Acaricidal efficacy of a new combination of fipronil and permethrin against *Ixodes ricinus* and *Rhipicephalus sanguineus* ticks

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Abstract

**Background:** Two blinded, controlled laboratory studies were conducted to assess the acaricidal efficacy of a new combination of fipronil and permethrin (Frontline Tri-Act®/Frontect®) against two tick species. Study A evaluated the efficacy of the product against both *Ixodes ricinus* and *Rhipicephalus sanguineus* and Study B evaluated the efficacy against *R. sanguineus* only.

**Methods:** 16 (Study A) and 12 (Study B) healthy adult dogs were allocated to two groups in each study. Dogs in Group 1 served as untreated controls. Dogs in Group 2 were treated with a new topical spot-on formulation containing 6.76% (w/v) fipronil + 50.48% (w/v) permethrin once on Day 0. Each dog of study A was infested with 50 unfed adult ticks of each species and each dog of study B was infested with 50 unfed adult *Rhipicephalus sanguineus* prior to treatment (Day 2 in Study A, Day 1 in Study B) and post treatment on Days 7, 14, 21 and 28. The ticks were removed and counted 48 h after treatment (Day 2) or subsequent infestations (Days 9, 16, 23 and 30). Acaricidal efficacy was defined as the percent reduction in the number of live ticks in the treated group compared to the untreated control group.

**Results:** The percent efficacy in the treated group for *R. sanguineus* was 100%, 100%, 100% and 96.7% in Study A, and 94.4%, 100%, 100%, 98.7% and 98.0% in Study B, for counts performed on Days 2, 9, 16, 23 and 30, respectively. For *I. ricinus*, in Study A, the percent efficacy of the treatment was 100%, 100%, 100% and 99.2% for counts performed on Days 2, 9, 16, 23 and 30, respectively. There was a significant difference of the geometric mean numbers of live ticks between the treated and control groups at each time point in each study (\(p = 0.005\) for every day in Study A, and \(p < 0.005\) for every day in Study B).

**Conclusions:** A single topical administration of a combination of fipronil and permethrin provides excellent acaricidal efficacy against both *I. ricinus* and *R. sanguineus* for at least 4 weeks.

**Keywords:** Ticks, *Ixodes ricinus*, *Rhipicephalus sanguineus*, Permethrin, Fipronil, Dog, Acaricide, Frontline Tri-Act®/Frontect®

Abrégé

**Contexte:** Deux études expérimentales contrôlées et randomisées ont été conduites afin de mesurer l’efficacité acaricide d’une nouvelle combinaison de fipronil et de perméthrine (Frontline Tri-Act®/Frontect®) contre deux espèces de tiques. L’étude A a évalué l’efficacité du produit vis-à-vis de *Ixodes ricinus* et *Rhipicephalus sanguineus* et l’étude B a évalué l’efficacité vis-à-vis de *R. sanguineus* seul.

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Pour chaque étude, 16 (étude A) et 12 (étude B) chiens adultes en bonne santé ont été répartis en deux groupes. Les chiens du groupe 1 servaient de témoins non traités. Les chiens du groupe 2 étaient traités une fois au jour 0 avec une nouvelle formulation spot-on topique contenant 6,76% (w/v) de fipronil + 50,48% (w/v) de perméthrine. Chaque chien de l’étude A a été infesté par 50 tiques adultes à jeun de chaque espèce et chaque chien de l’étude B a été infesté par 50 *Rhipicephalus sanguineus* adultes à jeun, avant le traitement (jour −2 dans l’étude A, jour −1 dans l’étude B) et après traitement, aux jours 7, 14, 21 et 28. Les tiques ont été retirées et comptées 48 h après traitement (jour 2) ou après chaque infestation (jours 9, 16, 23 et 30). L’efficacité acaricide est définie comme le pourcentage de réduction du nombre de tiques vivantes dans le groupe traité par rapport au groupe témoin non traité.

