Laboratory evaluation of the efficacy of lotilaner (Credelio™) against *Amblyomma cajennense* (sensu lato) infestations of dogs

Pedro Veloso Facury Lasmar¹, Martin Murphy², Steve Nanchen³, Jason Drake³*, Katherina Coumendouros⁴, Debora Azevedo Borges⁴, Priscila Cardim de Oliveira⁴ and Fábio Barbour Scott⁴

**Abstract**

**Background:** The ixodid tick *Amblyomma cajennense* (sensu lato) complex, widespread throughout South and Central America, is also present in Mexico, Texas and Florida. As a vector of *Rickettsia rickettsii*, and potentially of other pathogens, infestations with *A. cajennense* present a substantial health risk to humans, dogs and other mammals. Oral administration of lotilaner flavored chewable tablets (Credelio™, Elanco) to dogs was previously shown to rapidly provide killing activity of infesting ticks. This study investigated lotilaner’s efficacy against *A. cajennense* (s.l.).

**Methods:** Twenty purpose-bred Beagles (10 male and 10 female) were ranked by Day -5 burdens of nymphal *A. cajennense* (s.l.) and randomized to either treatment with lotilaner or to a sham-treated control group. On Day 0, dogs were fed within approximately 30 min prior to oral lotilaner administration at as close as possible to 20 mg/kg, the minimum dose rate. For efficacy assessments, tick counts were completed 48 h post-treatment or 48 h after experimental challenge infestations with 200 nymphal *A. cajennense* (s.l.) on Days -7, -2, 7, 14, 21 and 28.

**Results:** Tick infestations in the control group dogs ranged from a low of 43 to 95, with the average infestation remaining above 25% at each assessment, thereby meeting the requirement for efficacy comparison with the treated group. Lotilaner efficacy was 100% within 48 h post-treatment, and at nine days post-treatment. Efficacy was greater than 99% at all subsequent assessments through Day 30. No treatment-related adverse events were observed.

**Conclusion:** The results demonstrate that lotilaner, administered orally to dogs at a minimum dose of 20 mg/kg is well tolerated, provides rapid reduction of existing *A. cajennense* (s.l.) tick infestations, and provides sustained residual protection for at least 30 days against subsequent infestation by *A. cajennense* (s.l.).

**Keywords:** Canine, Ticks, Isoxazoline, Effectiveness, Cayenne tick

---

**Background**

The ixodid tick *Amblyomma cajennense* (s.l.) is important as a vector of *Rickettsia rickettsii*, the cause of Brazilian/Rocky Mountain spotted fever. This is a disease of dogs and humans which is potentially fatal, particularly in Brazil where the strain of *R. rickettsii* is reported to be of greater pathogenicity than in the United States [1–4]. *Amblyomma cajennense* species complex is widely distributed throughout tropical and sub-tropical regions of South and Central America, is present in Mexico, Texas and Florida, and on the Caribbean islands including Cuba [3–10]. In Brazil, capybaras act as the primary host of *A. cajennense* (s.l.) and most endemic areas of *R. rickettsii* infections are those with large, free living populations of this rodent [3, 4]. While dogs are not seen as being a primary host for *A. cajennense* (s.l.), nymphal stages are less host specific than the adult stage and are reported to be aggressive feeders on humans and dogs [11, 12]. As such, infestations with *A. cajennense* (s.l.) present a significant risk to human and canine health across a broad geography [1–6]. Ectoparasiticides introduced to treat and control canine tick infestations in affected areas should be shown to be effective against this tick species.
Targeting ligand-gated GABA receptors, the isoxazoline compounds have a novel mode of action, distinct from earlier ectoparasiticide treatments, that causes paralysis and death of fleas and ticks [13, 14]. Building on the efficacy against fleas and tick of the first isoxazoline, afoxolaner, which was first approved in 2013, subsequent members of the family that have become available are fluralaner and sarolaner, first approved in 2014 and 2016, respectively, and most recently, lotilaner, which was first approved in 2017. Following oral administration under fed conditions, peak lotilaner concentrations are reached within two hours, with a corresponding rapid onset of activity against fleas and ticks [15–17]. Lotilaner efficacy has been demonstrated against a number of tick species including, but not limited to, *Rhipicephalus sanguineus* (sensu lato), *Dermacentor reticulatus*, *Dermacentor variabilis*, *Ixodes scapularis*, *Ixodes ricinus* and *Amblyomma americanum* [18, 19]. With a half-life of 30 days, lotilaner has been shown to provide at least a month of activity against infesting ticks [15, 17–19]. To further expand the understanding of the activity of lotilaner flavored chewable tablets against ticks, a study was initiated to investigate efficacy in eliminating existing burdens of *A. cajennense* (s.l.) and in maintaining efficacy against challenge through the month following oral administration at a minimum dose rate of 20 mg/kg.

