took one pipette with 0.4 mL; dogs weighing from 4.5 to 9.1 kg took one pipette with 1.0 mL; dogs weighing from 9.1 to 25 kg took one pipette containing 2.5 mL; meanwhile, for those weighing more than 25 kg, the dose was one pipette contained 4.0 mL.

These drugs were used as spot-on against fleas and ticks along the scoreline. First, the snap-off top of the pipette was broken. Next, part the dog's hair at the base of the neck, between the shoulder blades, until the skin is visible, and place the pipette tip on the skin, then gently squeeze to empty the contents onto the skin in one or two spots. Each product was applied topically to dogs on day 0 and again on day 30.

The counts took place 24-48 hrs after therapy, on days 7, 14, 21, 30, and 45 post-treatment. The proportion of ticks, adult fleas, and their egg reductions in treated groups were assessed in combination with the calculation of the effectiveness concerning the untreated control. A clinical examination of the dog's hair was done with a flea comb to confirm the existence or non-existence of adult fleas and their eggs as well as thumb tick counts on days 0 (treatment day), 1, 2, 7, 15, 21, 30, and 45 post-treatment. The intensity of tick infestation was represented as the number of ticks per unit area of 4 square inches. In contrast, the intensity of flea infestation (adult and egg) was detected by passing the flea comb five times from head to tail, and the number of fleas trapped in the comb during the process was counted and expressed as the intensity of fleas on the body. The occurrence of adverse reactions was monitored throughout the study. This experimental design was applied according to previous studies (19, 28).

Statistical analysis

Data was statistically analyzed using the SPSS program version for Windows, Version 23.0. IBM Corporation, Armonk, New York, 2015 (<https://www.ibm.com/support/pages/downloading-ibm-spss-statistics-23>). The two-way ANOVA test was performed for comparisons between studied factors (i.e., groups "G1, G2, G3 and G4" and days post-treatment "0, 24h, 48h, 7, 15, 21, 30, and 45) and followed by a post hoc test using the Duncan multiple range (DMR) test for comparisons between means of groups, days post-treatment or the interaction between them. *P*-values less than 0.05 were considered statistically significant.

Assessment of efficacy

Efficacy was evaluated following the World Association for the Advancement of Veterinary Parasitology (WAAVP) guideline as described previously (29).

Efficacy percentage (%) = $100 \times (MC - MT) / MC$

Where MC is the mean of live ticks/fleas and flea eggs in the control group, and MT is the mean of live ticks/fleas in the treatment groups.

Results

The clinical examination of forty dogs revealed average body temperature, pulse rate, and pale mucous membranes. In addition, these dogs displayed clinical symptoms of alopecia, reduced food intake, restlessness, and scratching, in addition to rubbing themselves against hard objects (Figure 1A, B, C). Furthermore, our results revealed that twenty dogs were infested with ticks and twelve dogs had external fleas on their skin. While mixed infestations of ticks and fleas were detected in eight dogs. The detected ticks, and fleas are usually found around the shoulders, necks, backs, ears, heads, and over the tails. Moreover, the ticks were identified as brown dog ticks, *R. sanguineus* (Figure 2A, B). Whereas, the detected fleas were microscopically confirmed as *C. felis*, which were characterized morphologically by the presence of both genal and prortal ctenidia (combs), with the first genal spine in the genal ctenidia being nearly as long as the second one (Figure 2 C, D).

Ticks and fleas (adult and eggs) were counted on days 0, 1, 2, 7, 15, 21, 30, and 45 post-treatment and identified as *R. sanguineus* and *C. felis*, respectively. Dogs are checked regularly, especially near the end of the treatment period, to ensure any ticks and/or fleas, either adult or eggs; no signs of skin irritation were observed in the treated groups. In comparison to the treated groups, the control group experienced significant and continuous detection of ticks and fleas, either adult or egg, throughout the trials. In general, the weight differences between the treated and untreated group revealed a slight increase. In addition, the results showed that the medication was statistically insignificant ($P > 0.05$). Therefore, the weight of individual dogs in all treated groups was slightly higher than untreated groups at the end of this study (Table 1). It was also observed that the treated dogs improved their coat and physical

condition compared to the untreated control group that always maintained restlessness. In addition, none of the treated dogs showed any adverse symptoms after treatment. Furthermore, none of the workers mentioned adverse insecticide reactions (handlers and kennel workers).