**Méthodes:** Pour chaque étude, 16 (étude A) et 12 (étude B) chiens adultes en bonne santé ont été répartis en deux groupes. Les chiens du groupe 1 servaient de témoins non traités. Les chiens du groupe 2 étaient traités une fois au jour 0 avec une nouvelle formulation spot-on topique contenant 6,76% (w/v) de fipronil + 50,48% (w/v) de perméthrine. Chaque chien de l’étude A a été infesté par 50 tiques adultes à jeun de chaque espèce et chaque chien de l’étude B a été infesté par 50 *Rhipicephalus sanguineus* adultes à jeun, avant le traitement (jour −2 dans l’étude A, jour −1 dans l’étude B) et après traitement, aux jours 7, 14, 21 et 28. Les tiques ont été retirées et comptées 48 h après traitement (jour 2) ou après chaque infestation (jours 9, 16, 23 et 30). L’efficacité acaricide est définie comme le pourcentage de réduction du nombre de tiques vivantes dans le groupe traité par rapport au groupe témoin non traité.

**Résultats:** Le pourcentage d’efficacité contre *R. sanguineus* dans le groupe traité a été de 100%, 100%, 100% et 96,7% dans l’étude A, et 94,4%, 100%, 98,7% et 98,0% dans l’étude B, pour les comptages réalisés aux jours 2, 9, 16, 23 et 30, respectivement. Pour *I. ricinus*, dans l’étude A, le pourcentage d’efficacité du traitement était de 100%, 100%, 99,2% pour les comptages réalisés aux jours 2, 9, 16, 23 et 30, respectivement. Une différence significative entre les moyennes géométriques de tiques vivantes entre les groupes traité et témoin, a été retrouvée à chaque point de comptage dans les deux études (p = 0,005 pour chaque jour dans l’étude A, et p < 0,005 pour chaque jour dans l’étude B).

**Conclusions:** Une administration topique unique de la combinaison fipronil et perméthrine offre une excellente efficacité acaricide à la fois contre *I. ricinus* et *R. sanguineus* pour au moins 4 semaines.
each dog after 48 h. The two male and two female dogs with the lowest tick counts were dropped from Study A and the two dogs (regardless of sex) with the lowest tick counts were dropped from Study B. The remaining dogs were ranked within sex by descending tick counts and assigned to blocks of two dogs each. Within blocks, each dog was randomly allocated to the treated and untreated groups. The dogs were managed with due regard for their well-being in accordance with Merial, South African and Irish Institutional Animal Care and Use Committee requirements. The dogs were housed individually. A veterinary examination performed prior to the start of each study ensured that all dogs were healthy and suitable for inclusion, and the dogs were observed daily for any health changes throughout the study.

Treatment
Dogs in Group 1 of Study A and B served as untreated controls. Dogs in Group 2 of both studies were treated once on Day 0 with a topical formulation containing 6.76% (w/v) fipronil and 50.48% (w/v) permethrin with a total volume corresponding to the appropriately sized pipette based upon body weight such that dogs weighing less than or equal to 10 kg received 1.0 mL, dogs weighing greater than 10 but less than 20 kg received 2.0 mL, and dogs weighing greater than 20 but less than 40 kg received 4.0 mL. The total volume of the product was divided into two approximately equal fractions and placed on the skin on the midline of the neck. One fraction was applied between the base of the skull and the shoulder blades and the other was applied at the front of the shoulder blades. All of the animals were observed hourly for any adverse reaction for 4 h following the treatment of the last animal.

Ticks
The ticks used in the studies were unfed adult *I. ricinus* ticks (50 females with 4–5 added males for each challenge) and unfed adult *R. sanguineus* ticks (approx. equal sex ratio) that were not known to be resistant to any ectoparasiticide. The ticks originated from European tick populations, now bred under experimental conditions. The *I. ricinus* ticks originated from natural populations from the United Kingdom, Slovakia, and Ireland. The *R. sanguineus* ticks used in Study A originated from natural populations from Oxford, UK, and for Study B the ticks originated from field collections in France.

