Universidade Federal do Maranhão
Centro de Ciências Biológicas e da Saúde
Programa de Pós-Graduação em Ciências da Saúde
Doutorado

CONTROLE DOS PARASITOS DO CÃO E AVALIAÇÃO DO EFEITO CARRAPATICIDA DO CARVACROL

TÁSSIA LOPES DO VALE

São Luís
2023

TÁSSIA LOPES DO VALE

CONTROLE DOS PARASITOS DO CÃO E AVALIAÇÃO DO EFEITO CARRAPATICIDA DO CARVACROL

Tese apresentada ao Programa de Pós-Graduação em Ciências da Saúde da Universidade Federal do Maranhão como requisito para obtenção do título de Doutora em Ciências da Saúde.

.

Orientador: Profº. Drº Livio Martins Costa Junior.

São Luís
2023

Ficha gerada por meio do SIGAA/Biblioteca com dados fornecidos pelo(a) autor(a).
Diretoria Integrada de Bibliotecas/UFMA

Lopes do Vale, Tássia.
CONTROLE DOS PARASITOS DO CÃO E AVALIAÇÃO DO EFEITO CARRAPATICIDA DO CARVACROL / Tássia Lopes do Vale. - 2023.
117 p.

Orientador(a): Livio Martins Costa Junior.
Tese (Doutorado) - Programa de Pós-graduação em Ciências da Saúde/ccbs, Universidade Federal do Maranhão, São Luís, 2023.

1. Camundongos. 2. Carrapaticidas. 3. Isoxazolinas. 4. Monoterpenos. 5. R. sanguineus s.l. I. Martins Costa Junior, Livio. II. Título.

TÁSSIA LOPES DO VALE

CONTROLE DOS PARASITOS DO CÃO E AVALIAÇÃO DO EFEITO CARRAPATICIDA DO CARVACROL

Tese apresentada ao Programa de Pós-Graduação em Ciências da Saúde da Universidade Federal do Maranhão como requisito para obtenção do título de Doutora em Ciências da Saúde.

Aprovada em: 29/03/2023

BANCA EXAMINADORA

gov.br Documento assinado digitalmente
LIVIO MARTINS COSTA JUNIOR
Data: 30/05/2023 10:12:39-0300
Verifique em https://validar.iti.gov.br

_____

Profº. Drº Livio Martins Costa Junior (Orientador)
Universidade Federal do Maranhão

_____

1º Drº Caio Márcio de Oliveira Monteiro
Universidade Federal da Goiás

_____

2º Profº Drº Fábio Barbour Scott
Universidade Federal Rural do Rio de Janeiro

_____

3º Profº. Drª Denise Fernandes Coutinho
Universidade Federal do Maranhão

_____

4º Profº Drº Hermes Ribeiro Luz
Universidade Federal do Maranhão

“Não há fatos eternos, como não há verdades absolutas.”

Friedrich Nietzsche

Aos meus filhos, Maria Luíza e Carlos Alberto por serem a fonte da minha felicidade e amor. E ao Geovane, amigo e companheiro de trabalho...

Dedico

AGRADECIMENTOS

Em primeiro lugar, a Deus por ter permitido que essa jornada fosse iniciada e concluída.

Em segundo, a três pessoas muito especiais que cruzaram o meu caminho, pois sei que sem a ajuda deles a conclusão dessa etapa não seria possível, Naylene Carvalho, Geovane Ferreira e Jhone Costa, obrigada pela parceria infinita, vocês se tornaram minha família, não existe palavras que possam expressar o que sinto por vocês.

Ao meu orientador, professor Dr. Livio Martins Costa Junior, não só pelas oportunidades concedidas, mas por todas as palavras de incentivos, e principalmente por ter acreditado na minha capacidade, mesmo quando surgia os desânimos e incertezas. Gratidão por todo o tempo oferecido, paciência e ensinamentos científicos.

A minha família, que com todo o amor, compreendeu as minhas ausências, durante essa etapa profissional. Principalmente a minha filha Maria Luíza Carvalhal, que tão pequena soube incentivar para que eu vencesse todos os desafios e buscasse sempre o crescimento profissional, mesmo que isso custasse a minha presença. Ao meu filho Carlos Alberto Carvalhal Neto, o meu presente no final do doutorado, que me ensinou sobre o tempo. E, especialmente, ao meu marido Carlos Carvalhal Junior, que em muitos momentos foi responsável por suprir minha ausência.

