de Oliveira Filho, Jaires Gomes, Ferreira, Lorena Lopes, Sarria, André Lucio Franceschini, Pickett, John , Birkett, Michael A., Mascarin, Gabriel Moura, de León, Adalberto A. Pérez and Borges, Lígia Miranda Ferreira 2017. Brown dog tick, Rhipicephalus sanguineus sensu lato, infestation of susceptible dog hosts is reduced by slow release of semiochemicals from a less susceptible host. Ticks and Tick-borne Diseases 8 (1) , pp. 139-145. 10.1016/j.ttbdis.2016.10.010

Publishers page: http://dx.doi.org/10.1016/j.ttbdis.2016.10.010

Brown dog tick, Rhipicephalus sanguineus sensu lato, infestation of susceptible dog hosts is reduced by slow release of semiochemicals from a less susceptible host

Jaires Gomes de Oliveira Filho [a,1] , Lorena Lopes Ferreira [a,1] ,
André Lucio Franceschini Sarria [b] , John A. Pickett [b] , Michael A. Birkett [b] ,
Gabriel Moura Mascarin [c] , Adalberto A. Pérez de León [d] , Lígia Miranda Ferreira Borges [e,*]
[a] Escola de Veterinária e Zootecnia, Universidade Federal de Goiás. Campus Samambaia, Avenida Esperança, s/n, Campus Universitário, CEP: 74690-900, Goiânia, Goiás, Brazil
[b] Biological Chemistry and Crop Protection Department, Rothamsted Research, West Common, Harpenden, Hertfordshire, AL5 2JQ, United Kingdom
[c] EMBRAPA Arroz e Feijão, Rodovia GO-462, Km 12, Zona Rural, Caixa Postal: 179, CEP: 75375-000, Santo Antônio de Goiás, Goiás, Brazil
[d] USDA, ARS, Knipling-Bushland U.S. Livestock Insects Research Laboratory and Veterinary Pest Genomics Center, 2700 Fredericksburg Road, Kerrville, TX, 78028, USA
[e] Instituto de Patologia Tropical e Saúde Pública. Universidade Federal de Goiás, Rua 235s/n, Setor Universitário, CEP: 74605050, Goiânia, Goiás, Brazil

article info

abstract

Domestic dog breeds are hosts for the brown dog tick, Rhipicephalus sanguineus sensu lato, but infestation levels vary among breeds. Beagles are less susceptible to tick infestations than English cocker spaniels due to enhanced production of 2-hexanone and benzaldehyde that act as volatile tick repellents. We report the use of prototype slow-release formulations of these compounds to reduce the burden of R. sanguineus s. l. on English cocker spaniel dogs. Twelve dogs were randomly assigned to two groups with six dogs each. The treated group received collars with slow-release formulations of the compounds attached, while the control group received collars with clean formulations attached. Five environmental infestations were performed, with the number of ticks (at all stages) on the dogs being counted twice a day for 45 days. The counts on the number of tick stages found per dog were individually fitted to linear mixed effects models with repeated measures and normal distribution for errors. The mean tick infestation in the treated group was significantly lower than in the control group. For larvae and nymphs, a decrease in tick infestation was observed at the fifth count, and for adults, lower average counts were observed in all counts. The compounds did not interfere with the distribution of the ticks on the body of the dogs, as a similar percentage of ticks was found on the anterior half of the dogs (54.5% for the control group and 56.2% for the treated group). The biological and reproductive parameters of the ticks were not affected by the repellents. This study highlights for the first time the potential use of a novel allomone (repellent)-based formulation for reduction of tick infestation on susceptible dogs.

Keywords:
Non-host
Allomone
2-Hexanone
Benzaldehyde
Formulation
SLM’s

1. Introduction

The brown dog tick, Rhipicephalus sanguineus sensu lato, has a cosmopolitan host distribution and, in addition to parasitizing domestic dogs, can parasitize birds, livestock and human beings (Borges and Silva, 1994; Louly et al., 2006; Rodríguez-Vivas et al., 2016; Szabó et al., 2012). R. sanguineus s.l. comprises a species com-plex (Nava et al., 2015; Szabó et al., 2005), and is of veterinary and public health importance due to its obligate blood feeding habit and role as a vector of pathogens such as Babesia vogeli, Ehrlichia canis, Anaplasma platys, Rickettsia rickettsii and R. conorii (Cardoso et al., 2010; Eremeeva et al., 2011; Pacheco et al., 2011; Socolovicvhi et al., 2009).

Tick infestation levels can vary within a single host species according to host breed, age, immunological state, and individ-ual semiochemical production (Bunnell et al., 2011; Pickett et al., 2010; Weldon, 2010). Our hypothesis, relating to the ecological

---

[*] Corresponding author.
E-mail addresses: jaires filho@hotmail.com (J.G. de Oliveira Filho), loren4 lopes@hotmail.com (L.L. Ferreira), andre.sarria@rothamsted.ac.uk (A.L.F. Sarria), john.pickett@rothamsted.ac.uk (J.A. Pickett), mike.birkett@rothamsted.ac.uk (M.A. Birkett), gabriel.mascarin@embrapa.br (G.M. Mascarin), beto.perezdeleon@ars.usda.gov (A.A.P. de León), borges.ligia@gmail.com, ligia@ufg.br (L.M.F. Borges).

[1] These authors contributed equally to this work.

basis of ectoparasite repellency in hosts (Pickett et al., 2010), is that individual hosts, either closely related taxonomically or from a single taxon, differ in their interaction with ectoparasites due to differences in the production and emission of non-host chemical signaling. Furthermore, individual signal components emitted by each host can act synergistically or can exert antagonism to other ectoparasites (Nielsen et al., 2015; Weldon, 2010). For example, a blend of volatiles produced by the non-host waterbuck, Kobus defassa, was identified as being repellent to tsetse flies, Glossina morsitans, (Bett et al., 2015; Gikonyo et al., 2002, 2003). However, little is known about host natural odors that are repellent for ticks.

In our previous work where it was shown that beagles were less infested with R. sanguineus s. l. than the English cocker spaniel, it was suggested that a host factor could determine differential tick load (Louly et al., 2009, 2010). Our subsequent work identified two volatile small lipophilic molecules (SLMs), 2-hexanone and ben-zaldehyde, which are produced in greater amounts in beagles, and which act as natural repellents against R. sanguineus s. l. (Borges et al., 2015), and demonstrated constitutive release of these two SLMs in the odor of beagles (Oliveira Filho et al., 2016).

