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Original Article

Comparative efficacy of afoxolaner and ivermectin in dogs naturally infested with *Rhipicephalus sanguineus* sensu lato: A clinical field study conducted in Thailand

Ganyanat Tinkruejeen[a], Phanchalee Meesaimongkon[b], Sahatchai Tangtrongsup[c,d], Niyada Thitaram[c], Nuttawan Srifawattana[a], Fred Beugnet[e], Saruda Tiwananthagorn[b,f,*]

[a] Small Animal Hospital, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai 50100, Thailand
[b] Department of Veterinary Biosciences and Veterinary Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai 50100, Thailand
[c] Department of Companion Animal and Wildlife Clinic, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai 50100, Thailand
[d] Research Center of Producing and Development of Products and Innovations for Animal Health and Production, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai 50100, Thailand
[e] Boehringer Ingelheim, Lyon 69007, France
[f] Center of Excellence in Veterinary Bioscience, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai 50100, Thailand

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ABSTRACT

Afoxolaner is a novel insecticidal and acaricidal of the isoxazoline family, which is used in veterinary practice to control infestation of dogs by fleas, ticks, and mites (NexGard, Boehringer Ingelheim). Ivermectin is an avermectin administered at microdoses to prevent infection of dogs with *Dirofilaria immitis* and is used off-label to control *Rhipicephalus sanguineus* infestation of dogs in numerous countries, including Thailand. Here we conducted two trials to assess the efficacy of afoxolaner for treating natural *R. sanguineus* sensu lato (s.l.) infestations of dogs residing in the Chiang Mai area of Thailand. The first trial compared the efficacies of afoxolaner and ivermectin in dogs infested with < 500 ticks. A randomized, investigator-blinded, controlled study was conducted of 16 dogs, allocated into the groups as follows: afoxolaner (2.7–6.9 mg/kg, PO, group 1; $n = 8$), ivermectin (300 µg/kg, SC, group 2; $n = 5$), untreated (group 3; $n = 3$). Tick counts and drug administration were performed on days 0, 28, and 56. Mean numbers of ticks on day 0 in groups 1, 2, and 3 were not significantly different (225, 169, and 123, respectively; $p = .36$). The mean number of ticks (%efficacy to control) in groups 1, 2 and 3 on day 28 were 7 (97.05%), 230 (2.95%), and 237, respectively; on day 56 were 4 (96.11%), 93 (9.71%), and 103, respectively; and on day 84 were 1 (98.65%), 44 (40.54%), and 74, respectively. The efficacy of afoxolaner was > 96%, whereas the efficacy of ivermectin was significantly lower compared with that of afoxolaner ($p < .05$) and never achieved the 90% efficacy threshold claimed by registration agencies. The second trial assessed the efficacy of afoxolaner for treating dogs with heavy tick infestations (> 500 ticks/dog), including four dogs from two households. The dogs were treated monthly with Afoxolaner. The mean values of the numbers of ticks on dogs in the 2 households were not significantly different (913 and 800 on day 0, $p = .18$). The numbers of ticks significantly decreased thereafter, and the efficacy of afoxolaner was > 99% on days 28, 56, and 84. Adverse reactions were not observed in either trial. In conclusion, this study confirms the efficacy of afoxolaner against adult *R. sanguineus* s.l. that naturally infests dogs that inhabit Thailand.

1. Introduction

Rhipicephalus sanguineus (brown dog tick), which is of major medical and veterinary significance, functions as a vector for numerous animal and human pathogens. These pathogens include *Babesia vogeli*, *Ehrlichia canis*, *Hepatozoon canis*, *Rickettsia conorii*, *R. massiliae*, and *R. rickettsii* (Dantas-Torres et al., 2012). *R. sanguineus* has three hosts, and its three stages are adapted to dogs, although it occasionally infests humans, and thus represents a risk to human health. The tick's life cycle takes 63–91 days (Dantas-Torres, 2008; Gray et al., 2013), it is the most widely distributed tick worldwide, and it is prevalent year-long in the tropics and subtropics, for example, in Thailand where the environment is optimal for supporting the tick's life cycle (Irwin and Jefferies, 2004).

The numerous cases of human otoacariasis caused by *R. sanguineus*

* Corresponding author.
E-mail address: por.parn.vetmed@gmail.com (S. Tiwananthagorn).