**Methods**

The study was designed and completed during 2015 in accordance with the second edition guidelines from the World Association for the Advancement of Veterinary Parasitology (WAAVP) for evaluating the efficacy of parasiticides for the treatment, prevention and control of flea and tick infestations of dogs and cats and with current local regulatory standards [20]. According to Labruna & Pereira [10], the highest prevalence of infestation of *A. cajennense* (s.l.) in domestic dogs in Brazil occurs during its larval and nymphal stages. Infestations with adult *A. cajennense* (s.l.) ticks is less prevalent in dogs [10]. In addition, the attachment rate of adult ticks of this species in laboratory dogs is very low, not allowing for the minimum attachment rate of 25% recommended by international guidelines [10, 12]. For these reasons, nymphal stages were used for infestation of dogs in this study.

**Animals and housing**

Twenty-six purpose-bred Beagle dogs (male and female) were acclimatized, and of these 20 (10 male and 10 female) were retained in the study. Each dog was identified with a subcutaneous microchip identification number. A dog was eligible to be enrolled in the study if it was at least six months of age and 6.8 kg on Day -7, clinically healthy with no pre-existing conditions (e.g. injury, trauma, disease) that could have affected the study, and if it sustained a pre-treatment tick infestation of at least 50 ticks (25% of the infesting dose) (Fig. 1). Dogs were excluded if pregnant or lactating, or if treated with an ectoparasiticide in the eight weeks preceding selection for the study (or within six months for isoxazoline compounds). For the study, dogs were housed individually in concrete-floored pens measuring approximately 1.5 × 1.5 m and for socialization and exercise were placed into a fenced corridor daily, except on days when tick infestations were completed. For socialization, dogs within the same treatment group were separated by sex and socialized together, with separate designated areas for each treatment group to avoid any risk of between-group cross-contamination. Animals remained in the same socialization group throughout the study. At least one toy/chew was made available at all times to each dog when housed individually. Dogs were fed a commercially available high quality complete canine dry food diet except on Day 0, when canned food was offered to all dogs, mixed with their dry ration to encourage consumption. Water was provided *ad libitum*.

**Randomization and treatments**

Within sex, dogs that met all inclusion and exclusion criteria, including at least a 25% tick attachment rate, were ranked in order of descending Day -5 tick count. The 10 dogs of each sex with the highest tick counts were then included in the randomization process. The first two animals of each sex were allocated to form a block, the next two a second block, and so on. Dogs within each block were assigned, using random order numbers, to either treatment with lotilaner or to be a sham-treated control. Within approximately 30 min before dosing, all dogs had consumed a partial daily food ration.

Lotilaner was administered orally (whole tablets) at as close as possible to the targeted minimum dose rate of 20 mg/kg. Each dose was calculated based on bodyweight, to the nearest 0.1 kg, measured on Day -2. Control group dogs were sham-dosed on Day 0 using a process that matched the handling of the lotilaner-treated dogs, including removing the dog from its individual run and opening and massaging the dog’s throat.

**Tick infestations and counts**

Ticks had been field-collected from horses located in the state of Rio de Janeiro in the southeast region of Brazil during 2015 and were subsequently maintained in the Laboratory of Experimental Chemotherapy in Veterinary Parasitology of the Federal Rural University of Rio de Janeiro, Brazil. Ticks were reared on feeders of white leghorn chickens and kept in separation of each sex. In this study, both nymphal and adult ticks were obtained from the laboratory collection. The sex of nymphs and adults was confirmed by the morphology of ticks. Adult female ticks were sexed by examining the reproductive organs and the sex of nymphs was confirmed by dissection.