Acaricidal treatments remain the most common practice to con-trol R. sanguineus s. l. However, the indiscriminate use of products containing synthetic acaricides represents a strong selective pres-sure that results in the emergence of resistant tick populations to those active ingredients (Borges et al., 2007; Eiden et al., 2015; Miller et al., 2001). Considering the role of non-host chemical sig-naling in host selection by ectoparasites, it has been suggested that such signaling, produced by resistant hosts, may form the basis of ecologically-based repellents to control ectoparasite infestation (Pickett et al., 2010; Weldon, 2010), and may also reduce the risk of exposure to vector-borne pathogens. Following our earlier work on biting fly repellents from non-preferred hosts (Birkett et al., 2004; Logan et al., 2008, 2009), we hypothesized that tick numbers on a susceptible animal host could be reduced by application of natu-ral repellents discovered in the odor of less preferred conspecifics. Here, we investigate the effect of a prototype system, delivering 2-hexanone and benzaldehyde via slow release from polyethy-lene sachets, upon R. sanguineus s. l. infestation on English cocker spaniels, following artificial exposure under environmentally con-trolled conditions.

2. Material and methods

2.1. Animals

The use of animals (dogs and rabbits) in this study was approved by the Committee on Ethical Animal Use of the Federal Univer-sity of Goiás (CEUA/UFG, protocol number 024/2014). The care and use of the animals during this study were undertaken according to bioethics and animal welfare guidelines required by CEUA/UFG. Eight male and four female English cocker spaniel dogs, with ages varying from 50 to 116 days old, were obtained from dif-ferent breeders. They were treated for intestinal worms (Drontal Puppy – Bayer®), received all applicable vaccinations (Vanguard Plus and Defensor – Pfizer®), and were not treated with acaricides for 30 days before the study was initiated. All the dogs had their pelage cut to the same length prior to the initiation of the study, in order to offer the same conditions for tick infestation and also to facilitate tick counts. Dogs were housed in a kennel at the Vet-erinary and Animal Husbandry School of the Federal University of Goiás, Brazil. The kennel has a total area of 26.6 m^2 , with an internal area of 9.8 m^2 that was used to inspect the dogs and six individual stalls each one with 2.4 m^2 being a cover area with 1.28 m^2 and an open area with 1.12 m^2 . The floor is cemented and the walls of the kennel are glazed for easy cleaning. A week prior to the beginning of the experiment, all areas of the kennel were sanitized with a flamer three times in a week. During the progress of the experiment, the animals were fed twice a day (Golden Filhotes-Premier®), following the amounts recommended by the manufacturer and given water ad libitum, while the floor was cleaned daily with water and neutral detergent. The health of the animals was monitored daily and clin-ical pathology exams were conducted when necessary. Dogs were randomly divided composing two groups (treated and control) with six animals each (four males and two females per group). The dogs were randomly divided into three per stalls in order to facilitate the handling of animals and cleaning facilities.

2.2. Ticks

Engorged females of R. sanguineus s.l. were harvested from nat-urally infested dogs for the municipality of Goiânia, Goiás, Brazil, the establishment of a colony. The ticks were maintained in an acclimatized chamber (27 ± 1 ° C and 80% R.H.) and fed on rabbits (Oryctolagus cuniculus) to obtain differing life stages for use in dog infestations (Louly et al., 2010). The ticks used in the experiments were aged between one and three weeks old. Rabbits were removed from use after two consecutive infestations. During infestations, rabbits were examined daily and none showed symptoms of dam-age due to tick parasitism.

2.3. Preparation and use of prototype non-host semiochemical delivery system

Samples of analytical grade 2-hexanone (Aldrich Chemical Co. Ltd., Dorset, UK) and benzaldehyde (Sigma-Aldrich Co. Ltd., Stein-heim, Germany) were used in this study. Sheets of cellulose sponge (0.5 cm thick, code 0032 6865 J. Sainsbury plc), previously soaked in chloroform overnight then washed in chloroform 3 times before drying in a fume cupboard, were cut into pieces (~2.5 × 2 cm) and either treated with 400 L of benzaldehyde or 2-hexanone, or left untreated. Polyethylene sachets were prepared by heat sealing the sponges inside polyethylene sheeting (~3 × 3 cm, Al Packag-ing Ltd., London film type LFT size 50 mm gauge and 62.5 mic lift). The mean release rate per day of the compounds from the sachet formulations was determined over several weeks using dynamic headspace collection (air entrainment) and GC analysis. Thus, three sachets filled with either benzaldehyde or 2-hexanone were enclosed in a glass vessel (700 mL). Air was pumped through an activated charcoal filter into the vessel (1 L/min) and was then drawn (500 cc/min) into tubes containing the adsorbent Porapak Q (50 mg). After one hour, volatiles collected on the Porapak Q were eluted with 750 L of redistilled diethyl ether and the samples were stored at −20 ° C until analysis. Extracts (1 L) were analysed on an Agilent 6890 N GC fitted with a 10 m × 0.32 mm i.d. HP-1 col-umn. The oven temperature was maintained at 30 ° C for 0.1 min, then programmed at 10 ° C min^{-1} to 250 ° C and held for 30 min. The quantity of compound captured per sample was expressed in g/day using the analytical method described by Oliveira Filho et al. (2016). For 15 days, the release rates were 30.44 ± 1.12 g/day for benzaldehyde and 14.38 ± 1.26 g/day for 2-hexanone, decreas-ing sharply to 11.18 g/day and 1.29 g/day, respectively, after the 15th day. The ratio of released 2-hexanone and benzalde-hyde broadly matched the ratio of naturally release from beagles (Oliveira Filho et al., 2016), and so experiments were conducted with these prototype formulations. The sachets were stored at −20 ° C until required for tests. Sachets were attached to dog collars using a stapler shooter containing light duty staples (5/16” 8 mm – Stanley) and covered with gauze to prevent injury to the dogs’ necks. A single 2-hexanone sachet and a single benzaldehyde sachet was attached per dog collar. A collar with one untreated sachet was used in each of the untreated control group dogs.

2.4. Tick infestation, evaluation, and sampling

Five artificial environmental infestations were established each time by positioning breeding vials against the stall walls approxi-mately 80 cm above the floor to release 2000 larvae, 100 nymphs, and 60 adults (30 males and 30 females) per stall. The first infesta-tion was held 10 days before the attachment of the first collar, and afterwards four more infestations were carried out, every seven days. After the first infestation dogs were shaved. The collars were changed after two weeks, and were removed one week after the fifth infestation and a count followed by an additional week. The dogs were rotated among the stalls on a weekly basis and in a clock-wise fashion, which promoted standard experimental conditions to the animals.