Agradeço aos meus pais, sogros e minhas irmãs Janne Lopes e Thayanny do Vale pelas orações, conselhos e incentivos. Amar e ser amado faz a vida ser suportada de todas as formas.

Com o coração muito grato, gostaria de agradecer à família LCP. Principalmente à Carolina Rocha, Isabella Chaves, Aldilene Lima, Davi, Ellen Vale, Henrique Costa Junior, Arnon, Dauana Mesquita, Caio Tavares e demais, pois a família é numerosa. Obrigada pela amizade, risadas e companhia.

Ao Matheus Nobate, por toda a parceria, jamais me deixando sozinha nos momentos que mais precisei.

À Irlla Licá, pela amizade construída durante o isolamento da pandemia do COVID19, o apoio nos experimentos e principalmente na vida fora da pós-graduação.

Ao Dr. Hermes Luz, pelos ensinamentos e a sua alegria.

À dona Maria Raimunda Costa, Maria Dinalva Nogueira e Sônia Maria Sousa, pelo café de todos os dias adoçado com amor, boas conversas e acolhimento.

Aos senhores Josuel Araújo e Hilton Mota, por me receberem todos os dias no prédio da Pós-graduação com uma palavra amiga e um sorriso no rosto.

À FAPEMA pela bolsa concedida.

Ao Programa de Pós-graduação em Ciências da Saúde, que muito contribuiu na minha formação.

E por fim, aos animais que foram uma parte fundamental da minha vida profissional e científica. Gratidão.

RESUMO

Rhipicephalus sanguineus sensu lato (s.l.) é um carrapato importante para a saúde animal e humana, sendo vetores de vários patógenos. Diante deste cenário, é essencial o controle desse ectoparasito, que é realizado essencialmente com moléculas sintéticas. Todavia, apesar dos *guidelines* sobre como realizar o controle de parasitos, muitas vezes essas moléculas são utilizadas de forma errônea, além de a maioria ter uma toxicidade elevada para os mamíferos e haver relatos de seleção de carrapatos resistentes. Nesse contexto, o uso de moléculas naturais, como os terpenos, são uma ferramenta importante para o controle de carrapatos. Ademais, as informações sobre as recomendações para o controle de parasitos no cão pelos médicos veterinários no Brasil são escassas. Assim, os objetivos deste estudo foram investigar como o médico veterinário recomenda o controle de parasitos de cães no Brasil e avaliar o efeito carrapaticida do carvacrol sobre *R. sanguineus* s.l e sua capacidade de alterar a morfologia do tegumento de ninfas. Para o estudo investigativo sobre os métodos de controles de parasitos dos cães usados no Brasil, foram aplicados questionários on-line para médicos veterinários de todo o Brasil. As questões abordaram os métodos de controle para helmintos e ectoparasitos, bem como medicamentos, frequência de administração e percepção de ineficácia dos medicamentos. Entre esses veterinários, 71,4% (n = 288) afirmaram que os proprietários estão preocupados com o controle de parasitos. O tratamento preventivo contra ectoparasitos caninos é o mais recomendado pelos veterinários, e 46,6% (n = 188/403) trocaram com frequência a classe química do antiparasitário. A profilaxia para ectoparasitos com regime mínimo mensal (sistêmico, tópico e/ou coleiras) foi recomendada por 21,5% dos veterinários (n = 87/403). Esses resultados mostram que o uso indiscriminado de compostos antiparasitários e a percepção sobre a ineficácia exigem a necessidade de orientações para tratamento e controle de parasitoses caninas nas clínicas veterinárias. Melhorar a conscientização do tutor do cão requer a atenção dos veterinários e um esforço conjunto de todos os profissionais para executar estratégias eficazes no controle de parasitos. Adicionalmente, investigamos o efeito carrapaticida *in vitro* e *in vivo* do composto natural carvacrol, através de bioensaio *in vitro* sobre larvas e ninfas de *R. sanguineus* s.l. e da administração oral em camundongos, buscando alterações sobre o tegumento das ninfas de *R. sanguineus* s.l. após o tratamento. Para encontrar a concentração letal média CL_{50} foi realizado a imersão de larvas e ninfas em carvacrol sob diferentes concentrações. Já o bioensaio *in vivo* foi realizado mediante administração oral do carvacrol em camundongos Swiss infestados artificialmente com ninfas de *R. sanguineus* s.l. Os animais foram alocados em três grupos de acordo com o tratamento: controle negativo (Sorbitol), carvacrol (60 mg/kg) e controle positivo (Lotilaner 20 mg/kg). Os resultados do bioensaio *in vitro* demonstraram que carvacrol apresentou uma CL_{50} de 0,94 mg/mL para imersão de larvas e 1,81 mg/mL para ninfas. No bioensaio *in vivo*, o carvacrol exibiu, em 48h após o tratamento, uma mortalidade das ninfas de 17%. Foram observadas alterações histológicas no tegumento das ninfas que se alimentaram dos animais tratados com carvacrol (60 mg/mL), pois as células cúbicas da epiderme apresentavam uma possível agregação de grânulos de proteínas entre a camada epitelial e subcuticular. Nosso estudo demonstrou pela primeira vez que o carvacrol administrado através da via oral induz alterações no tegumento, podendo interferir no desenvolvimento do carrapato *R. sanguineus* s.l.