**Probability of success**

Ticks were collected from wild horses on a property located in the state of Rio de Janeiro in Brazil. Ticks were reared on feeders of white leghorn chickens and kept in separation of each sex. In this study, both nymphal and adult ticks were obtained from the laboratory collection. The sex of nymphs and adults was confirmed by the morphology of ticks. Adult female ticks were sexed by examining the reproductive organs and the sex of nymphs was confirmed by dissection. In the study, ticks were collected on a daily basis from horses, and the sex and stage of each tick were recorded. The ticks were then placed in separate containers, and tick counts were performed on a daily basis. Ticks were kept in the laboratory at moderate humidity and temperature, and were fed on mice to ensure their survival for the experiment. Ticks were collected on a daily basis from horses, and the sex and stage of each tick were recorded. The ticks were then placed in separate containers, and tick counts were performed on a daily basis. Ticks were kept in the laboratory at moderate humidity and temperature, and were fed on mice to ensure their survival for the experiment.
Janeiro, Brazil. The nymphal stage of *A. cajennense* (s.l.) was used for the study challenges because the highest prevalence of infestation of *A. cajennense* (s.l.) in domestic dogs in Brazil occurs during its larval and nymphal stages, while infestations with adult ticks is less prevalent [10]. In addition, the attachment rate of adult ticks of this species in laboratory dogs is very low, not allowing for the minimum attachment rate of 25% recommended by international guidelines. Each dog was experimentally infested in its pen with 200 viable unfed nymphs on Days -7, -2, 7, 14, 21 and 28 (Fig. 1). Dogs were not sedated for either tick application or counting. The ticks were applied gently to the dorsal rump area and allowed to crawl into the hair coat to select an attachment site. Ticks applied on Day -7 were counted and removed on Day -5, 48 (± 2) h after the first infestation. Ticks applied on Day -2 were counted and removed on Day 2, 48 (± 2) h after lotilaner administration. Since Day 2 tick counts were 4 days after initial infestation, the kennel floors were also inspected for engorged ticks since nymphal ticks may complete feeding in 3–4 days. No engorged nymphs were found on kennel floors. On Days 9, 16, 23 and 30 the ticks were counted and removed, 48 (± 2) h post-infestation. For counting ticks on treatment and control group dogs, each dog’s hair was pushed manually against its natural lie so that the skin and ticks were visible. A systematic examination for ticks included exploration of each dog’s head, limbs and all dorsal and ventral areas.

### Assessment of efficacy

Efficacy was determined by the reductions in live, attached tick counts on lotilaner-treated dogs, relative to control dogs, 48 h after treatment on Study Day 0 and 48 h after each subsequent infestation on Days 9, 16, 23 and 30. Arithmetic and geometric mean group efficacies were calculated according to Abbott’s formula as follows:

\[
\text{Efficacy} (\%) = 100 \times \frac{(M_c - M_t)}{M_c}
\]

where *Mc* is the untreated control group’s mean number of live ticks on dogs, and *Mt* is the treated group’s mean number of live ticks on dogs. The Shapiro-Wilk test was used to evaluate data normality. As this showed substantial deviation from normality, the Mann-Whitney test was utilized to compare the mean values between the live nymph counts of the groups. Calculation of geometric means was performed using logarithm transformed counts (count +1) with one (1) subtracted, subsequently, from the result. All analyses were performed using SAS version 9.2 software. Lotilaner was considered effective if the average live tick attachment rate in the control group was at least 25%, if there was a statistically significant difference (*P* < 0.05) between the treated group and the control group, and if the treated group had a calculated efficacy of at least 90%.

### Results and discussion

The doses of lotilaner administered to study dogs ranged from 20.3–25.0 mg/kg, which was as close to 20 mg/kg as possible using commercially available tablets without dropping below the minimum approved dose of 20 mg/kg. There were no treatment-related adverse events. Tick infestations in the control dogs ranged from a low of 43 up to a maximum of 95, with the average infestation rate remaining above 25% at each assessment, thereby meeting the requirement for adequate infestation and efficacy comparison with the treated group (Table 1).
Within 48 h post-treatment, no live ticks were found attached to any lotilaner-group dogs, and efficacy remained at, or close to 100% at every post-treatment assessment (Table 1, Fig. 2). From each infesting dose of 200 ticks, the maximum number of live ticks found on any lotilaner-group dog in the post-treatment period was three. Only one dog was infested on Day 16 (three ticks), two dogs on Day 23 (each with two ticks) and three on Day 30 (with one, two and three ticks). Between-group differences in mean counts of live *A. cajennense* (s.l.) were significant ($P = 0.0002$) at every post-treatment assessment.