No significant difference was found at the start of the trial (0 days) among the treated groups ($P > 0.05$). However, the number of ticks in the control group was significantly lower than that reported in the treated groups, but adult and egg of flea counts were the same for treated groups and the control. Regarding tick count, 100% efficiency in G2, G3, and G4 after the first assessment was recorded on days 2 and 1 and 15 post-treatment, respectively. However, the efficacy of counting ticks was reduced to 99.12% in G2 and 92.82% in G4 at 21 days post-treatment, while starting later in G3 (30 days, 96.35%). In addition, significantly lower efficacy was recorded in G4 (59.5%) at 30 days post-treatment than other treated groups. The efficiency in all treated groups at 45 days after the second treatment application was 100% (Table 2).

Adult fleas were collected on day 0 and started to sustain on day 1 in all treated groups. G2 and G3 showed 100% efficacy at 48 hrs post-treatment compared to G4 (in which 100% efficacy started later, 21 days) and returned on days 21, 30, 30 for G2, G3 and G4, respectively (Table 3). All dogs of G3 remained free of adult fleas from two days after the first assessment up to one month, and slightly fell at one month post-treatment (96.93%) and returned to 100% at six weeks post-treatment (end of the trial). On day 45 after G4 treatment, there were significantly fewer adult fleas recorded, but not to the same extent (100%) as in other treated groups at the same time.

Meanwhile, 100% efficiency was observed in fleas' eggs on the 1st and 7th day after the G2 and G3 therapies. The same mentioned groups returned to 100% in 45 days after falling on days 21 and 30. Whereas the efficacy of flea eggs was not 100% in G4 throughout the trials, the maximum effectiveness (99.29%) was recorded on day 15 of the first assessment (Table 4). The combination of fipronil/ (S)-methoprene outperformed fipronil alone in controlling ticks and fleas in naturally infested dogs. Compared to the other two topical spot-on formulations used in this study, the combination of imidacloprid/permethrin was the least effective, particularly against fleas.



Figure 1: Clinical picture of alopecia and tick infestations in dogs. Local alopecia and itching in the belly (A), Dorsal of the back (B), and Dog's ear (C)