Palavras-chave: carrapaticidas; camundongos; *R. sanguineus* s.l.; monoterpenos e isoxazolinas.

ABSTRACT

Rhipicephalus sanguineus sensu lato (s.l.) is an important tick for animal and human health, being vectors of several pathogens. Given this scenario, it is essential to control this ectoparasite, which is essentially carried out with synthetic molecules. However, despite the guidelines on how to control parasites, these molecules are often used incorrectly, in addition to the fact that most have high toxicity for mammals and there are reports of selection of resistant ticks. In this context, the use of natural molecules, such as terpenes, is an important tool for tick control. Furthermore, information on recommendations for the control of parasites in dogs by veterinarians in Brazil is scarce. Thus, the objectives of this study were to investigate how the veterinarian recommends the control of parasites in dogs in Brazil and to evaluate the acaricidal effect of carvacrol *on R. sanguineus* s.l and its ability to alter the morphology of the tegument of nymphs. For the investigative study on the methods of controlling parasites in dogs used in Brazil, online questionnaires were applied to veterinarians throughout Brazil. The questions addressed control methods for helminths and ectoparasites, as well as medications, frequency of administration, and perception of medication ineffectiveness. Among these veterinarians, 71.4% (n = 288) stated that owners are concerned about parasite control. Preventive treatment against canine ectoparasites is the most recommended by veterinarians, and 46.6% (n = 188/403) frequently changed the chemical class of the antiparasitic. Prophylaxis for ectoparasites with a minimum monthly regimen (systemic, topical, and/or collars) was recommended by 21.5% of veterinarians (n = 87/403). These results show that the indiscriminate use of antiparasitic compounds and the perception of their ineffectiveness require guidelines for treating and controlling canine parasitic infections in veterinary clinics. Improving dog owner awareness requires the attention of veterinarians and a concerted effort by all professionals to implement effective parasite control strategies. Additionally, we investigated the in vitro and in vivo acaricidal effect of the natural compound carvacrol, through an *in vitro* bioassay on larvae and nymphs of *R. sanguineus* s.l. and oral administration in mice, looking for alterations on the tegument of the nymphs of *R. sanguineus* s.l. after treatment. To find the average lethal concentration CL_{50}, larvae, and nymphs were immersed in carvacrol at different concentrations. The *in vivo* bioassay was performed by oral administration of carvacrol in Swiss mice artificially infested with *R. sanguineus* s.l. nymphs. The animals were allocated into three groups according to treatment: negative control (Sorbitol), carvacrol (60 mg/kg), and positive control (Lotilaner 20 mg/kg). The results of the *in vitro* bioassay showed that carvacrol had a LC_{50} of 0.94 mg/mL for immersion larvae and 1.81 mg/mL for nymphs. In the *in vivo* bioassay, carvacrol exhibited, 48h after treatment, nymph mortality of 17%. Histological alterations were observed in the tegument of the nymphs that fed on the animals treated with carvacrol (60 mg/mL), as the cubic cells of the epidermis showed a possible aggregation of protein granules between the epithelial and subcuticular layer. Our study demonstrated for the first time that carvacrol administered orally induces changes in the tegument, which may interfere with the development of the tick *R. sanguineus* s.l.

Keywords: acaricides; mice; *R. sanguineus* s.l.; monoterpenes and isoxazolines.

SUMÁRIO

LISTA DE TABELAS

Capítulo 1.

LISTA DE FIGURAS

Capítulo 1.

Capítulo 2.

