

# Efficacy of a Single Spot-on Administration of Fipronil and Permethrin (Frontline Tri-act®) Against *Haemaphysalis longicornis* Tick Infestation in Dogs

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**KEY WORDS:** *Haemaphysalis longicornis*, fipronil, permethrin, Frontline Tri-Act, dog, efficacy.

## ABSTRACT

A blinded controlled laboratory study was conducted to assess the acaricidal efficacy of the fipronil/permethrin spot-on formulation (Frontline Tri-Act®, Boehringer Ingelheim) against *Haemaphysalis longicornis* ticks. Fourteen healthy dogs were included in this study. Dogs in Group 1 served as untreated controls while dogs in Group 2 were treated with Frontline Tri-Act on Day 0 at the minimum recommended dose of 0.1 mL/kg, corresponding to 6.76 mg/kg fipronil, and 50.48 mg/kg permethrin. All dogs were experimentally infested with 50 viable unfed female *H. longicornis* ticks on days -2, 7, 14, 21, and 28. Thumb counts were per-

formed at 24 hours post-infestations on Days 8, 15, 22, and 29, and ticks were removed and counted at 48 hours post-treatment (on Day 2) and after each tick infestation on Days 9, 16, 23, and 30. Acaricidal efficacies for 24h thumb-counts were 100%, 98.8%, 91.7%, and 84.4% on Days 8, 15, 22, and 29, respectively. Efficacies of 98.1%, 100%, 100%, 98.1%, and 94.7% were determined based on removal counts performed on Days 2, 9, 16, 23, and 30, respectively. There were significant differences of the number of live ticks between treated and control group at each time-point ( $p < 0.005$ ). A single topical administration of the combination of fipronil plus permethrin was highly effective against *H. longicornis* for four weeks.

## INTRODUCTION

*Haemaphysalis longicornis*, also called the

‘longhorn tick’ or ‘bush tick,’ is the most frequently encountered tick species on cattle in Asia, New Zealand, Australia, and, the Pacific Islands,<sup>1,2,3</sup> but is also commonly observed on dogs and cats in other countries like in Japan.<sup>4,5</sup> In 2017, it was first reported on a sheep in United States (New Jersey),<sup>6</sup> while retrospective tick collections revealed that populations of *H. longicornis* existed as early as 2013.<sup>7</sup> Now, the tick has been reported in several US states and is considered well established.<sup>8</sup>

This tick species is characterized by having both bisexual and parthenogenetic populations.<sup>1,3</sup> Both males and females are commonly observed in their native habitats all over Asia, whereas only parthenogenetic females are present in invaded countries like Australia, New Zealand, the Pacific Islands, and the US. Like other three-host ixodid tick species, *H. longicornis* can infest a wide range of wild and domestic animals, even though the adults have a tropism towards wild and domestic ruminants. *H. longicornis* is known as a vector of several bacteria, protozoans, and viruses such as *Anaplasma* spp.,<sup>9,10</sup> *Ehrlichia* spp.,<sup>11</sup> *Babesia gibsoni* (the agent of canine babesiosis in Asia),<sup>12</sup> *Theileria orientalis* (agent of cattle theileriosis),<sup>3</sup> Flavivirus (causing tick-borne encephalitis),<sup>13</sup> Rickettsia japonica (causing Japanese spotted fever),<sup>14</sup> and Phlebovirus (causing severe fever with thrombocytopenia syndrome/ SFTS).<sup>15</sup>

Due to the medical and economic importance of *H. longicornis*, successful control of tick infestations is essential to reduce the risk of pathogen transmission and its geographical expansion.