This is the second report describing the efficacy of a treatment against *A. cajennense* (s.l.) infestations in dogs. The first such report described a study that used a similar protocol to that of our study to investigate the efficacy of sarolaner [21]. That study produced 100% efficacy for sarolaner by 48 h following Day 12 infestation and $\geq 99.6\%$ by 48 h following Day 33 infestation, similar to those that we report for lotilaner [21]. While the results of the two studies suggest that efficacy against *A. cajennense* (s.l.) may be an isoxazoline class effect, other studies suggest that different members of the class do not necessarily provide the same degree of efficacy, particularly against *Amblyomma* spp. For instance, there are only two reports of afoxolaner efficacy against *A. americanum* - in one of these at 24 h post-infestation afoxolaner efficacy was less than 80% from Day 14 through Day 30 while under the same conditions sarolaner efficacy remained at greater than 90% until Day 28 [22]. Studies assessing lotilaner efficacy against *A. americanum* at 48 h post-treatment and post infestation have demonstrated efficacy of 100% throughout, except at nine days post-treatment when efficacy was 99% [19].

For lotilaner, the results of this study align with earlier reports of efficacy against ticks in laboratory and field studies. One study demonstrated efficacy of 70% against existing infestations with *I. ricinus* within four hours post-treatment [17]. A report of a series of studies

### Table 1 Summary and efficacy of live attached tick counts 48 hours post-treatment and following post-treatment infestations

| Day of tick count | -5  | 2   | 9   | 16  | 23  | 30  |
|-------------------|-----|-----|-----|-----|-----|-----|
| Control           |     |     |     |     |     |     |
| Arithmetic mean ± SD | 60.6 ± 7.1 | 60.0 ± 7.0 | 62.4 ± 13.7 | 62.0 ± 12.7 | 62.1 ± 14.8 | 63.2 ± 12.5 |
| Geometric mean    | 60.2 | 59.6 | 61.2 | 60.9 | 60.7 | 62.1 |
| Lotilaner         |     |     |     |     |     |     |
| Arithmetic mean ± SD | 61.1 ± 9.7 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.3 ± 0.9 | 0.4 ± 0.8 | 0.6 ± 1.1 |
| Efficacy (%)      | na  | 100 | 100 | 99.5 | 99.4 | 99.1 |
| Geometric mean    | 60.5 | 0.0 | 0.0 | 0.1 | 0.2 | 0.4 |
| Efficacy (%)      | na  | 100 | 100 | 99.8 | 99.6 | 99.4 |

**Comparison**

- $Z = 0.11, P = 0.8951$
- $Z = 4.00, P < 0.0001$
- $Z = 3.92, P < 0.0001$
- $Z = 3.87, P < 0.0001$
- $Z = 3.82, P < 0.0001$

**Abbreviations**: SD, standard deviation; na, not applicable.

![Fig. 2 Percent efficacy in geometric mean *A. cajennense* tick counts of lotilaner-treated dogs compared to untreated control group dogs](image-url)
involving experimental infestations with commonly occurring European ticks showed that lotilaner efficacy remained at least 95% through 35 days post-treatment and a field study across three European countries subsequently confirmed lotilaner effectiveness against field challenge [18, 23]. Similar efficacy was reported following induced infestations of commonly occurring ticks in North America [24]. As in this study, experimental infestation studies typically involve assessments at 48 h post-treatment, and 48 h after subsequent weekly challenges. As tick transmission of some protozoal pathogens may not occur until up to 72 h post-infestation, the demonstration of lotilaner efficacy against *A. cajennense* (s.l.) at 48 h after challenge provides assurance that the risk of disease from a tick bite can be substantially reduced [25, 26]. For lotilaner, this was shown in an earlier study in which treatment successfully prevented the transmission of *Babesia canis* to dogs exposed to infected *D. reticulatus* ticks [27].

**Conclusions**

This study demonstrates that lotilaner given orally at a minimum dose rate of 20 mg/kg is well tolerated, provides a rapid reduction of existing nymphal *A. cajennense* (s.l.) tick infestations, and provides a sustained residual protection for at least 30 days against infestation by *A. cajennense* (s.l.) ticks in dogs. This level of activity indicates that lotilaner can be a valuable tool to help prevent the transmission of tick-borne diseases to treated dogs [27].

**Abbreviations**

GABA: γ-aminobutyric acid; Mc: mean number of live ticks on dogs in the untreated control group; Mt: mean number of live ticks on dogs in the treated group; na: not applicable; SD: standard deviation; WAAVP: World Association for the Advancement of Veterinary Parasitology

**Acknowledgements**

The authors would like to thank Dr Bill Ryan of Ryan Mitchell Associates LLC for assistance with the manuscript.