Tick infestation and counting
Dogs in both studies were infested prior to treatment (Day –2 in Study A, Day –1 in Study B) and post treatment on Days 7, 14, 21 and 28 with 50 unfed adult *R. sanguineus* (approx. equal sex ratio). In Study A each dog was also infested with 50 unfed adult female *I. ricinus* ticks (with at least an additional 10 male *I. ricinus* ticks to stimulate female attachment) at the same time points.

Live ticks on the dogs were removed and counted on Days 2, 9, 16, 23 and 30 (48 h after treatment or infestation). *Ixodes ricinus* and *R. sanguineus* ticks were counted and recorded separately. Only female *I. ricinus* were counted while for *R. sanguineus*, both females and males were counted.

For tick infestations and counting in Study A, dogs were anesthetized with intramuscular injections of ketamine (Narketan®, Vetoquinol; approx. 10.0 mg/kg) and xylazine (Chanazine®, Chanelle; approx. 2.0 mg/kg). In Study B, dogs were sedated with medetomidine (Domitor®, Pfizer; 0.06 mg/kg) for tick infestations only.

Data analysis
For each tick species, total counts of live ticks were transformed to the natural logarithm of (counts + 1) for calculation of geometric means (GM) by treatment group at each time point. As described in the WAAVP guidelines, the use of geometric means allow to describe a central tendency whereas arithmetic means maintains the same weight to extreme data. Percent efficacy of the treated group compared to the control group was calculated at every post-treatment time point using the formula 100×[(C−T)/C], where C is the GM for the control group and T is the GM for the treated group.

In Study A the treated group was compared to the control group at every post-treatment time using the Friedman rank test with blocks defined as the allocation blocks. The testing was two-sided and used a significance level of 5%. All analyses were performed using SAS® Version 9.1.3. In Study B the groups were compared by a non-parametric analysis using the Mann–Whitney test. SAS® Version 9.3 TS Level 1 M2 was used for the statistical analysis.

Results
No adverse reactions to treatment were observed in any dog in either study, including during the 4 h after treatment.

A summary of the tick counts and efficacy results are shown in Table 1 (Study A) and Table 2 (Study B).

The percent efficacy of the treated group for *R. sanguineus* was 100%, 100%, 100%, 100% and 96.7% in Study A, and 94.4%, 100%, 100%, 98.7% and 98.0% in Study B, for counts performed on Days 2, 9, 16, 23 and 30, respectively. There was a significant difference of the geometric mean number of live ticks between the treated and control groups in both studies at each time point (*p* = 0.005 for every day in Study A, and *p* < 0.005 for every day in Study B).

For *I. ricinus* the percent efficacy of the treated group was 100%, 100%, 100%, 100% and 99.2% for counts performed on Days 2, 9, 16, 23 and 30, respectively. There was a significant difference of geometric mean number of live
ticks between the treated and control groups at each time point ($p = 0.005$ for every day).

In addition, the majority of dogs remained free of live ticks in the treated groups. All of the dogs treated with the tested spot-on were not infested with ticks at the counts performed for I. ricinus on Days 2, 9, 16 and 23; on Day 30, 6 out of 8 treated dogs did not harbour tick (data not shown). In Study A, no ticks were found on all of the treated dogs at the counts performed for R. sanguineus on Days 2, 9, 16 and 23; on Day 30, only 2 of 8 dogs were found to be infested with ticks. In Study B all of the dogs in the treated group were not infested with ticks. In Study B all of the dogs treated with the combination of fipronil and permethrin in either study for the Day 9 and Day 16 counts and only a very low number of ticks found on Days 2, 23, and 30 (Tables 1 and 2). These results are in the range to what has already been published with other acaricidal spot-on formulations like Frontline® Combo or Advantix® [8,12,13,16-18] and exceed the European regulatory threshold of more than 90% of efficacy counted at 48 h to get a claim.