Dogs were thoroughly inspected every day for larvae, nymphs and adults in the morning and afternoon, starting two days after the initial infestation and until seven days after the second set of repellent delivery collars were removed. A map of 24 body areas, including R. sanguineus s. l. sites, was used to standardize tick counts (Otranto et al., 2005). The final number of ticks per day was deter-mined by the maximum number of ticks per area found in one day, and the counts representing the total of ticks found per dogs during a week, except at the first count that lasted 10 days.

During the counts, the dogs were brushed twice a day to col-lect all engorged stages of R. sanguineus s. l. Male ticks were not removed during the brushes. The ticks collected from the dogs were placed in a climate chamber (27 ± 1 ° C and 80% R.H.) to assess the engorged female weight (EFW), egg mass weight (EMW), conver-sion of body weight on eggs (CBWE), conversion of body weight on larvae (CBWL), percentage of larval-hatchability (LH), percentage of larval ecdysis (LE), and percentage of nymphal ecdysis (NE). EFW was obtained after manual detachment of females while EMW was determined 15 days after the onset of egg laying. LH was evaluated by averaging the counts of two evaluators according to the estimate of the percentage of larvae that hatched from eggs (Bechara et al., 1994).

2.5. Statistical analysis

Count data on the number of larvae, nymphs and adults found per dog were individually fitted to linear mixed effects models with repeated measures and normal (Gaussian) distribution for errors. Normality assumptions were previously checked based on Shapiro-Wilk and Bartlett tests. In addition, goodness-of-fit of the models were assessed using residual plots. These models were implemented using the “lme4” package (Bates et al., 2015, https:// CRAN.R-project.org/package=lme4) from the free statistical soft-ware R (R Core Team, 2014; http://www.R-project.org/.). The linear predictors were represented by treatment (control vs. treated dogs wearing the repellent collar), infestations, and their interaction term. Since all dogs were repeatedly evaluated for all four weeks, dog was included as a random effect in models (i.e., to allow for non-independence of each time measurement from a given dog). Therefore, the model may be written as:

$$Y_{ijn} = \check{}_{0} + X_{i} + T_{j} + X_{*} T_{ij} + {}_{n} + \varepsilon_{ij}$$

Where Y_{ijn} is the value of the outcome variable (number of lar-vae, nymphs or adults per dog) for the i-th treatment of the j-th infestation at the n-th replicate (i.e., dog), $\check{}_0$ is the intercept, X_i is the treatment effect, T_j is the infestation time, $X*T_{ij}$ is the interaction term, n is the random effect for dogs, and ε_{ij} is the error for treatment i in time j. Then, type 3 F-tests with Satterthwaite’s approximation for degrees of freedom was employed to assess significance (P-values) of fixed effects using the “lmerTest” package from R (Kuznetsova et al., 2015, http://CRAN.R-project. org/package=lmerTest). When the interaction term was significant, multiple pairwise comparisons were performed based on differ-ences of least squares means at $P < 0.05$.

The number of engorged larvae, nymphs and females recov-ered during the brushes was insufficient to evaluate the biological and reproductive parameters over the successive infestations. The Student t test at $P < 0.05$ was used to compare these parameters between treated and control groups adding the ticks recovered from all infestations, during the time the dogs were wearing the repellent collars.

3. Results

During the first infestation of R. sanguineus s.l. on dogs, con-ducted before the attachment of repellent-treated collars, the number of ticks (larvae, nymphs and adults) in the two groups was virtually similar, ranging from a rate of 30 ticks found on treated group and 27 ticks on the control group (larvae: $t = 0.2774$, $df = 5$, $P = 0.7926$; nymphs: $t = 0.0$, $df = 5$, $P = 1.0$; adults: $t = 0.3974$, $df = 5$, $P = 0.7075$). In the last count (6th week) realized without artificial infestation and in the absence of collars, a total (larvae, nymphs and adults) of 203 and 204 ticks in treated and control groups was observed, respectively. No larvae were found in either group, only five nymphs were found in the treated group vs. six in control group ($t = 0.3492$, $df = 5$, $P = 0.7412$), and the average number of adults was 198 for untreated dogs vs. 198 for treated dogs ($t = 0.1661$, $df = 5$, $P = 0.8746$).

According to the F-test, treatment and infestation time (or sampling date) had a significant effect on the number of larvae, nymphs and adults of R. sanguineus s. l. found on dogs, as their interaction term was significant ($P = 0.0110$, $P = 0.0430$, $P = 0.0373$, respectively). This indicates that all life stages of this tick species considerably decreased across time in the presence of the repel-lent formulation (Fig. 1). The compounds did not interfere with the distribution of the ticks on the body of the dogs, as a similar per-centage of ticks was found on the anterior half of the dogs (54.5% for the control group and 56.2% for the treated group).

A more pronounced reduction in tick infestation due to the repellent effect was noted for larvae at the 5th count, with averages of 4.67 larvae in the treated group against 10.67 larvae in the control group ($P < 0.0001$). Likewise, the counts of nymphs showed a sig-nificant difference in the same interval with average loads of 3.33 and 10.67 nymphs in the control and treated groups respectively ($P < 0.0001$). Averages for adult counts always showed significant reductions in infestation upon artificial infestations in the four con-secutive weeks for animals wearing the repellent collar compared to those wearing untreated collars. For instance, in the 2nd infes-tation, the means of adult ticks found were 12.67 and 21.33 in treated and control groups respectively ($P = 0.002$). In the 3rd infes-tation, the mean was 34.5 in treated group vs. 39.67 in control group ($P = 0.006$), in the 4th infestation the number was 41 in treated and 49.67 in control group ($P < 0.0001$), and in 5th infestation the mean of adults reached 39.67 and 48 in treated and control, respectively ($P < 0.0001$) (Fig. 1).