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infestation of the external ear canal (public communication News in Thailand) have encouraging pet owners to seek effective strategies to control ticks. The effective acaricidal control can prevent direct blood loss that causes anemia and ameliorates skin irritation that decreases the accumulation of ticks to reduce the risk of tick-borne transmission of pathogens. The numerous ectoparasiticides are administered topically, orally, or are injectable (Beugnet and Franc, 2012).

Ivermectin (IVM) is a macrocyclic lactone that engages the arthropod gamma-aminobutyric acid (GABA) and glutamate receptors, paralyzing and killing an arthropod. IVM is approved for veterinary use for treating dogs and cats on a monthly schedule to prevent disease caused by heartworms; the oral dose for dogs, 6 μg/kg, provides a very high margin of safety. In Thailand, IVM (subcutaneous, 200–400 μg/kg) is widely used to control *Rhipicephalus (Boophilus)* ticks that infest livestock. However, IVM is used off-label to control ixodid ticks in pets in Egypt (Morsy and Haridy, 2000) and Thailand (in conversations with small-animal practitioners), mainly because of its expense. However, off-label administration of IVM to dogs is of concern because of safety considerations, particularly there is insufficient information about the MDR-1 mutation. Furthermore, the efficacy of IVM for killing parasitic ticks of dogs and cats using the approved dosing protocol is unknown.

Afoxolaner is a new insecticide/acaricide of the new isoxazoline class that acts on the insect GABA and glutamate receptors, which causes excess neuronal stimulation and death of the arthropod (Beugnet et al., 2015a, 2015b). The receptors located within the chloride ion channels of neurons function at the synaptic level and differ from the receptors for avermectins, milbemycins, and fipronil (Shoop et al., 2014). The soft, chewable formulation of afoxolaner rapidly dissolves and is quickly absorbed after oral administration of the minimum effective dose (2.5 mg/kg), with maximum plasma concentrations (C_{max}) (1655 ± 332 ng/mL) achieved from 2 to 6 h (T_{max}). Afoxolaner is slowly metabolized in the liver and binds abundantly and tightly to plasma proteins, leading to sustained activity for ≥1 month (Letendre et al., 2014).

A single dose of afoxolaner achieves > 90% efficacy against *R. sanguineus* sensu lato (s.l.) within 48 h and for as many as 28 days (EMA, 2014; Kunkle et al., 2014), and efficacies range from 86.4% to 99.5% at 24 h up to 4 weeks (Beugnet et al., 2015a). However, these studies were conducted using controlled laboratory conditions, and field studies are required to assess actual efficacies. Multicenter field studies of afoxolaner were conducted in Europe, the United States, and Australia (EMA, 2014) to assess its ability to control fleas and ticks, although such trials have not been conducted in Asian countries. Thus, these studies should be performed in regions with different climates, particularly to determine the drug's efficacy for treating heavy infestation with fleas or ticks. To fill this gap in our knowledge, here we aimed to assess the activity of afoxolaner for treating dogs living in a challenging environment in Thailand, that are heavily infested with *R. sanguineus* s.l. We also compared the efficacy of afoxolaner with that of IVM.

2. Materials and methods

2.1. Study design and ethical approval

Two randomized, controlled field efficacy trials were conducted using dogs naturally infested with *R. sanguineus* s.l. during May to August 2017. Trial 1 aimed to compare the efficacy of afoxolaner and ivermectin against adult *R. sanguineus* s.l. ticks in moderately to heavily infested dogs (< 500 ticks per dog). Trial 2 assessed the efficacy of afoxolaner against *R. sanguineus* s.l. ticks in very heavily infested dogs (> 500 ticks per dog). Study procedures were in accordance with the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines for evaluating the efficacy of parasiticides for the treatment, prevention, and control of flea and tick infestation on dogs and cats (Marchiondo et al., 2013). The protocol was reviewed and approved by the local Committee on the Ethics of Animal Experiments of the Faculty of Veterinary Medicine, Chiang Mai University (Permit Number: R1/2560).

2.2. Animals

Nineteen naturally infested dogs were selected for Trial 1. To assess the efficacy of afoxolaner and ivermectin against adult tick infestation, dogs had to be infested with > 25 *R. sanguineus* ticks (Marchiondo et al., 2013). We collected 5–10 attached, unfed adult male or female ticks (2 days ± 1 before treatment; day −2) to identify the tick species according to the identification key by Walker et al. (2014).