LISTA DE ABREVIATURAS E SIGLAS

ABINPET	Associação Brasileira da Indústria de Produtos para Animais de Estimação
EUA	Estados Unidos da América
Rhipicephalus sanguineus s.l	*Rhipicephalus sanguineus sensu lato*
Rhipicephalus sanguineus s.s	*Rhipicephalus sanguineus sensu stricto*
Fig.	Figura
LM	Lactona macrocíclica
PS	Piretróide sintético
GABA	Ácido gama aminobutírico
AChE	Acetilcolinesterase
OMS	Organização mundial de saúde
WHO	Word Health Organization
APRD	Arthropod Pesticide Resistance
IRAC	Comitê de Ação à Resistência a Inseticidas
OEs	Óleos essenciais

Capítulo 1

CAPC	Companion Animal Parasite Council
ESCCAP	European Scientific Council Companion Animal Parasites
TroCCAP	Tropical Council for Companion Animal Parasites
BCSAC	Brazilian Conference of Small Animals Clinic
IBGE	Brazilian Institute of Geography and Statistics

Capítulo 2

GRAS	Generally Recognized As Safe
FDA	Food and Drug Administration
CEUA	Comissão de Ética no Uso de Animais
%	Porcentagem
mL	Mililitro
BIL	Bioensaio de imersão larval
BIN	Bioensaio de imersão de ninfas

UR%	Umidade Relativa %
3R’s	Redução, substituição e refinamento
UFMA	Universidade Federal do Maranhão
UESC	Universidade Estadual de Santa Cruz
Neg	Negativo
Carv	Carvacrol
Pos	Positivo
n amostral	Número de animais incluídos nos grupos
GT	Grupo tratado
GC	Grupo controle
$CL_{50\%}$	Concentração letal média a 50%
IC 95%	Intervalo de confiança de 95%
R^2	Coeficiente de Correlação de Regressão
HE	Hematoxilina e eosina
ep	epitélio
epc	epicutícula
exc	procutícula
n	núcleo
va	vacúolos

1. INTRODUÇÃO

O crescimento populacional e industrial foram os principais responsáveis pelas mudanças ambientais, que promoveram a expansão de vetores de doenças, a dispersão de patógenos e uma maior proximidade na relação homem-animal-patógeno. À vista disso, uma abordagem *One Health* que tem como estratégia mundial reconhecer a ligação entre a saúde pública, animal e ambiental é primordial para vigilância e controle de doenças (BOWSER; ANDERSON, 2018), principalmente no Brasil, país que possui uma população canina de aproximadamente 52,2 milhões, perdendo apenas para os Estados Unidos da América (ABINPET, 2019). Todavia, essa proximidade expõe os tutores e quem tem contato com esses animais a diversos parasitos, uma vez que podem ser transmitidos direta ou indiretamente através dessa proximidade (OVERGAAUW *et al.*, 2020).

Em virtude do potencial zoonótico desses parasitos altamente prevalentes, o controle parasitário é fundamental à saúde humana e animal (CHOMEL, 2011). Um exemplo é o carrapato *Rhipicephalus sanguineus* sensu lato, principal ectoparasito que acomete o cão, podendo parasitar outros mamíferos domésticos e silvestres, e o homem. Além disso, *R. sanguineus s.l.* também é vetor de *Babesia canis, Babesia vogeli, Hepatozoon canis, Mycoplasma haemocanis, Erlichia canis, Rickettsia rickettsii* e *Rickettsia conorii* (DANTAS-TORRES, 2006, 2010, 2008; DEMMA *et al.*, 2005; GRAY *et al.*, 2013; RODRÍGUEZ-VIVAS *et al.*, 2016; SILVA et al., 2017).

Rhipicephalus sanguineus s.l. é um problema à saúde dos cães; e o controle desse carrapato deve ser realizado de forma racional, de modo que um conjunto de fatores deve ser investigado e levado em consideração, como sua presença no ambiente, seu ciclo epidemiológico, além do princípio ativo mais adequado frente à eficácia e ao desenvolvimento de resistência aos carrapaticidas (ALHO *et al.*, 2018). O Brasil é um país de grandes dimensões e diferentes realidades socioeconômicas e sanitárias, dificultando o controle do carrapato do cão. Até então, apesar do *Guideline* da Trocap para controle de parasitos em países tropicais (DANTAS-TORRES *et al.*, 2020), os dados sobre como esse controle de parasitos é recomendando pelos médicos veterinários eram desconhecidos. Esse dado é crucial para corroborar a instrução do uso racional dos produtos carrapaticidas, principalmente diante dos relatos de populações de *R. sanguineus* s.l. resistentes às diferentes bases químicas (BORGES *et al*., 2007; RODRÍGUEZ-VIVAS *et al.*, 2016).