Regardless of the treatment, all tick life stages tended to increase with time, and population peaks were noted at the 4th or 5th week of evaluation (Fig. 1). Overall, tick infestation loads in dogs wearing the collar containing the repellent formulation exhibited signifi-cantly lower numbers of larvae ($P = 0.00049$), nymphs ($P = 0.00027$) and adults ($P < 0.0001$), when compared to untreated dogs from the control group (Fig. 2). The weight of the female ticks, egg con-version, larval hatchability and ecdysis were statistically similar in

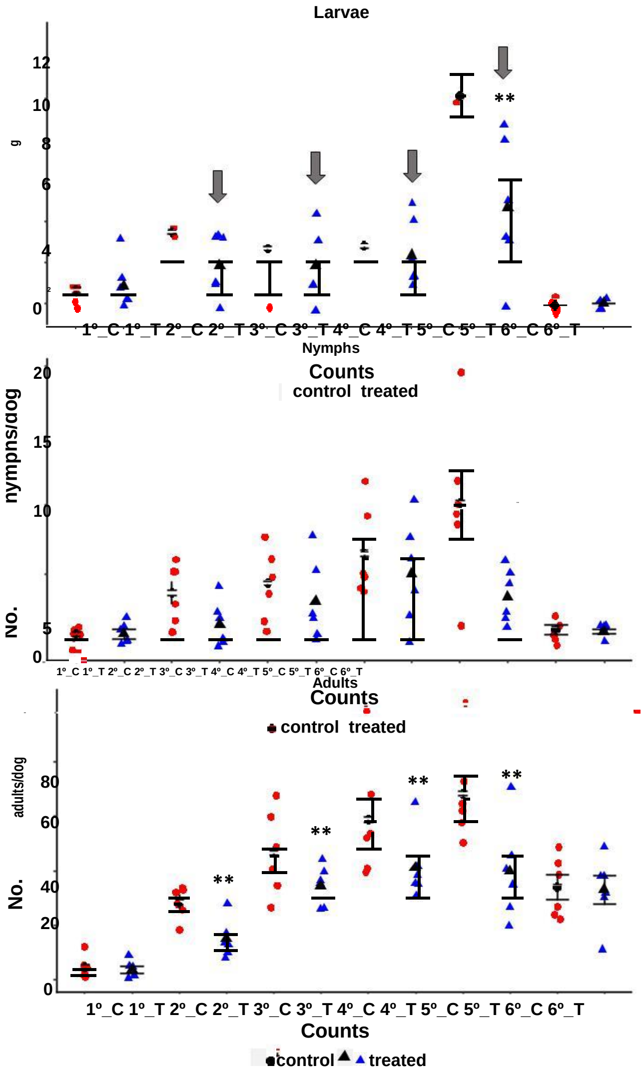

Fig. 1. Mean (±SE, standard error) number of ticks (larvae, nymphs, and adults) retrieved from untreated (control) and treated (repellent collar) dogs across infes-tation periods. Vertical arrows indicate when dogs received the repellent collar. At 4th infestation interval, the repellent collar was replaced by a new one and in the 5th the repellent collar was removed. The counts during a week, except the first count during 10 days. Significant difference between control and treated dogs is indicated by $P < 0.05$ (*) or $P < 0.01$ (**).

treated and control groups (Table 1), suggesting that the repellent formulations had no effect on the biological parameters of this tick species.

4. Discussion

The results presented here support the hypothesis that non-host unsuitability can be conferred to a suitable host by treatment with non-host semiochemicals (Borges et al., 2015; Pickett et al., 2010; Weldon, 2010; Weldon, 2013). Our findings document, for the first time, in vivo activity of a natural repellent blend containing odor components of a resistant host, which could be regarded in general as a safer tool to be used to manage R. sanguineus s. l. infestations on dogs, and perhaps other susceptible host species. The doses of the two compounds tested are well below the median lethal dose (LD_{50}) for each compound. Furthermore, they are benign, are used as flavorings, perfume components and are present in food (ATSDR, 1995; FDA, 2013; Sigma Aldrich, 2015). Experimental data showed that the release of 2-hexanone and benzaldehyde using the prototype delivery system prevented infestation levels in a dog breed known to be susceptible to parasitism by R. sanguineus s. l. The artificial environmental infestations were established to resemble more closely field conditions of dog exposure to R. sanguineus s. l. (Hansford et al., 2015; Rodríguez-Vivas et al., 2016).

Table 1
Mean (±SD) of biological parameters of Rhipicephalus sanguineus sensu lato ticks obtained along successive infestations on dogs treated or non- treated with 2-hexanone and benzaldehyde.

Parameters	Groups	
	Treated	Control
EFW (g)	93.00 ± 30.00^a (n = 10)	81.00 ± 34.00^a (n = 10)
EMW (g)	50.00 ± 25.00^a (n = 10)	40.00 ± 21.00^a (n = 10)
LH (%)	83.55 ± 31.64^a	83.33 ± 31.72^a
CBWE (%)	51.99 ± 16.41^a	52.61 ± 20.72^a
CBWL (%)	42.87 ± 22.39^a	49.45 ± 19.96^a
LE (%)	64.08 ± 21.27 ^{a} (n = 23)	79.16 ± 24.57 ^{a} (n = 39)
NE (%)	77.77 ± 40.36 ^{a} (n = 9)	75.17 ± 41.83 ^{a} (n = 7)

All parameters evaluated were statistically similar between treated and control groups by t-Student test ($P > 0.05$). EFW: engorged female weight, EMW: egg mass weight, LH: larval-hatchability rate, CBWE: conversion body weight on eggs, CBWL: conversion body weight on larvae, LE: percentage of larval ecdysis, NE: percentage of nymphal ecdysis.

Several non-host compounds deter parasitism of animals by hematophagous arthropods (Birkett et al., 2004; Borges et al., 2015; Douglas et al., 2004; Gikonyo et al., 2002, 2003; Pageat, 2005). In several cases, however, their evaluation as the basis for innova-tive control technologies remains to be accomplished. Saini et al. (2013) tested a collar prototype with repellents identified on the non-host Kobus defassa by Gikonyo et al. (2003) in association with a synthetic repellent against Glossina morsitans. A 90% reduction in the transmission of trypanosomiasis was observed after the use of this prototype. Refinement of the blend constituents showed the potential to deploy the repellent-based technology to protect cat-tle from tsetse and the risk of exposure to Trypanosoma spp. (Bett et al., 2015). Pageat (2005) reported the presence of allomones bis (2-ethylhexyll) adipate and 2,2,4-trimethyl-1,3-pentanediol diisobutyrate from the secretion of uropygial gland of ducks that repel red poultry mites, Dermanyssus gallinae, and a product com-prising these compounds is being manufactured and distributed in France for use in the control of D. gallinae on poultry farms. The use of a prototype system to deliver repellent compounds pro-vides an opportunity to develop an optimal formulation, in terms of compound quantity and ratio and matrix for formulation stabil-ity, for efficient sustained release over several weeks, as has been attempted with other efforts to develop new formulations to con-trol R. sanguineus s. l. infestations on dogs (Bhoopathy et al., 2014; Stanneck et al., 2012).