Trial 1 included 19 dogs from six households. Each household was required to have > 3 dogs, which were randomly allocated into the groups as follows: afoxolaner-treated (group 1; $n = 8$), ivermectin-treated (group 2; $n = 6$), and untreated (group 3; $n = 5$). Trial 2 included 4 very heavily infested dogs living in two households (> 500 ticks per dog), which were treated with afoxolaner. These households were located in Muang Chiang Mai, Hang Dong, Mae On, Mae Wang, Mae Rim, and Saraphi districts, Chiang Mai Province.

Inclusion criteria were as follows: males and females of mixed breeds (except for those carrying an MDR1 mutation) (Gramer et al., 2011), age > 6 months, and weight between 10 kg and 25 kg. The owners were required to sign a consent form and agreed to the visits stated in the protocol. All dogs lived semi-outdoors and were not previously treated with a long-acting topical or systemic insecticide/acaricide during the 12 weeks preceding day 0 (Beugnet et al., 2015a). The dogs were clinically healthy with normal hematological and chemical profiles. Pregnant or lactating females were excluded. Concomitant ectoparasiticide treatments were excluded, as well as environmental products with activity against ticks.

2.3. Treatment protocols

The treatment protocols are shown in Table 1. The study period was 84 days. Chewable afoxolaner tablets (NexGard®, Boehringer Ingelheim) were administered orally on days 0, 28, and 56. The commercial dose was selected in accordance with registration and weight intervals. Tablets (3 g) containing 68 mg of afoxolaner were administered to dogs weighing 10.1–25 kg to achieve doses of 2.7–6.9 mg/kg (EMA, 2014). IVM (300 μg/kg; 10% *w/v* injectable solution for ruminants (Baymec®, Bayer) was subcutaneously administered on days 0, 28, and 56 (Morsy and Haridy, 2000).

Table 1
Study protocol.

Protocol	Group		
	1 (Afoxolaner)	2[a] (Ivermectin)	3[a] (Untreated Control)
Preceding at day −2 (± 1) the 3 groups	Physical examination, Health check (CBC,ALT, CR)		
Treatments	Afoxolaner, PO, 3 times, at day 0, 28 & 56	Ivermectin SC, 3 times, at day 0, 28 & 56	–
Efficacy assessments	Tick count on days 1, 2, 7 (± 2), 14 (± 2), 21(± 2), 28 (± 2), 42 (± 2), 56 (± 2), 70 (± 2) and 84 (± 2) post-treatment		

[a] Only in trial 1. No Ivermectin treated group, neither negative control in the trial 2.

2.4. Tick count

Tick count was conducted at each visit (Table 1). Visual inspection and palpation (thumb counting) were performed to count ticks before and after treatment (Marchiondo et al., 2013). Systematically pushing the hair against the hair growth was executed (Navarro et al., 2016) so the ticks became visible. The attached adult ticks were then removed, counted, and recorded as alive at days 0, 28 and 56 (Marchiondo et al., 2013). We examined the face, ears, pinna and pinnal fold; frontal area; neck; dorsal strip from shoulder blades to base of the tail; lateral areas of left and right shoulders and forearms; left and right abdominal area; left and right hind legs; inside hind legs; perineal and anal areas; and feet and interdigital spaces (Marchiondo et al., 2013; Beugnet et al., 2015a). The ticks were then preserved in absolute alcohol (Navarro et al., 2016). Since the number of adult ticks per animal exceeded a hundred of ticks, the maximum of 30 randomly selected ticks were identified for the species under a light stereomicroscope according to the key (Walker et al., 2014). As no genetic analysis was performed on the collected ticks, knowing that *R. sanguineus* is a complex of species (Nava et al., 2015; Coimbra-Dores et al., 2016). In the present study, ticks morphologically compatible with the description of Walker et al. (2014) for "*R. sanguineus*" were referred to as "*R. sanguineus* sensu lato (s.l.)" (Dantas-Torres et al., 2013; Dantas-Torres et al., 2017). The ticks in the study areas were only *R. sanguineus* s.l.

2.5. Adverse events

Full physical examinations were conducted that included measuring rectal temperature, observations to detect abnormal signs, and abnormalities at the application site. The dogs were observed for adverse events for 24 h after they were administered the drug.