**Funding**

The study was funded by Elanco.

**Availability of data and materials**

Data not included in this manuscript can only be made available to bona fide researchers, subject to a non-disclosure agreement due to commercial confidentiality of this research.

**Authors’ contributions**

All authors participated in one or more of design, completion and reporting of the studies and were involved in the drafting of the manuscript. All authors read and approved the final manuscript.

**Ethics approval and consent to participate**

The protocol was approved by the Veterinary Institute of Universidade Federal Rural do Rio de Janeiro (UFRJR) Animal Welfare Ethics Committee (Comissão de Ética no Uso de Animais (CEUA)) and by Novartis Animal Health Global Animal Welfare.

**Consent for publication**

Not applicable.

**Competing interests**

PL, MM, SN and JD are employees of Elanco Animal Health.

**Publisher’s Note**

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**Author details**

1. Elanco Animal Health, Av. Morumbi, 8264, São Paulo, SP 04703–002, Brazil.
2. Elanco Animal Health, Mattenstrasse 24a, CH-4058 Basel, Switzerland.
3. Elanco Animal Health, 2500 Innovation Way, Greenfield, IN 46140, USA.
4. Rural Federal University of Rio de Janeiro, BR 465 km 7 UFRJ, Seropédica, RJ, Brazil.

**Received:** 15 June 2018 **Accepted:** 18 September 2018

**Published online:** 03 October 2018

**References**

1. Drexler NA, Yaglom H, Casal M, Fierro M, Kriner P, Murphy B, et al. Fatal Rocky Mountain spotted fever along the United States - Mexico border, 2013–2016. Emerg Infect Dis. 2017;23:1621–4.
2. Levin ML, Killmaster LF, Zemtsova GE, Ritter JM, Langham G. Clinical presentation, convalescence, and relapse of rocky mountain spotted fever in dogs experimentally infected via tick bite. PLoS One. 2014;9:e115105.
3. Guedes E, Leite RC, Pacheco RC, Silveira I, Labruna MB. Ricketsia species infecting Amblyomma ticks from an area endemic for Brazilian spotted fever in Brazil. Rev Bras Parasitol Vet. 2011;20:308–11.
4. Krawczak FS, Nieri-Bastos FA, Nunes FP, Soares JF, Moraes-Filho J, Labruna MB. Rickettsial infection in Amblyomma cajennense ticks and capybaras (Hydrochoerus hydrochaeris) in a Brazilian spotted fever-endemic area. Parasit Vectors. 2014;7.
5. Billings AN, Yu XJ, Teel PO, Walker DH. Detection of a spotted fever group rickettsia in Amblyomma cajennense ticks in a Brazilian spotted fever-endemic area. Parasit Vectors. 2006;22.
6. Guedes E, Leite RC, Prata MC, Pacheco RC, Walker DH, Labruna MB. Detection of Rickettsia rickettsii in the tick Amblyomma cajennense in a new Brazilian spotted fever-endemic area in the state of Minas Gerais. Mem Inst Oswaldo Cruz. 2011;105:283–8.
7. Camus E, Barre N. Vector situation of tick-borne diseases in the Caribbean Islands. Vet Parasitol. 1995;57:167–76.
8. Estrada-Peña A, Guglielmone AA, Mangold AJ. The distribution and population dynamics in the Tamaulipan Biotic Province in Texas. J Vector Ecol. 1998;35:474–8.
9. Guedes E, Leite RC, Prata MC, Pacheco RC, Walker DH, Labruna MB. Detection of Rickettsia rickettsii in the tick Amblyomma cajennense in a new Brazilian spotted fever-endemic area in the state of Minas Gerais. Mem Inst Oswaldo Cruz. 2001;105:283–8.
10. Camus E, Barre N. Vector situation of tick-borne diseases in the Caribbean Islands. Vet Parasitol. 1995;57:167–76.
11. Lopes CM, Leite RC, Martins J, Figueiroa PR, Rodrigues ZB, et al. Host specificity of Amblyomma cajennense (Acari: Ixodidae) with comments on the drop-off rhythm. Mem Inst Oswaldo Cruz. 1998;93:347–51.
12. Freitas CM, Leite RC, Bastianetto E, Cunha AP, Belo AC. Possible acquired resistance of dogs successively infested by Amblyomma cajennense (Acari, Ixodidae) with comments on the drop-off rhythm. Mem Inst Oswaldo Cruz. 1998;93:347–51.
13. Gassel M, Wolf C, Noack S, Williams H, Ilg T. The novel isoxazoline ectoparasiticide fluralaner: selective inhibition of arthropod gamma-aminobutyric acid- and L-glutamate-gated chloride channels and insecticidal/acaricidal activity. Insect Biochem Mol Biol. 2014;45:111–24.
14. Rufener L, Danelli V, Betrand D, Sager H. The novel isoxazoline ectoparasiticide lotilaner (Credelio™): a non-competitive antagonist specific to invertebrates γ-aminobutyric acid-gated chloride channels (GABACs). Parasit Vectors. 2017;10:530.
15. Toutain CE, Seewald W, Jung M. The intravenous and oral pharmacokinetics of lotilaner and the effect of food in dogs. Parasit Vectors. 2017;10:532.
16. Cavalli D, Murphy M, Seewald W, Drake J, Nanchen S. Assessment of the onset of lotilaner (Credelio™) speed of kill of fleas on dogs. Parasit Vectors. 2017;10:531.
17. Murphy M, Cavalleri D, Seewald W, Drake J, Nanchen S. Laboratory evaluation of the speed of kill of lotilaner (Credelio™) against *Ixodes ricinus* ticks on dogs. Parasit Vectors. 2017;10:541.