Frontline Tri-Act®/Frontect® has also been shown to have repellent and parasiticidal efficacy against Dermacentor ticks [23], fleas [24,25], mosquitoes and plebotomine sandflies [26,27], indicating that the combination of fipronil and permethrin can be an important component in the reduction of the risk of transmission of most canine vector-borne diseases.

**Conclusions**

In conclusion, a single topical administration of a combination of fipronil and permethrin provides excellent acaricidal efficacy against both I. ricinus and R. sanguineus for at least 4 weeks. The product is safe and can also be used to reduce the risk of transmission of tick-borne pathogens in dogs.

**Competing interests**

The work reported herein was funded by Merial Limited. All authors were employees or contractors of Merial.

**Authors’ contributions**

PO, STC, BG, MS and FB participated in the design of the studies and protocols, and PD, BG, JF and FB carried out the studies. STC compiled and analysed the data. All authors read and approved the final manuscript.

**Acknowledgements**

The authors are sincerely grateful to all monitors, investigators and the staff of the study locations (Ireland and South Africa) either linked to the authors or serving as independent CROs who took part in the studies and ensured that high GCP standards were adhered to. Frontline Tri-Act® and Frontect® are registered trademarks of Merial in France and pending registration in other countries. All other marks are the property of their respective owners.

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Received: 20 January 2015 Accepted: 21 January 2015