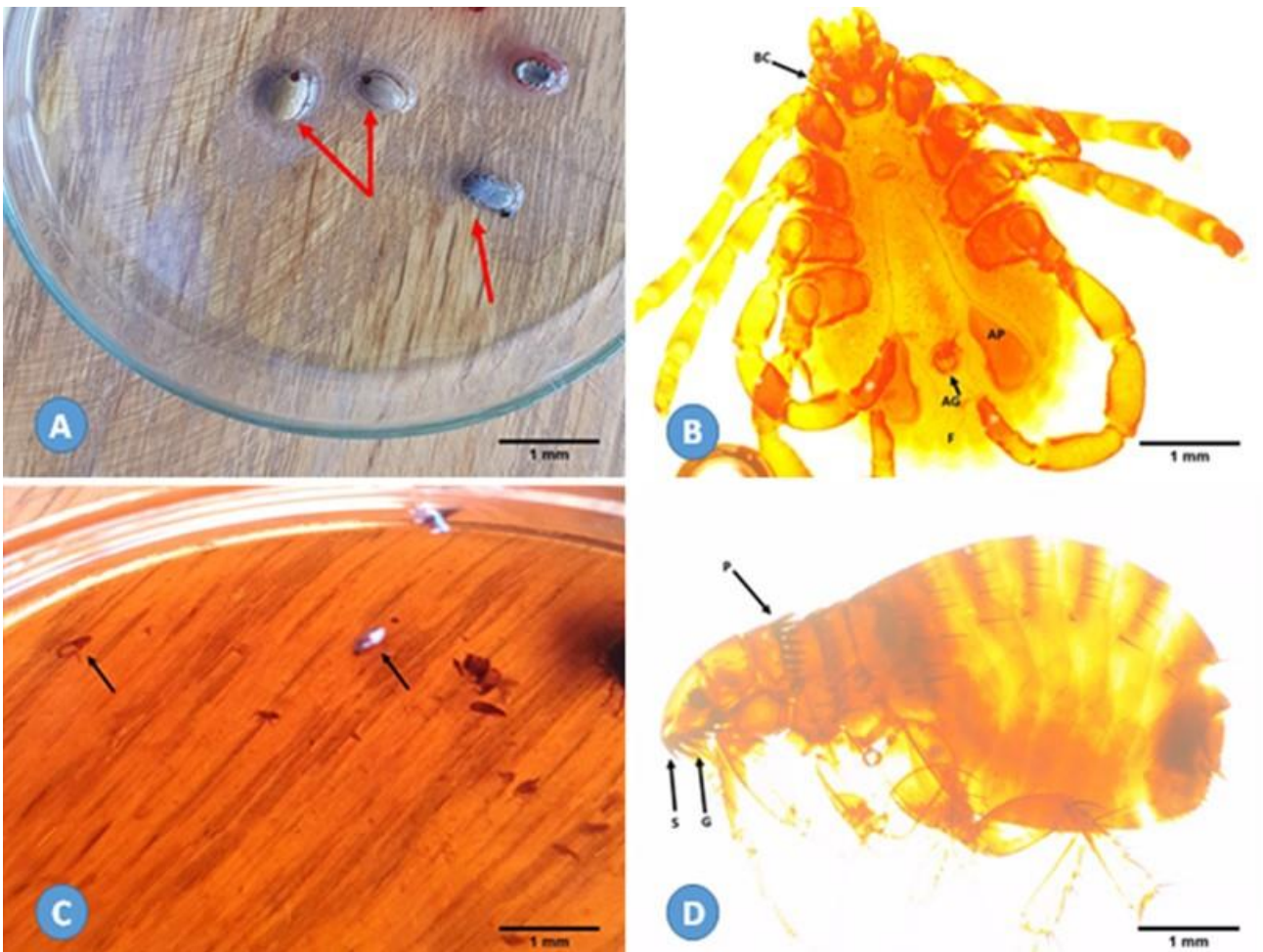


Figure 2: Adult *R. sanguineus* and adult *C. felis* isolated from infested dogs. (A) Isolated adult *R. sanguineus* (Red arrows); (B) Light microscope identification of an adult *R. sanguineus* male (ventral view); (BC: Basis capitulum is hexagonal in shape; AP: Adanal plate; AG: Anal groove below the anus; F: Festoon); (C) Adult fleas isolated from infested dogs (black arrows); and (D) Light microscope identification of adult *C. felis*, (P: Pronotal ctenidia; G: Genal ctenidia; and S: The length of the first spine of the genal ctenidia is nearly equal to the length of the second spine (scale bar, 1 mm)

Table 1: The variation in body weight of dogs all over the experimental period

Groups (G)	Days post treatment \pm SE						Groups effect
	0	7	15	21	30	45	
G1(control)	16.81 \pm 2.22	16.59 \pm 2.21	16.33 \pm 2.22	15.25 \pm 2.06	14.34 \pm 2.11	13.91 \pm 2.07	15.54 \pm 0.85
G2	16.00 \pm 1.86	16.18 \pm 1.86	16.37 \pm 1.88	16.72 \pm 1.88	17.08 \pm 1.91	17.70 \pm 1.94	16.68 \pm 0.74
G3	16.50 \pm 3.00	16.58 \pm 3.01	16.87 \pm 3.05	17.25 \pm 3.03	17.55 \pm 3.01	18.20 \pm 3.01	17.16 \pm 1.18
G4	16.30 \pm 3.2	16.43 \pm 3.09	16.67 \pm 3.12	16.95 \pm 3.13	17.24 \pm 3.11	18.10 \pm 3.28	16.95 \pm 1.24
Days post treatment effect	16.40 \pm 1.26	16.45 \pm 1.25	16.56 \pm 1.26	16.54 \pm 1.25	16.55 \pm 1.26	16.98 \pm 1.3	