18. Cavalleri D, Murphy M, Gorbea RL, Seewald W, Drake J, Nanchen S. Laboratory evaluations of the immediate and sustained effectiveness of lotilaner (Credelio™) against three common species of ticks affecting dogs in Europe. Parasit Vectors. 2017;10:527.

19. Murphy M, Garcia R, Karadzovska D, Cavalleri D, Snyder DE, Seewald W, et al. Laboratory evaluations of the immediate and sustained effectiveness of lotilaner (Credelio™) against four common species of ticks affecting dogs in North America. Parasit Vectors. 2017;10:523.

20. Marchiondo AA, Holdsworth PA, Fourie LJ, Rugg D, Hellmann K, Snyder DE, et al. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.). 2nd ed.: Guidelines for evaluating the efficacy of parasiticides for the treatment, prevention and control of flea and tick infestations on dogs and cats. Vet Parasitol. 2013;194:84–97.

21. Scott F, Franz L, Campos DR, Azevedo TRC, Cunha D, Six RH, et al. Efficacy of sarolaner (Simparic™) against induced infestations of *Amblyomma cajennense* on dogs. Parasit Vectors. 2017;10:390.

22. Six RH, Everett WR, Chapin S, Mahabir SP. Comparative speed of kill of sarolaner (Simparica) and afoxolaner (NexGard) against induced infestations of *Amblyomma americanum* on dogs. Parasit Vectors. 2016;9:98.

23. Cavalleri D, Murphy M, Seewald W, Drake J, Nanchen S. A randomized, controlled study to assess the efficacy and safety of lotilaner (Credelio™) in controlling ticks in client-owned dogs in Europe. Parasit Vectors. 2017;10:531.

24. Murphy M, Garcia R, Karadzovska D, Cavalleri D, Snyder D, Seewald W, et al. Laboratory evaluations of the immediate and sustained efficacy of lotilaner (Credelio™) against four common species of ticks affecting dogs in North America. Parasit Vectors. 2017;10:523.

25. Little SE. Changing paradigms in understanding transmission of canine tick borne diseases: the role of interrupted feeding and intrastadial transmission. In: 2nd Canine Vector-Borne Disease (CVBD) Symposium. Mezara del Vallo, Sicily, Italy; 2007. p. 30–4. http://www.cvbd.org/fileadmin/media/cvbd/Proceedings_CVBD_2007_FINAL23042007.pdf. Accessed 24 Jul 2017.

26. Salinas LJ, Greenfield RA, Little SE, Voskuhl GW. Tick-borne infections in the southern United States. Am J Med Sci. 2013;346:194–201.

27. Cavalleri D, Murphy M, Seewald W, Drake J, Nanchen S. Two randomized, controlled studies to assess the efficacy and safety of lotilaner (Credelio™) in preventing *Dermacentor reticulatus* transmission of *Babesia canis* to dogs. Parasit Vectors. 2017;10:520.