Our results indicated that the counts of larvae (0.47%) and nymphs (12.15%) are a fraction of the total released into the environment where the dogs were housed. Various sources of vari-ability contributed to our inability to recover all the ticks released. Despite our methodical approach, it is possible some ticks were missed during visual and tactile inspection, and during brushing for ectoparasite enumeration, because of the small size of larvae and nymphs, immature ticks can also perish due to desiccation and predation (Apanaskevich and Oliver, 2014; Troughton and Levin, 2007). Host grooming is a preponderant behavior that can result in the removal of up to 80% of the ticks, mainly larvae, because they can attach several times before feeding, which is accompanied by itching that triggers grooming (Hart, 2000; Mooring et al., 1996).

Higher counts for adults (180%) were observed parasitizing the dogs than those released in the environment. When the adults were counted, males and unengorged females were not removed from the dogs. Therefore, the higher number of adults found on dogs

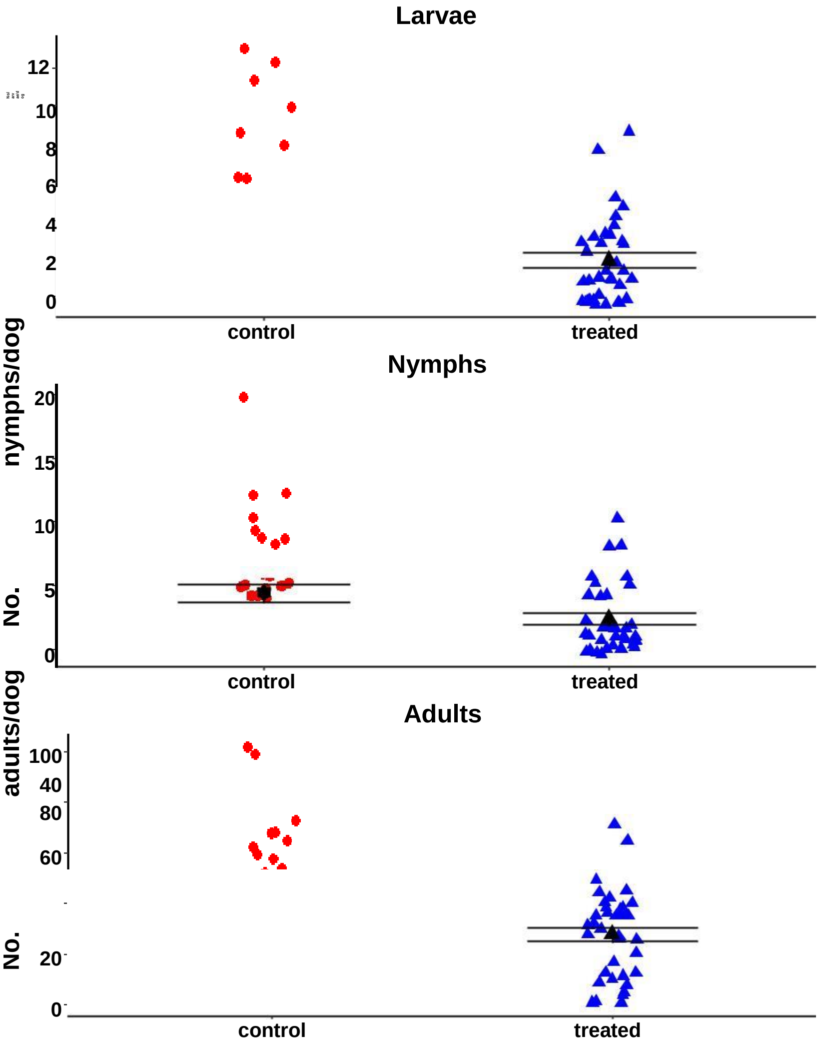

Fig. 2. Overall mean (±SE, standard error) number of ticks (larvae, nymphs, and adults) retrieved from untreated (control) and treated (repellent collar) dogs. Significant difference between control and treated dogs is indicated by P < 0.05 (*) or P < 0.01 (**).

could be related to the fact that males of R. sanguineus s. l. can survive in the environment for up to 568 days and seek the host repeatedly for feeding, or finding a mate thus moving between hosts and throughout the environment (Hooker et al., 1912; Little et al., 2007; Troughton and Levin, 2007). It is possible that male ticks may have been counted on the same dog in different areas or even on different dogs through time because our prototype formulation was repellent and not acaricidal.

Adult tick numbers on treated dogs were lower than on the con-trol animals across all counts. However, this trend was observed for larvae and nymphs only in the fifth count. Synthetic compounds like DEET promote significant repellency against all R. sanguineus s. l. developmental stages, but the rate and duration of repellency tends to decrease with time when this compound was tested against adults (Kumar et al., 1992). The natural repellents 2-hexanone and benzaldehyde were observed to repel adult R. sanguineus s. l. in vitro as strongly as DEET (Borges et al., 2015). Further research is required to determine the relative repellency of 2-hexanone and benzalde-hyde against R. sanguineus s. l. larvae and nymphs.

The recovery rate for engorged larvae, nymphs, and females was 0.17, 0.80 and 3.33%, respectively. Notwithstanding the number of engorged tick that may have been missed during brushing, some ticks could have engorged and detached from the dogs before the animals were inspected to quantify the ectoparasite load. Engorged larvae detach from their hosts during the day and night, while engorged nymphs and females detach mainly during the night (Paz et al., 2008). Therefore, the majority of engorged ticks may have dropped off at night before the dogs were brushed during the day.

A lack of effect on the biological and reproductive parameters of R. sanguineus s. l. was noted for 2-hexanone and benzalde-hyde. However, R. sanguineus s. l. that fed on a resistant dog breed had their biological parameters impaired (Louly et al., 2009). R. sanguineus s. l. apparently senses repellent SLMs to distinguish between resistant and susceptible hosts, but also is deterred by contact compounds produced by resistant animals (Louly et al., 2010). In comparison to chemosensation in congeneric ticks, it was hypothesized that R. microplus could perceive not only phagostimulants known to be present in host blood, but also anti-feeding substances that could exist in cattle serum associated with decreased susceptibility to tick infestation (Ferreira et al., 2015). Additional experiments are needed to determine if the deleteri-ous effects observed in R. sanguineus s. l. upon feeding on resistant dogs are caused by compounds other than 2-hexanone and ben-zaldehyde, which could be present on host skin or circulating in the blood.

The specificity of SLM perception and susceptibility to their effects are linked to subtle ratios of the compounds produced by hosts and non-host species (Bruce et al., 2005; Logan et al., 2008;

Weldon, 2010). The release rate adopted here roughly matched that observed by Oliveira Filho et al. (2016), where it was shown, using analytical chemistry experiments, that the mean ratio of 2-hexanone: benzaldehyde was ~1:2 in odor samples collected from beagles. Further work is needed to determine optimal doses and formulations for the two compounds, which will underpin our attempts to develop a commercial product.