Frontline Tri-Act, a spot-on formulation which combines 6.76 % w/v fipronil, and 50.48 % w/v permethrin, is effective for the treatment and prevention of flea infestations, including *Ctenocephalides felis* and *Ctenocephalides canis*,<sup>16,17</sup> and ticks, including *Dermacentor reticulatus*, *Rhipicephalus sanguineus* (*sensu lato*) and *Ixodes ricinus*.<sup>18,19,20,21,22</sup> The product has shown repellent and insecticidal activities against

mosquitoes, stable flies, and sandflies under laboratory conditions.<sup>23,24</sup> Moreover, its ability to reduce the risk of transmission of canine babesiosis and ehrlichiosis as well as leishmaniosis has been demonstrated in experimental studies.<sup>21,25</sup>

Recently, the concomitant use of fipronil/permethrin (Frontline Tri-Act) with afoxolaner/milbemycin oxime (Nexgard Spectra®) has shown to provide a safe and effective prevention strategy against both endo- and ectoparasites, including vector-borne diseases under field conditions.<sup>26</sup>

This study was conducted to assess the immediate and persistent efficacy of Frontline Tri-Act against *H. longicornis* in experimentally infested dogs.

## MATERIALS AND METHODS

This study was designed in accordance with the Committee for Medicinal Products for Veterinary use (CVMP) “Guideline for the testing and evaluation of the efficacy of antiparasitic substances for the treatment and prevention of tick and flea infestation in dogs and cats”, EMEA/CVMP/EWP/005/2000-Rev.3,<sup>27</sup> as well as the “World Association for the Advancement of Veterinary Parasitology” (WAAVP) second edition guidelines for evaluating the efficacy of parasiticides for the treatment, prevention and control of flea and tick infestation on dogs and cats.<sup>28</sup>

It was a parallel group, randomized, blinded, and controlled laboratory study which complied with the Good Clinical Practices (GCP) as described in the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH).<sup>29</sup> The containment of the dogs complied with the South African National Standard SANS 10386: latest edition “The care and use of animals for scientific purposes” and the protocol was approved by the Institutional Animal Care and Use Committee (IACUC) prior to conduct the study.

## Animals

Eighteen purpose-bred Mongrel and Beagle dogs were initially selected and acclimatized

to the study conditions for 7 days. During the acclimation period, each dog was examined by a veterinarian. Weighing of all dogs and measuring of hair length were also performed. All dogs were confirmed to be healthy. In addition, all dogs were dewormed prior to the initiation of the study and did not harbor resident ticks or fleas. None of the dogs used in this study had been treated with any long acting topical or systemic acaricide/insecticide in the 12 weeks prior to the acclimation period. An initial *H. longicornis* tick infestation was conducted to determine individual tolerance of tick infestations and for random allocation to the study groups.

After allocation, 14 healthy dogs, 8 males and 6 females, aged of 13 months to 5.4 years and weighing 11.4 to 18.6 kg were included in the study. The animals were kept individually in cages with visual and auditory contact with conspecifics. At least one toy was made available to each dog (replenished weekly). The dog cages were part of an indoor animal unit, environmentally controlled for temperature ( $20\text{ }^{\circ}\text{C} \pm 4\text{ }^{\circ}\text{C}$ ). Standard commercially available diet was provided once a day at the recommended manufacturer's rates. Potable water was supplied by the local municipality and provided in stainless steel bowls and replenished at least twice daily. All the animals were observed daily from Day -7 to Day 30 for their general health.

### **Allocation to Treatment Groups**

The study followed a randomized block design based on pre-treatment tick count. Eighteen dogs were infested and the 14 dogs with the highest live attached tick counts were ranked within sex in descending order of individual pre-administration live, counts, and subsequently blocked into seven blocks of two dogs each. Within blocks, dogs were randomly allocated to the two study groups: Group 1, untreated control; Group 2 treated with Frontline Tri-Act (fipronil, permethrin).

### **Infestations, Treatment, and Tick Counts**

Each dog was experimentally infested with 50 ( $\pm 4$ ) viable, adult, unfed female *H. lon-*

*gicornis* ticks on Day -7 for randomization purposes, on Day -2 to assess immediate curative efficacy and on Days 7, 14, 21, and 28 to assess sustained (i.e. persistent) efficacy. The tick strain originated from a parthenogenetic laboratory colony of *H. longicornis* (Okayama strain, Japanese origin). Tick infestations were performed by sedating the dogs with medetomidine, placing the sedated animals in an infestation chambers for approximately 4 hours following tick infestation. Ticks were released on multiple sites of the dogs' flank, being careful to avoid application sites. In addition, the light in the room was turned off during the tick infestation procedure to encourage attachment.