2.6. Data and statistical analysis

Trial 1: Statistical analyses (one-way ANOVA/Tukey's multiple comparison test) were performed using GraphPad Prism software to calculate the significance of the differences in the average numbers of ticks among the three groups. We used Abbott's formula (Marchiondo et al., 2013) to compare the percentage efficacies of the treated groups compared with the control group. The %Efficacy post-treatment each assessment day = $\mathbf{100 \times (Mc\text{-}Mt)/Mc}$, where Mc = arithmetic mean of the live tick count (control group), Mt. = arithmetic mean of the live tick count (treated group: groups 1 or 2).

Trial 2: The efficacy of afoxolaner against adult *R. sanguineus* s.l. was defined as the reduction in the average viable tick count compared with the day 0 baseline count during the entire treatment and was calculated using the formula as follows: % Efficacy post-treatment each assessment day = $\mathbf{100 \times (Mc_{(Day0)} - Mt_{(post\text{-}treatment)}/(Mc_{(Day0)}}$ (Stanneck et al., 2012; Becskei et al., 2016).

3. Results

3.1. Efficacies of afoxolaner and ivermectin for treating moderate to heavy tick infestations (trial 1)

Three infested dogs (1 from the ivermectin-treated group and 2 from the control group) were excluded, because the owners removed the ticks and treated the dogs or the environment with an acaricide between observations. The mean number of ticks infesting groups 1, 2, and 3 on day 0 were 225, 169, and 123, respectively. There was no significant difference ($P > .05$) in the average number of ticks among the groups on day 0.

The average number of ticks infesting the afoxolaner-treated group was significantly lower compared with the ivermectin-treated group on days 1, 2, 7, 21, 28, 42, 56 (Table 2). The efficacy against adult *R. sanguineus* s.l. remained high in the afoxolaner-treated group throughout treatment. After day 2, the efficacy of afoxolaner remained > 90% for the next 84 days. In the ivermectin-treated group, the efficiency remained < 50% (Table 2).

Table 2
Average tick number and %efficacy of afoxolaner and ivermectin against adult *R. sanguineus* s.l. in moderate to heavy tick infestations (the study trial 1), observed for 84 days.

Days post treatment	Arithmetic mean of *R. sanguineus* counts		
	Afoxolaner-treated group (%efficacy)	Ivermectin-treated group (%efficacy)	Control group
Day 0[a]	224.75	169.00	122.67
Day 1	29.75[1] (78.57%)	177.20 (0%)	139.67
Day 2	24.63*,[1] (81.88%)	149.60 (0%)	138.00
Day 7	27.88*,[1] (79.41%)	124.20 (8.82%)	136.00
Day 14	12.38 (88.99%)	168.80 (0%)	109.00
Day 21	8.13[1] (92.98%)	117.80 (0%)	114.**00**
Day 28[a]	6.75*,[1] (97.05%)	229.60 (2.95%)	236.67
Day 42	13.88[1] (91.86%)	309.80 (0%)	171.67
Day 56[a]	3.75**,[1] (96.12%)	92.60 (9.71%)	102.67
Day 70	2.50* (97.22%)	84.20 (22.22%)	108.33
Day 84	0.88* (98.65%)	44.00 (40.54%)	74.33

*p < .05; **$p < .01$ from control: one-way ANOVA & Tukey's multiple comparison test.

[1] **(bold)**: Statistically significantly difference ($p < .05$) were recorded between treated groups.

[a] Afoxolaner tablet has been administered.

3.2. Efficacy of afoxolaner for treating heavy tick infestations (trial 2)

The average number of ticks before treatment was > 800. After the first week of treatment, the average number of tick decreased to 25–35 and further decreased to 10 ticks during the third week. Thereafter, the tick number was < 10 (Table 3). The efficacy of afoxolaner against adult *R. sanguineus* s.l. ticks was > 96% from the first day after treatment and during the 84-day trial (Table 3).

3.3. Adverse drug reactions

Adverse drug reactions or other health problems were not experienced by any dog in the three groups.

Table 3
Average tick number and efficacy of afoxolaner against adult *R. sanguineus* s.l. in very heavy tick infestations (the study trial 2), observed for 84 days.

Days post treatment	Afoxolaner-treated dogs
	Arithmetic mean of *R. sanguineus* count (%efficacy)
Day 0[a]	856.00
Day 1	33.00 (96.14%)
Day 2	26.25 (96.90%)
Day 7	26.25 (96.93%)
Day 14	2.25 (99.74%)
Day 21	10.00 (98.83%)
Day 28[a]	2.75 (99.68%)
Day 42	1.25 (99.77%)
Day 56[a]	0.25 (99.94%)
Day 70	0.25 (99.94%)
Day 84	0.00 (100%)

[a] Afoxolaner tablet has been administered.