Problems with the control of ticks such as R. sanguineus s. l. and the pathogens they transmit are increasing in complexity (Dantas-Torres, 2015; Esteve-Gassent et al., 2016). The One Health approach, which is the synthesis of strategies aimed at enhancing animal and human health that take into consideration our envi-ronment, has been proposed to address the problem of tick and tick-borne diseases as a way to facilitate the development of sus-tainable control solutions (Dantas-Torres et al., 2012; Pérez de León et al., 2010; Vayssier-Taussat et al., 2015). Work on natural prod-ucts that repel arthropod vectors, including ticks, merits attention for development because they could be used as part of integrated pest management programs, and as tools to enable the rational use of acaricides (Guerrero et al., 2014; Mencke, 2013; Pérez de León et al., 2014).

Natural semiochemicals such as those tested here are ubiquitous and form part of the adaptive behavioral ecology of R. sanguineus s. l. (Nielsen et al., 2015; Pickett et al., 2010; Weldon, 2010). Further research will help understand the mode of repellency of 2-hexanone and benzaldehyde against R. sanguineus s. l. As has been noted with other species, knowledge gaps on the anatomy and molecular biology of chemosensation and chemoreception in ticks remain to be elucidated (Borges et al., 2016; Esteve-Gassent et al., 2016; Ferreira et al., 2015; Renthal et al., 2016). An enhanced under-standing of how ticks detect and process chemical cues to find hosts or avoid unsuitable but potential hosts could offer further innova-tive technologies to mitigate the burden of these ectoparasites and vectors of pathogens that affect humans, domestic animals, and wildlife.

Acknowledgments

We thank the National Council of Technological and Scien-tific Development (CNPq), Brazil, for its financial support (Grant no. #/2013-9) and for supply a Scholarship to LMFB (#/2015-0). Rothamsted Research receives grant-aided support from the Biotechnology and Biological Sciences Research Council (BBSRC) of the United Kingdom. USDA is an equal opportunity provider and employer.

References

ATSDR – Agency for Toxic Substances and Disease Registry., 1995. 2-Hexanone CAS # 591-78-6. www.atsdr.cdc.gov/toxfaqs/tfacts44.pdf. Last access: January 2016.

Apanaskevich, D.A., Oliver Jr, J.H., 2014. Life cycles and natural history of ticks. In: Sonenshine, D.E., Roe, M. (Eds.), Biology of Ticks, vol. 1. Oxford University Press, Oxford and New York, pp. 59–73.

Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. lme4: Linear mixed-effects models using Eigen and S4 . R package version 1. 1–9, https://CRAN.R-project. org/package=lme4>. Last access: January 2016.

Bechara, G.H., Szabó, M.P.J., Mukai, L.S., Rosa, P.C.S., 1994. Immunization of dogs, hamsters and guinea pigs against Rhipicephalus sanguineus using crude unfed adult tick extracts. Vet. Parasitol. 52, 79–90.

Bett, M.K., Saini, R.K., Hassanali, A., 2015. Repellency of tsetse-refractory waterbuck (Kobus defassa) body odor to Glossina pallidipes (Diptera: glossinidae): assessment of relative contribution of different classes and individual constituents. Acta Trop. 146, 17–24.

Bhoopathy, D., Latha, B.R., Uma, T.S., Sreekumar, C., Leela, V., 2014. A novel approach to control brown dog tick, Rhipicephalus sanguineus using sustained release poly- -caprolactone-pheromone microspheres. Acta Parasitol. 59, 153–157.

Birkett, M.A., Agelopoulos, N., Jensen, K.M.V., Jespersen, J.B., Pickett, J.A., Prijs, H.J., Thomas, G., Trapman, J.J., Wadhams, L.J., Woodcock, C.M., 2004. The role of volatile semiochemicals in mediating host location and selection by nuisance and disease-transmitting cattle flies. Med. Vet. Entomol. 18, 313–322.

Borges, L.M.F., Silva, C.R.F., 1994. Ixodídeos parasitos de bovinos e equinos da microrregião de Goiânia, Goiás. Rev. Parasit. Trop. 23, 69–74.

Borges, L.M.F., Soares, S.F., Fonseca, I.N., Chaves, V.V., Louly, C.C.B., 2007. Resistência acaricida em larvas de Rhipicephalus sanguineus (Acari ixodidae) de goiânia-GO, Brasil. Rev. Pat. Trop. 36, 87–95.

Borges, L.M.F., Oliveira Filho, J.G., Ferreira, L.L., Louly, C.C.B., Pickett, J.A., Birkett, M.A., 2015. Identification of non-host semiochemicals for the brown dog tick, Rhipicephalus sanguineus sensu lato (Acari Ixodidae), from tick-resistant beagles, Canis lupus familiaris. Ticks Tick Borne Dis. 6, 676–682.

Borges, L.M.F., Li, A.Y., Olafson, P.U., Renthal, R., Bauchan, G.R., Lohmeyer, K.H., Pérez de León, A.A., 2016. Neuronal projections from the Haller's organ and palp sensilla to the synganglion of Amblyomma americanum. Rev Brasil. Parasitol. Vet. 25, 217–224.

Bruce, T.J.A., Wadhams, L.J., Woodcock, C.M., 2005. Insect host location: a volatile situation. Trends Plant. Sci. 10, 269–274.

Bunnell, T., Hanisch, K., Hardege, J.D., Breithaupt, T., 2011. The fecal odor of sick hedgehogs (Erinaceus europaeus) mediates olfactory attraction of the tick Ixodes hexagonus. J. Chem. Ecol. 37, 340–347.

Cardoso, L., Tuna, J., Vieira, L., Yisaschar-Mekuzas, Y., Baneth, G., 2010. Molecular detection of Anaplasma platys and Ehrlichia canis in dogs from the North of Portugal. Vet. J. 183, 232–233.

Dantas-Torres, F., Chomel, B.B., Otranto, D., 2012. Ticks and tick-borne diseases: a one health perspective. Trends Parasitol. 28, 346–437.

Dantas-Torres, F., 2015. Climate change, biodiversity, ticks and tick-borne diseases: the butterfly effect. Int. J. Parasitol. Parasites Wildl. 4, 452–461.

Douglas, H.D., Co, J.E., Jones, T.H., Conner, W.E., 2004. Interspecific differences in Aethia spp: auklet odorants and evidence for chemical defense against ectoparasites. J. Chem. Ecol. 30, 1921–1935.