Neste sentido, nosso grupo de pesquisa busca formulações que promovam uma maior eficácia e ainda possam aumentar a vida útil dos compostos sintéticos. E uma fonte para alcançar esse objetivo é o uso de terpenos os quais já demonstraram a eficiência carrapaticida *in vitro* (MONTEIRO *et al.*, 2009; SOUSA *et al.*, 2022; VALE *et al.*, 2021). Alguns óleos essenciais (EOs) já tiveram sua capacidade repelente comprovada através de estudo *in vivo* em cães, como o EOs de açafrão (GOODE et al., 2018). Já para monoterpenos que são moléculas extraídas de EOs, uma microemulsão de timol e eugenol reduziu a carga parasitária de larvas de *R. sanguineus* s.l. em cães (MONTEIRO *et al*., 2021). Apesar dos estudos que documentam a atividade carrapaticida de monoterpenos, ainda podemos considerar que os ensaios *in vivo* são escassos (NWANADE *et al*., 2020).

Os estudos *in vivo* em cães têm se tornado cada vez mais inviáveis devido aos custos e às mudanças na sociedade, portanto é necessária a busca por novos modelos. E uma alternativa encontrada para avaliar os produtos carrapaticidas foi a utilização de modelos experimentais, como ratos. Esses animais foram infestados com *Amblyomma americanum* para avaliar a atividade carrapaticida de diversas moléculas sintéticas, o que, no final, foi considerado um teste *in vivo* robusto (GUTIERREZ *et al.*, 2006). Assim, outro modelo animal que pode ser promissor é o camundongo da linhagem Swiss, já amplamente utilizado na pesquisa científica para avaliar as diferentes atividades de terpenos como o carvacrol. Esse modelo já foi empregado com carvacrol em estudos com bactérias como *Campylobacter jejuni* e o trematódeo *Schistosoma mansoni* (Mousavi *et al.*, 2020; Xavier *et al.*, 2022). O camundongo da linhagem Swiss pode ser um modelo para teste carrapaticida *in vivo* para *R. sanguineus* s.l. e até mesmo para outras espécies de carrapato, como *Amblyomma* spp., sendo necessário padronizar esse tipo de teste, devido às especificidades dos carrapatos e do modelo animal.

Diante do exposto, esta pesquisa visa a: 1) Investigar como é realizado o controle de parasitos em cães no Brasil; 2) Subsidiar informações sobre quais as moléculas mais recomendadas pelos médicos veterinários brasileiros para o controle de parasitos em cães; 3) Propor um novo bioensaio em camundongos Swiss como teste de eficácia *in vivo*; e 4) Avaliar a eficácia carrapaticida e as alterações histológicas provocadas por carvacrol e molécula convencional sobre o tegumento do carrapato do cão.

2. REFERENCIAL TEÓRICO

2.1 *Rhipicephalus sanguineus*

Os carrapatos são uma causa comum de desordens pruriginosas, importantes não só pela elevada casuística, mas também pelo sofrimento causado ao hospedeiro e pelo potencial zoonótico de alguns destes (DANTAS-TORRES; OTRANTO, 2011; EISEN *et al.*, 2018). Os Ixodídeos são os principais vetores de patógenos que causam doença aos animais e aos humanos. Neste contexto, está inserido o complexo *Rhipicephalus sanguineus*, que possuem ampla distribuição e são responsáveis pela transmissão de patógenos como protozoários e bactérias (DANTAS-TORRES; OTRANTO, 2015; WALKER *et al.*, 2000).

O carrapato marrom do cão, *R. sanguineus* sensu stricto (s.s.), foi descrito pela primeira vez em 1806 por Latreille como *Ixodes sanguineus*. Todavia, apesar da sua ampla distribuição global, a identificação de carrapatos considerados com *R. sanguineus* vem sendo questionada devido ao progresso dos estudos em filogenia e às inúmeras diferenças morfológicas e biológicas. Até bem pouco tempo atrás, a comunidade científica ainda não havia chegado a um consenso sobre sua classificação taxonômica, sendo proposto que o nome específico para *R. sanguineus* s.s. não fosse utilizado (DANTAS-TORRES; OTRANTO, 2015; NAVA *et al.*, 2015; SANCHES *et al.*, 2016).