SE refers to standard error

Table 2: Reduction in the mean of tick counts all over the experiment

Groups (G)	Days post treatment								Groups effect
	0	24 h	48h	7	15	21	30	45	
G1	180.71 fgh \pm 25.49	199.86 fg \pm 33.13	245.143 ef \pm 33.7	291.000 cde \pm 31.49	345.714 bcd \pm 33.36	362.000 bc \pm 38.18	411.143 b \pm 54.15	494.429 a \pm 59.17	316.250 a \pm 18.99
G2	342.14 bcd \pm 21.33	17.14 j \pm 11.28	0.000 j \pm 0	0.000 j \pm 0	0.000 j \pm 0	3.000 j \pm 3	14.286 j \pm 14.29	0.000 j \pm 0	47.071 c \pm 15.42
G3	313.29 cde \pm 33.52	0.00 j \pm 0	0.000 j \pm 0	0.000 j \pm 0	0.000 j \pm 0	0.000 j \pm 0	11.429 j \pm 11.43	0.000 j \pm 0	40.589 c \pm 14.51
G4	278.43 de \pm 31.11	147.86 gh \pm 18.53	49.000 ij \pm 11.91	2.857 j \pm 2.86	0.000 j \pm 0	20.000 j \pm 13.63	112.857 hi \pm 28.43	0.000 j \pm 0	76.375 b \pm 13.73
Days post treatment effect	278.64 a \pm 17.74	91.214 cd \pm 18.81	73.536 d \pm 21.2	73.464 d \pm 25.29	86.429 d \pm 29.86	96.250 cd \pm 31.08	137.429 b \pm 34.83	123.607 bc \pm 43.5	
Efficacy percentage %									
G1	0.00	-10.59	-35.65	-61.03	-91.30	-100.32	-127.51	-173.60	0.00
G2	0.00	94.99	100.00	100.00	100.00	99.12	95.82	100.00	85.12
G3	0.00	100.00	100.00	100.00	100.00	100.00	96.35	100.00	87.17
G4	0.00	46.90	82.40	98.97	100.00	92.82	59.47	100.00	75.85
Days post treatment effect	0.00	67.26	73.61	73.64	68.98	65.46	50.68	55.64	

SE refers to standard error. Values are represented as mean \pm SEM. Means carrying different superscripts are significant at $P < 0.05$ **Table 3:** Reduction in the mean of adult fleas counts all over the experiment