Eiden, A.L., Kaufman, P.E., Allan, S.A., Miller, R.J., 2015. Detection of permethrin resistance and fipronil tolerance in Rhipicephalus sanguineus (acari: ixodidae) in the United States. J. Med. Entomol. 52, 429–436.

Eremeeva, M.E., Zambrano, M.L., Anaya, L., Beati, L., Karpathy, S.E., Santos-Silva, M.M., Salceda, B., Macbeth, D., Olguin, H., Dasch, G.A., Aranda, C.A., 2011. Rickettsia rickettsii in Rhipicephalus Ticks, Mexicali, Mexico. J. Med. Entomol. 48, 418–421.

Esteve-Gassent, M.D., Castro-Arellano, I., Feria-Arroyo, T.P., Patino, R., Li, A.Y., Medina, R.F., Pérez de León, A.A., Rodríguez-Vivas, R.I., 2016. Translating ecology, physiology, biochemistry, and population genetics research to meet the challenge of tick and tick-borne diseases in North America. Arch. Insect Biochem. Physiol. 92, 38–64.

FDA – Food and Drug Administration, 2013. Everything Added to Food in the United States. FDA – Food and Drug Administration (www.accessdata.fda.gov/ scripts/fcn/fcnNavigation.cfm?rpt=eafusListing Last access: January 2016).

Ferreira, L.L., Soares, S.F., de Oliveira Filho, J.G., Oliveira, T.T., de Leon, A.A.P., Borges, L.M.F., 2015. Role of Rhipicephalus microplus cheliceral receptors in gustation and host differentiation. Ticks Tick Borne Dis. 6, 228–233.

Gikonyo, N.K., Hassanali, A., Njagi, P.G.N., Gitu, P.M., Midiwo, J.O., 2002. Odor composition of preferred (Buffalo and Ox) and nonpreferred (Waterbuck) hosts of some savanna tsetse flies. J. Chem. Ecol. 28, 969–981.

Gikonyo, N.K., Hassanali, A., Njagi, P.G.N., Saini, R.K., 2003. Response of Glossina morsitans to blends of electroantennographically active compounds in the odor of its preferred (buffalo and ox) and nonpreferred (waterbucks) hosts. J. Chem. Ecol. 29, 2331–2345.

Guerrero, F.D., Pérez de León, A.A., Rodriguez-Vivas, R.I., Jonson, N., Miller, R.J., Andreotti, R., 2014. Acaricide research and development, resistance and resistance monitoring. In: Sonenshine, D.E., Roe, M. (Eds.), Biology of Ticks, vol.
2. Oxford University Press, Oxford and New York, pp. 353–381. Hansford, K.M., Pietzsch, M., Cull, B., Medlock, J.M., 2015. Brown dog tick infestation of a home in England. Vet. Rec. 176, 129–130.

Hart, B.L., 2000. Role of grooming in biological control of ticks. Ann. N.Y. Acad. Sci. 916, 565–569.

Hooker, W.A., Bishopp, F.C., Wood, H.P., 1912. The life history and bionomics of some North American ticks. USDA Bur. Ent. Bull. 106, 239.

Kumar, S., Prakash, S., Kaushik, M.P., Rao, K.M., 1992. Comparative activity of here repellents against the ticks Rhipicephalus sanguineus and Argas persicus. Med. Vet. Entomol. 6, 47–50.

Kuznetsova, A., Brockhoff, P.B., Christensen, R.H.B., 2015. lmerTest: Tests in Linear Mixed Effects Models. R package version 2. 0–29. http://CRAN.R-project.org/ package=lmerTest Last access: January 2016.

Little, S.E., Hostetler, J., Kocan, K.M., 2007. Movement of Rhipicephalus sanguineus adults between co-housed dogs during active feeding. Vet. Parasitol. 150, 139–145.

Logan, J.G., Birkett, M.A., Clark, S.J., Powers, S., Seal, N.J., Wadhams, L.J., 2008. Identification of human-derived volatile chemicals that interfere with attraction of Aedes aegypti mosquitoes. J. Chem. Ecol. 34, 308–322.

Logan, J.G., Seal, N.J., Cook, J.I., Stanczyk, N.M., Birkett, M.A., Clark, S.J., Gezan, S.A., Wadhams, L.J., Pickett, J.A., Mordue (Luntz), J.M., 2009. Identification of human-derived volatile chemicals that interfere with attraction of the scottish biting midge and their potential use as repellents. J. Med. Entomol. 46, 208–219.

Louly, C.C.B., Fonseca, I.N., Oliveira, V.F., Borges, L.M.F., 2006. Ocorrência de Rhipicephalus sanguineus em trabalhadores de clínicas veterinárias e canis do município de Goiânia. Go. Cienc. Anim. Bras. 7, 103–106.

Louly, C.C.B., Soares, S.F., Silveira, D.N., Neto, O.J., Silva, A.C., Borges, L.M.F., 2009. Differences in the susceptibility of two breeds of dogs, English cocker spaniel and beagle, to Rhipicephalus sanguineus (Acari: ixodidae). Int. J. Acarol. 35, 25–32.

Louly, C.C.B., Soares, S.F., Silveira, D.N., Guimarães, M.S., Borges, L.M.F., 2010. Differences in the behavior of Rhipicephalus sanguineus tested against resistant and susceptible dogs. Exp. Appl. Acarol. 51, 353–362.

Mencke, N., 2013. Future challenges for parasitology: vector control and 'One health' in Europe: the veterinary medicinal view on CVBDs such as tick borreliosis, rickettsiosis and canine leishmaniosis. Vet. Parasitol. 195, 256–271.

Miller, J.R., George, J.E., Guerrero, F., Carpenter, L., Welch, J.B., 2001. Characterization of acaricide resistance in Rhipicephalus sanguineus (Latreille, 1806) (Acari: ixodidae) collected from the corozal army veterinary quarantine center, Panama. J. Med. Entomol. 38, 293–302.

Mooring, M.S., Mckenzie, A.A., Art, B.L.H., 1996. Role of sex and breeding status in grooming and total tick load of impala. Behav. Ecol. Sociobiol. 39, 259–266.

Nava, S., Estrada-Pena,˜ A., Petney, T., Beati, L., Labruna, M.B., Szabó, M.P.J., Venzal, J.M., Mastropolo, M., Mangold, A.J., Guglielmone, A.A., 2015. The taxonomic status of Rhipicephalus sanguineus (Latreille, 1806). Vet. Parasitol. 208, 2–8.