On Day 0, dogs from Group 1 remained untreated while dogs from Group 2 were treated once topically with Frontline Tri-Act at the minimum effective dose of 0.1 mL/kg (equivalent to 6.76 mg/kg fipronil bodyweight and 50.48 mg/kg permethrin) according to label instructions. Dogs were observed hourly for four hours post treatment for any adverse reaction to treatment.

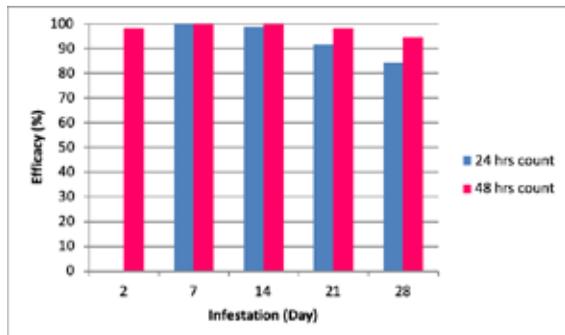
On Day 2, all ticks were removed and counted to evaluate the curative efficacy of Frontline Tri-Act.

In-situ tick thumb counts by palpation and visual observation of the ticks onto the dogs were performed at 24 hours after each tick exposure on Days 8, 15, 22, and 29. At 48 hours post-exposure, all ticks were removed and counted using forceps. The numbers of live attached, live free, dead attached, and dead free ticks were recorded.

### **Data Analysis**

To evaluate the acaricidal efficacy, arithmetic means of the live tick counts (free and attached) were calculated by group at each time-point. Percent effectiveness for the treated group was calculated using the Abott formula  $[(C - T) / C] \times 100$ , where C = arithmetic mean for the control group and T = arithmetic mean for the treated group. The groups were compared using an ANOVA (Proc GLM procedure in SAS) with a treatment effect. SAS Version 9.3 TS Level 1M2 was used for all the statistical analyses. All

**Figure 1.** Percent tick efficacy of Frontline Tri-Act against *H. longicornis* ticks at 24 and 48 hours, based on arithmetic means.



statistical comparisons used a two-sided 5% significance level. During acclimation, hair length, bodyweight and pre-treatment tick counts were also compared for group homogeneity at the time of inclusion.

## RESULTS AND DISCUSSION

No abnormal signs were observed during the daily observations or during the specific post-administration observations.

There were no statistically significant differences between the dogs in the two groups for animal body weights ( $p =$

0.7900), mean hair length ( $p = 0.8793$ ) and pre-treatment live tick counts ( $p = 0.7216$ ), indicating that groups were homogenous and comparable at baseline.

The average retention rates in the control group ranged from 10.7 to 22.3 (21.4 – 44.6%) at 24 hours and from 9.7 to 22.3 (19.4 – 44.6%) at 48 hours after administration or tick infestations which were above the 20% required rate by the WAAVP guideline for adequate infestations (except once on Day 16).<sup>28</sup> Although measures were taken to facilitate infestations (sedated

dog and infested in chambers with light turned off), variable counts were observed in individual control dogs, with few ticks attaching in some instances (Table 1). Such variability has previously been reported in a study assessing the efficacy of afoxolaner against *H. longicornis*.<sup>30</sup> Typically, this tick species is found on livestock and wild animals, and only occasionally infesting dogs. In order to increase retention rates, some experimental studies have used alternative measures such as holding ticks in perforated