Groups(G)	Days post treatment								Groups effect
	0	24 h	48h	7	15	21	30	45	
G1	137.800 c \pm 9.01	169.000 b \pm 14.9	171.400 b \pm 11.4	173.600 b \pm 10	169.200 b \pm 7.43	180.200 ab \pm 4.88	185.600 ab \pm 7.7	203.000 a \pm 3.9	173.725 a \pm 4.05
G2	134.600 c \pm 8.2	74.000 d \pm 10.94	0.000 g \pm 0	0.000 g \pm 0	0.000 g \pm 0	2.200 g \pm 2.2	15.400 fg \pm 12.41	0.000 g \pm 0	21.750 c \pm 7.26
G3	143.200 c \pm 10.16	21.800 fg \pm 0	0.000 g \pm 0	0.000 g \pm 0	0.000 g \pm 0	0.000 g \pm 0	4.400 g \pm 4.4	0.000 g \pm 0	18.450 c \pm 7.66
G4	126.000 c \pm 11.17	74.000 d \pm 11.09	46.200 e \pm 11.58	17.200 fg \pm 8.85	0.600 g \pm 0.6	0.000 g \pm 0	31.400 ef \pm 17.24	6.200 g \pm 2.71	37.700 b \pm 7.27
Days post treatment effect	135.400 a \pm 4.67	66.200 b \pm 15.74	54.400 cd \pm 16.52	47.700 cd \pm 17.03	42.450 d \pm 16.88	45.600 d \pm 17.87	59.200 bc \pm 17.69	52.300 cd \pm 20	
Efficacy percentage %									
G1	0.00	-22.64	-24.38	-25.98	-22.79	-30.77	-34.69	-47.31	0.00
G2	0.00	45.02	100.00	100.00	100.00	98.37	88.56	100.00	87.48
G3	0.00	84.78	100.00	100.00	100.00	100.00	96.93	100.00	89.38
G4	0.00	41.27	63.33	86.35	99.52	100.00	75.08	95.08	78.30
Days post treatment effect	0.00	51.11	59.82	64.77	68.65	66.32	56.28	61.37	

SE refers to standard error. Values are represented as mean \pm SEM. Means carrying different superscripts are significant at $P < 0.05$

Table 4: Reduction in the mean of fleas' egg counts all over the experiment

Groups(G)	Days post treatment								Groups effect
	0	24 h	48h	7	15	21	30	45	
G1	79.800 d ±16.6	102.400 cd ±19.85	118.000 bc ±19.55	125.400 b ±12.44	174.200 a ±4.77	181.800 a ±7.96	183.600 a ±7.37	187.400 a ±3.36	144.075 a ±7.62
G2	86.400 d ±11.53	8.400 f ±3.6	0.400 f ±0.4	0.000 f ±0	0.000 f ±0	0.600 f ±0.4	10.600 f ±6.65	0.000 f ±0	13.300 bc ±4.73
G3	88.000 d ±12.98	0.000 f ±0	0.000 f ±0	0.000 f ±0	0.000 f ±0	0.000 f ±0	5.000 f ±4.29	0.000 f ±0	11.625 c ±4.88
G4	84.200 d ±7.77	37.000 e ±6.57	25.200 ef ±9.97	2.800 f ±1.39	0.600 f ±0.6	0.800 f ±0.8	12.600 f ±2.69	1.200 f ±0.97	20.550 b ±4.65
Days post treatment effect	84.600 a ±5.83	36.950 cd ±10.43	35.900 cd ±12.21	32.050 d ±12.7	43.700 bcd ±17.32	45.800 bc ±18.11	52.950 b ±17.5	47.150 bc ±18.59	
Efficacy percentage %									
G1	0.00	-28.32	-47.87	-57.14	-118.30	-127.82	-130.08	-134.84	0.00
G2	0.00	90.28	99.54	100.00	100.00	99.31	87.73	100.00	90.77
G3	0.00	100.00	100.00	100.00	100.00	100.00	94.32	100.00	91.93
G4	0.00	56.06	70.07	96.67	99.29	99.05	85.04	98.57	85.74
Days post treatment effect	0.00	56.32	57.57	62.12	48.35	45.86	37.41	44.27	

SE refers to standard error. Values are represented as mean ± SEM. Means carrying different superscripts are significant at $P < 0.0$