Nielsen, B.L., Rampin, O., Meunier, N., Bombail, V., 2015. Behavioral responses to odors from other species: introducing a complementary model of allelochemics involving vertebrates. Front. Neurosci. 9, 1–11.

Oliveira Filho, J.G., Sarria, A.L.F., Ferreira, L.L., Caulfield, J.C., Powers, S.J., Pickett, J.A., Borges, L.M.F., 2016. Quantification of brown dog tick repellents, 2-hexanone and benzaldehyde, and release from tick-resistant beagles, Canis lupus familiaris. J. Chromatogr. B 1022, 1–6.

Otranto, D., Lia, R.P., Cantacessi, C., Galli, G., Paradies, P., Mallia, E., Capelli, G., 2005. Efficacy of a combination of imidacloprid 10% permethrin 50% versus fipronil 10% (S)-methoprene 12%, against ticks in naturally infected dogs. Vet. Parasitol. 130, 293–304.

Pérez de León, A.A., Strickman, D.A., Knowles, D.P., Fish, D., Thacker, E., de la Fuente, J., Krause, P.J., Wikel, S.K., Miller, R.S., Wagner, G.G., Almazán, C., Hillman, R., Messenger, M.T., Ugstad, P.O., Duhaime, R.A., Teel, P.D., Ortega-Santos, A., Hewitt, D.G., Bowers, E.J., Bent, S.J., Cochran, M.H., McElwain, T.F., Scoles, G.A., Suarez, C.E., Davey, R., Howell Freeman, J.M., Li, Lohmeyer. K., Guerrero, A.Y., Kammlah, F.D., Phillips, D.M., Pound, P., Group for Emerging Babesioses and One Health Research and Development in the U.S, 2010. One Health approach to identify research needs in bovine and human babesioses: workshop report. Parasit. Vectors 3, 36.

Pérez de León, A.A., Teel, P.D., Li, A., Ponnusamy, L., Roe, R.M., 2014. Advancing integrated tick management to mitigate burden of tick-borne diseases. Outlooks Pest Manage. 25, 382–389.

Pacheco, R.C., Moraes-Filho, J., Guedes, E., Silveira, I., Richtzenhain, L.J., Leite, R.C., Labruna, M.B., 2011. Rickettsial infections of dogs, horses and ticks in Juiz de Fora southeastern Brazil, and isolation of Rickettsia rickettsii from Rhipicephalus sanguineus ticks. Med. Vet. Entomol. 25, 148–155.

Pageat P., 2005. Allomone repulsive compositions for controlling arachnids. PCT/EP2003/007143.

Paz, G.F., Labruna, M.B., Leite, R.C., 2008. Drop off rhythm of Rhipicephalus sanguineus (Acari: ixodidae) of artificially infested dogs. Rev. Bras. Parasitol. Vet. 17, 139–144.

Pickett, J.A., Birkett, M.A., Dewhirst, S.Y., Logan, J.G., Omolo, M.O., Torto, B., Pelletier, J., Syed, Z., Leal, W.S., 2010. Chemical ecology of animal and human pathogen vectors in a changing global climate. J. Chem. Ecol. 36, 113–121.

R Core Team, 2014. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria, http://www.R-project.org/ (accessed 06.01.2016).

Renthal, R., Maghnani, L., Bernal, S., Qu, Y., Griffith, W., Lohmeyer, K., Guerrero, F., Borges, L.M.F., Pérez de León, A.A., 2016. The chemosensory appendage proteome of Amblyomma americanum (Acari: ixodidae) reveals putative odorant-binding and other chemoreception-related proteins. Insect Sci.

Rodríguez-Vivas, R.I., Apanaskevich, D.A., Ojeda-Chi, M.M., Trinidad-Martíneza, I., Ryes-Noveloc Esteve-Gassentd, M.D., Pérez de León, A.A., 2016. Ticks collected from humans, domestic animals, and wildlife in Yucatan, Mexico. Vet. Parasitol. 215, 106–113.

Saini, R., Andoke, J., Muasa, P., Mbuvi, D., Marete, T., Ngiela, J., Mbahin, N., Affogon, H., 2013. Cows in waterbuck clothing for protection against tsetse flies andenhanced productivity. In: Il'ichev, A.L., Cardé, R.T.D., Williams, G., Zalucki, M.P. (Eds.), Conference Program and Abstracts Handbook of International Chemical Ecology Conference. 19–23 August, 2013, Melbourne, p. 59.

Sigma Aldrich, 2015. Safety Data Sheet of 2- hexanone and benzaldehyde. Sigma Aldrich, http://www.sigmaaldrich.com/ (accessed 13.01.16).

Socolovicvhi, C., Bitam, I., Raoult, B., Parola, P., 2009. Transmission of Rickettsia conorii in naturally infected Rhipicephalus sanguineus. Clin. Microbiol. Infect. 15, 319–321.

Stanneck, D., Kruedewagen, E.M., Fourie, J.J., Horak, I.G., Davis, W., Krieger, K.J., 2012. Efficacy of an imidacloprid/flumethrin collar against fleas, ticks, mites and lice on dogs. Parasit. Vectors 5, 102.

Szabó, M.P.J., Mangold, A.J., Joao, C.F., Bechara, G.H., Guglielmone, A.A., 2005. Biological and DNA evidence of two dissimilar populations of the Rhipicephalus sanguineus tick group (Acari: ixodidae) in South America. Vet. Parasitol. 130, 131–140.

Szabó, M.P.J., Rossi, G.F., Cabral, D.D., Martins, M.M., Amorim, G.M.P., Tsuruta, S.A., 2012. Experimental evaluation of birds as disseminators of the cosmopolitantick Rhipicephalus sanguineus (Acari: ixodidae). Exp. Parasitol. 132, 389–393.

Troughton, D.R., Levin, M.L., 2007. Life cycles of seven ixodid tick species (Acari: ixodidae) under standardized laboratory conditions. J. Med. Entomol. 44, 732–740.

Vayssier-Taussat, M., Cosson, J.F., Degeilh, B., Eloit, M., Fontanet, A., Moutailler, S., Raoult, D., Sellal, E., Ungeheuer, M.N., Zylbermann, P., 2015. How a multidisciplinary 'One Health' approach can combat the tick-borne pathogen threat in Europe. Future Microbiol. 10, 809–818.

Weldon, P.J., 2010. Nuisance arthropods, nonhost odors, and vertebrate chemical aposematism. Nat. Wiss. 97, 443–448.

Weldon, P.J., 2013. Chemical aposematism. Chemoecology 23, 201–202.