**Table 1.** Acaricidal efficacy using arithmetic mean for *Haemaphysalis longicornis* ticks.

Time-point	Study Day	Untreated Control dogs (n=7)	Treated dogs (fipronil + permethrin) (n=7)		
		Mean (min-max)	Mean (min-max)	Efficacy (%)	p-value
24 hours	8	22.3 (15-32)	0.0 (0-0)	<b>100</b>	<.0001
	15	11.7 (6-20)	0.1 (0-1)	<b>98.8</b>	<.0001
	22	15.4 (11-20)	1.3 (0-3)	<b>91.7</b>	<.0001
	29	11.0 (1-19)	1.7 (0-5)	<b>84.4</b>	0.0020
Time-point	Study Day	Untreated Control dogs (n=7)	Treated dogs (fipronil + permethrin) (n=7)		
		Mean (min-max)	Mean (min-max)	Efficacy (%)	p-value
48 hours	2	14.9 (3-28)	0.3 (0-2)	<b>98.1</b>	0.0006
	9	22.3 (14-31)	0.0 (0-0)	<b>100</b>	<.0001
	16	9.7 (2-17)	0.0 (0-0)	<b>100</b>	0.0012
	23	15.1 (9-24)	0.3 (0-2)	<b>98.1</b>	<.0001
	30	10.9 (3-22)	0.6 (0-4)	<b>94.7</b>	0.0039

*p*-value: One-way ANOVA with a treatment effect.

plastic Petri dishes attached to the animal with medical adhesive,<sup>31</sup> placing Elizabethan collars and T-shirt on dogs for the 48h tick challenge,<sup>32</sup> or placing ticks on animal's ears to mimic the natural preferred attachment sites.<sup>33</sup>

Based on arithmetic mean values, fewer ticks were recorded in the treated group compared to the negative control group on all assessment days ( $p < 0.005$ ). The curative efficacy calculated on Day 2 after tick challenge on Day -2 was 98.1%. Percent efficacy at 24h post exposures was 100%, 98.8%, 91.7%, and 84.4% on Days 8, 15, 22, and 29, respectively (Figure 1, Table 1). After 48 h, efficacy was 100%, 100%, 98.1%, and 94.7% on Days 9, 16, 23, and 30, respectively (Figure 1, Table 1). In addition, at least six out of the seven dogs in the treated group remained free of ticks on Days 2, 9, 16, 23, and 30 and a very low number of ticks were found (<4) on each infested dog on Days 2, 23, and 30.

According to EU "Guideline for the testing and evaluation of the efficacy of antiparasitic substances for the treatment and prevention of tick and flea infestation in dogs and cats" (EMA/CVMP/EWP/005/2000-Rev.3)" repellent effect "sensu lato" is based on the attachment process (inhibition or disruption) and defined as "no tick should be on a treated animal after 24h following administration of the product". In this study, the term "repellency" was voluntarily not used, as a 24 h assessment would likely be related to a combination of both repellency and acaricidal activity.

Twenty-four hours could be considered as a late assessment of repellency, as previous published studies evaluated the activity of Frontline Tri-Act® against ticks at 4h and 6h after tick exposure.<sup>19, 20, 22</sup> For instance, acaricidal efficacy against *R. sanguineus* was reported  $\geq 94.7\%$  at 4h for 3 weeks and  $\geq 90.9\%$  at 6h for one month, and was  $\geq 91.1\%$  at 4h during the full month for *I. ricinus*. In the present study, efficacy at 24 h was  $\geq 95\%$  through Day 15 and decreased to 91.7% on Day 22, and 84.4% on Day

29. However, the lower attachment rates observed in control dogs on Days 29 and 30 likely negatively impacted the efficacy results in the treated group in the current study.

In conclusion, Frontline Tri-Act administered once topically at the minimum recommended dose was effective against *Haemaphysalis longicornis* infestations for at least 4 weeks. The fipronil/permethrin spot-on formulation combines a safe and reliable solution against ectoparasites and could be included in a prevention strategy to reduce the risk of transmission of vector-borne pathogens in dogs.

## CONFLICTS OF INTEREST

The work reported herein was funded by Merial SAS, now part of Boehringer Ingelheim. The authors are current employees or contractors of Boehringer Ingelheim.

Frontline Tri-Act is a registered trademark of Merial, now part of Boehringer Ingelheim. All other marks are the property of their respective owners.

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## ACKNOWLEDGEMENT

The authors would like to thank all the study personnel at Clinvet South Africa.

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