Discussion

In developing countries, the public health significance of ectoparasites of domestic animals and pets is rising due to zoonosis associated with the pathogens they transmit. There is limited information on pets' ectoparasites in Sharkia Governorate. The clinical symptoms exhibited by dogs in this study were the same as previously reported (30, 31). *R. sanguineus* was identified in all tick counts in our study. This high percentage (100%) matched the findings of Abdullah et al. (32), who demonstrated that the prevalence of *R. sanguineus* was 100% in dogs in Egypt. Also, Papazahariadou et al. (33), who found *R. sanguineus* in 89.3% of dogs, and Ayodhya (31), who reported *Rhipicephalus* spp. in 38 of the 48 ticks infested (79.16%). Furthermore, Adhikari et al. (34) discovered that only 46.39% of dogs were infested with three different ixodid tick species (*Boophilus*, *Rhipicephalus*, and *Haemaphysalis* spp.). The infestation rate of *C. felis* in this study was 100%, matching that of a previous study (35) that found *C. felis* to have the highest prevalence of all flea species on 756 (67.5%) of examined dogs. However, this contradicts the findings of another study (36) in which *C. canis* was found in 92.2% of the examined dogs and *C. felis* was found in 7.8% of the dogs. Furthermore, Klimpel et al. (37) reported that *C. canis* (39.1%) and *C. felis* (17.4%) in urban stray dogs in Fortaleza (Brazil) were the most common ecto-

parasites than in other species. The occurrence and coexistence of ticks and fleas and their proximity to human dwellings demonstrate possible implications for animal and human health in Sharkia Governorate, where information about ectoparasites of dogs is scarce. Regarding the variation in the weight of treated individual dogs observed throughout the trial duration, the mean body weight was not significantly different from controlled dogs, similar to earlier report (28). This interprets the possibility of keeping dogs on a good diet for a long time to restore body gain. Fipronil alone produces reasonable control of dogs against ticks and fleas, but less than the combination of fipronil/methoprene as have been documented in many reports (38-40). High efficacy was attributed to the speed of kill and efficacy of fipronil in combination with methoprene, which is responsible for sterilizing laid eggs with inhibition of survival of ectoparasites, leading to a long period of the clean environment (41, 42).

The results recorded in this study concerning the fipronil/methoprene combination against the control of fleas was in accordance with Nambi et al. (19) but contrary to the same authors concerning low efficacy in controlling ticks (34.0-65.84%) versus recorded in this trial (96.35-100%). On the contrary, Cavalleri et al. (43) recorded a subordinate role of the fipronil/ methoprene combination in controlling ticks in dogs than the role provided by oral administration of chewable tablets

(credelio). In this trial, the imidacloprid/permethrin combination achieved the lowest efficacy for controlling ticks and fleas in naturally infected dogs, which agreed with a previous study (44). The fewer efficacies of some drugs used in this trial compared to others may be due to innate resistance to used drugs and development of tolerant strains, being used at the wrong time, or not treating pets frequently enough (40,45) and permitting persistence of ectoparasites for an extended time and increasing environmental reservoirs (46). The combination of fipronil and methoprene had an excellent acaricidal effect against ticks and fleas in naturally infected dogs, which was initiated within 24 hrs post-application. It prevented infestation for about four weeks, followed by fipronil alone and, lastly, the combination of imidacloprid / permethrin. A monthly dosing interval (28) is necessary to reduce the reappearance and massive infestation of dogs with fleas and ticks and decrease the environmental reservoir, so remission of clinical signs was achieved (47,48).

Conclusion

Our findings provide a comprehensive overview of the anti-ectoparasite effects of fipronil and its synergistic effect with methoprene as a more effective topical spot-on against different ticks and fleas infesting dogs. Fipronil/(S)-methoprene outperformed fipronil alone in controlling ticks and fleas in naturally infested dogs. Overall, because these ectoparasites concern animal welfare and human health, this study underlines the importance of continued ectoparasite monitoring and educating pet owners about the impacts of flea and tick infestations as well as pathogenic hazards.

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All authors contributed to the conception and design of the study. HME, SGY, NEA, and YAE performed the practical part and monitored the animals. RR has performed laboratory parasitology

examinations. HME, EMF, SGY, and NEA analyzed the data. HME and EMF prepared the initial draft of the manuscript. HME, RR, SGY, and YAE wrote the reviews and editing. EMF, NEA, RR, and YAE reviewed the results. All authors had complete access to all the data in the study, analysis accuracy and approved the final version to be published.

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