Published online: 27 January 2015
References

1. Dantas-Torres F, Chomel BB, Otranto D. Ticks and tick-borne diseases: a One Health perspective. Trends Parasitol. 2012;28:437–46.
2. Wilhelmsson P, Lindblom P, Fryland L, Nyman D, Jaenson TG, Forsberg P, et al. *Ixodes ricinus* ticks removed from humans in Northern Europe: seasonal pattern of infestation, attachment sites and duration of feeding. Parasit Vectors. 2013;6:362.
3. Chomel B. Tick-borne infections in dogs—an emerging infectious threat. Vet Parasitol. 2011;179:294–301.
4. Claerebout E, Losson B, Cochez C, Casetta S, Delemans AC, De Cat A, et al. Ticks and associated pathogens collected from dogs and cats in Belgium. Parasit Vectors. 2013;6:183.
5. Beugnet F, Chalvet-Monfray K. Impact of climate change in the epidemiology of vector-borne diseases in domestic carnivores. Comp Immunol Microbiol Infect Dis. 2013;36:559–66.
6. Gray JS, Daulet H, Estrada-Pena A, Kahl O, Lindgren E. Effects of climate change on ticks and tick-borne diseases in Europe. Interdiscip Perspect Infect Dis. 2009;2009:592532.
7. Dantas-Torres F, Otranto D. Seasonal dynamics of *Ixodes ricinus* on ground level and higher vegetation in a preserved wooded area in southern Europe. Vet Parasitol. 2013;192:253–8.
8. Brianti E, Pennisi MG, Brucato G, Risitano AL, Gagli G, Lombardo G, et al. Efficacy of the fipronil 10% + (S)-methoprene 9% combination against *Rhipicephalus sanguineus* in naturally infested dogs: speed of kill, persistent efficacy on immature and adult stages and effect of water. Vet Parasitol. 2010;170:99–103.
9. Jongejan F, Uilenberg G. The global importance of ticks. Parasitology. 2004;129(Suppl):S3–14.
10. Shaw SE, Day MJ, Birtles RJ, Breitschwerdt EB. Tick-borne infectious diseases of dogs. Trends Parasitol. 2001;17:74–80.
11. Beugnet F, Franc M. Results of a European multicentric field efficacy study of fipronil-(S)-methoprene combination on flea infestation of dogs and cats during 2009 summer. Parasite. 2010;17:337–42.
12. Bonneau S, Gupta S, Cadiegues MC. Comparative efficacy of two fipronil spot-on formulations against experimental tick infestations (*Ixodes ricinus*) in dogs. Parasitol Res. 2010;107:735–9.
13. Kuzner J, Turk S, Grace S, Soni-Gupta J, Fournie JJ, Marchiondo AA, et al. Confirmation of the efficacy of a novel fipronil spot-on for the treatment and control of fleas, ticks and chewing lice on dogs. Vet Parasitol. 2013;193:245–51.
14. Brianti E, Falzone L, Napoli E, Prudente C, Gagli G, Gannetto S. Efficacy of a combination of 10% imidacloprid and 4.5% flumethrin (Seresto(R)) in slow release collar to control ticks and fleas in highly infested dog communities. Parasit Vectors. 2013;6:210.
15. Dantas-Torres F, Capelli G, Giannelli A, Ramos RA, LiP RP, Cantacessi C, et al. Efficacy of an imidacloprid/flumethrin collar against fleas, ticks and tick-borne pathogens in dogs. Parasit Vectors. 2013;6:245.
16. Doyle V, Beugnet F, Carithers D. Comparative efficacy of the combination fipronil-(S)-methoprene and the combination permethrin-imidacloprid against *Dermacentor reticulatus*, the European dog tick, applied topically to dogs. Vet Ther. 2005;6:303–10.
17. Endris RG, Everett R, Cunningham J, Katz TL, Thompson K. Efficacy of two 65% permethrin spot-on formulations against canine infestations of *Ctenocephalides felis* and *Rhipicephalus sanguineus*. Vet Therm. 2002;1:326–33.
18. Endris RG, Hair JA, Katz TL, Zobbe E, Pennington RG, Meyer JA. Efficacy of three dose volumes of topically applied 65% permethrin against *Ctenocephalides felis* and *Rhipicephalus sanguineus* on dogs weighing 30 kg or more. Vet Ther. 2002;3:435–40.
19. Nideleld N, Bouyer J, Stachurski F, Girmaud P, Belem AM, Moelle Mbaindijatoufam F, et al. Treating cattle to protect people? Impact of footbath insecticide treatment on tsetse density in Chad. PLoS One. 2013;8:e67580.
20. Bissinger BW, Apperson CS, Watson DW, Arellano C, Sonenshine DE, Roe RM. Novel field assays and the comparative repellency of *BioUD(R)*, DEET and permethrin against *Amblyomma americanum*. Med Vet Entomol. 2011;25:217–26.
21. Miller NJ, Rainone EE, Dyer MC, Gonzalez ML, Mather TN. Tick bite protection with permethrin-treated summer-weight clothing. J Med Entomol. 2011;48:327–33.
22. Marchiondo AA, Holdsworth PA, Fourie LJ, Rugg D, Kellmann K, Snyder DE, et al. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) second edition: guidelines for evaluating the efficacy of parasitcides for the treatment, prevention and control of flea and tick infestations on dogs and cats. Vet Parasitol. 2013;19:48–97.
23. Dumont P, Fournie JJ, Soll M, Beugnet F. Acaricidal and repellent efficacy of a new combination of fipronil and permethrin against the main vector of canine babesiosis in Europe: *Dermacentor reticulatus* ticks. Parasit Vectors (in press).
24. Fankhauser B, Dumont P, Halos L, Hunter IIIIS, Kunkle B, Everett WR, et al. Efficacy of a new combination of fipronil and permethrin against *Ctenocephalides felis* flea infestation in dogs. Parasit Vectors (in press).
25. Beugnet F, Soll MD, Boushira E, Franc M. Sustained speed of kill and ‘repellence’ of a new combination of fipronil and permethrin against *Ctenocephalides canis* flea infestation in dogs. Parasit Vectors (in press).
26. Fankhauser B, Dumont P, Hunter IIIIS, McGall IW, Kaufmann C, Mathis A, et al. Repellent efficacy of a new combination of fipronil and permethrin against several mosquitoes species (*Aedes sp., Culex sp.*) Parasit Vectors (in press).
27. Dumont P, Fankhauser B, Boushira E, Lienard E, Jacquiet P, Beugnet F, et al. Repellent efficacy of a new combination of fipronil and permethrin against the main vector of canine leishmaniosis in Europe (*Phlebotomus perniciosus*). Parasit Vectors (in press).