4. Discussion and conclusion

Here we aimed to determine the efficacy of afoxolaner by conducting a clinical field study in a tropical environment with a high incidence of tick infestations. We found that the afoxolaner group achieved > 90% efficacy against the adult stage of *R. sanguineus* s.l. that naturally infested dogs from day 21 to the end of the study (day 84). The results are consistent with those of laboratory studies reporting > 90% efficacy of a single dose for treating infestations by *R. sanguineus* s.l. within 48 h of application and for at least 28 days (EMA, 2014; Kunkle et al., 2014).

The efficacy of afoxolaner during the first 2 weeks of Trial 1 was < 90% (range, 78.6%–89%). This finding is likely explained by field conditions in which dogs were continuously reinfested by ticks in the environment (Becskei et al., 2016). The guidelines to assess flea and tick treatment efficacies under field conditions indicate that flea and tick counts should be performed no sooner than day 28, which considers the time to control environmental contamination (Marchiondo et al., 2013). In the present study, we allocated the dogs *en bloc* ($n = 3$) into the three treatment groups. Thus, an untreated dog may serve as a reservoir that perpetuates continuous infestation.

In our study of very severe tick infestation (Trial 2) of dogs treated with afoxolaner, > 96% efficacy was achieved 24 h post-treatment, and efficacy > 99% was maintained from days 14 through the end of the trial. Afoxolaner was orally administered each month at the minimum recommended dose of 2.5 mg/kg (range, 2–4 mg/kg), which we found safe and highly effective against natural tick infestations. The afoxolaner-treated dogs did not experience adverse events. A benefit of oral administration is that the efficacy is independent of exposure of treated sites to dirt, sun, or bathing, particularly in tropical climates.

The present study demonstrates that IVM (300 µg/kg, subcutaneous, monthly) did not effectively control infestations with *R. sanguineus* s.l. The risk to the safety of such a dose, particularly to dogs harboring MDR1 mutations, should be considered by veterinarians. Moreover, the low sustained efficacy was unable to control the tick burden and therefore was unable to decrease the risk of transmission of tick-borne pathogens to dogs.

Numerous risk factors associated with tick infestation include the following: geography, season, type of dog (modes of life), treatment history, individual levels of infestation, age, breed, and lifestyle. *Rhipicephalus sanguineus* ticks spend most of their lives in the environment and are thus directly influenced by temperature and relative humidity. The conditions in Thailand are ideal for propagating ticks, which likely explains the high risks of exposure and infestations of dogs (Dantas-Torres, 2008). Clinical field studies to assess the efficacy of engaging contemporary tick populations in different geographic and climatic regions should be conducted to confirm the efficacy of tick medications under various conditions for treating a variety of tick species. Unfortunately, dog owners in Chiang Mai Province do not pay sufficient attention to controlling ticks, despite the high risk of tick-borne diseases. We therefore strongly recommend the implementation of programs to educate such dog owners about the threat to human health associated with ticks and tick-borne diseases.

Ethical statement

The Committee on the Ethics of Animal Experiments of the Faculty of Veterinary Medicine, Chiang Mai University approved the study (Permit Number: R1/2560).

Contributions

Ganyanat Tinkruejeen & Phanchalee Meesaimongkon: Investigation, Methodology, Data curation; Formal analysis, Writing - original draft: Sahatchai Tangtrongsup: Investigation, Formal analysis, Writing - review & editing; Niyada Thitaram & Nuttawan Srifawattana: Resources, Investigation: Fred Beugnet: Data curation; Formal analysis, Writing - review & editing: Saruda Tiwananthagorn: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Validation; Visualization; Writing - original draft; Writing - review & editing.

Declaration of Competing Interest

The product Nexgard® was supported by Merial, now Boehringer Ingelheim (Thailand). Fred Beugnet is an employee of Boehringer Ingelheim. The others belonging to Faculty of Veterinary Medicine, Chiang Mai University are independent to conduct the study. Any reference to the brands or trademarks herein is for comparative scientific purposes only and it is not intended for a commercial purpose or to dilute the rights of the respective brands and trademarks.

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