Rhipicephalus sanguineus s.s. foi considerado o único táxon por mais de dois séculos (NAVA *et al.*, 2015), formando um grupo representado por mais de 11 espécies, que inclui *Rhipicephalus sulcatus* Neumann, 1908, *Rhipicephalus rossicus* Yakimov e Kohl-Yakimov, 1911, *Rhipicephalus schulzei* Olenev, 1929, *Rhipicephalus pumilio* Schulze, 1935, *Rhipicephalus pusillus* Gil Collado, 1936, *Rhipicephalus turanicus* Pomerantzev, 1940, *Rhipicephalus leporis* Pomerantzev, 1946, *Rhipicephalus guilhoni* Moreland Vassiliades, 1963, *Rhipicephalus moucheti* Morel, 1965, *Rhipicephalus bergeoni* Morel e Balis, 1976 e *Rhipicephalus camicasi* Morel, Mouchet e Rodhain, 1976 (PEGRAM *et al.*, 1987). Recentemente a identidade de *R. sanguineus* s.s. foi consolidada pela publicação de detalhes morfológicos e genéticos através das descrições de todos os ínstares, pela deposição de material de referência em coleções de museus respeitáveis e pela fixação de um neótipo (NAVA *et al.*, 2018).

O estabelecimento do neótipo de *R. sanguineus s.s.* sugere ainda que os carrapatos pertencentes a esse grupo sejam denominados *R. sanguineus* sensu lato (s.l.) (DANTAS-TORRES; OTRANTO, 2015; NAVA *et al.*, 2015), uma vez que populações

de carrapatos de diferentes morfologias, e até mesmo incompatíveis biologicamente e geneticamente, foram chamadas de *R. sanguineus* s.s. Assim, a aplicação do nome *R. sanguineus* s.l. deve ser utilizada a todo o complexo de espécies desse grupo (NAVA *et al.*, 2015; ŠLAPETA et al., 2021).

Rhipicephalus sanguineus s.l. é conhecido por duas linhagens distintas: uma encontrada em climas temperados, denominada de Temperada; outra que representa populações tropicais e subtropicais, que seria a linhagem Tropical (MORAES-FILHO *et al.*, 2011). Essa taxonomia também vem sendo questionada, e novos estudos foram divulgados a respeito dessa classificação (ŠLAPETA *et al.*, 2021). Do ponto de vista empírico, essa discriminação parece ser irrelevante; todavia, essa precisão sobre as espécies é determinante na compreensão da ecologia, epidemiologia e controle dos patógenos transmitidos por esses carrapatos (GRAY *et al.*, 2013).

2.1.1 Morfologia e aspectos biológicos

Popularmente chamado carrapato marrom do cão, *R. sanguineus* s.l., possui rostro curto com capítulo de base hexagonal. Não apresenta ornamentação, com palpos cônicos, olhos e festões estão presentes. Coxa I é profunda com as placas fendidas e espiraculares em forma de vírgula nos machos, escudo estreito na parte anterior com posterior aumento na região dos olhos. Os machos possuem placas adanais alongadas e subtriangulares, em número par, largas em seu aspecto posterior e ocasionalmente arredondadas (DANTAS-TORRES, 2008).

As fêmeas de *R. sanguineus* s.l. apresentam escudo que recobre metade da superfície dorsal do corpo, com o capítulo largo e longo, com a base apresentando ângulos laterais acentuados e áreas porosas pequenas. Os palpos são mais longos, estreitamente arredondado apicalmente. Os olhos, dispostos nos ângulos laterais, são medianamente convexos, geralmente margeados com algumas pontuações dorsalmente (COIMBRA-DORES *et al.*, 2016; WALKER et al., 2000). Antes da alimentação as fêmeas adultas se assemelham aos machos em tamanho (2,4–2,7 mm de comprimento por 1,44–1,68 mm de largura), forma e cor. Ao se alimentar de sangue, a fêmea aumenta vertiginosamente de tamanho, podendo alcançar até 11,5 mm de comprimento por 7,5 mm de largura (DANTAS-TORRES, 2008). Esse tamanho depende da origem do carrapato, pois estudos morfométricos demonstraram que a linhagem tropical apresenta

carrapatos fêmeas de porte menor quando comparadas ao grupo temperado, em relação ao comprimento e largura do idiossoma (SANCHES *et al.*, 2016).

Rhipicephalus sanguineus s.l. possui um ciclo biológico do tipo trioxeno e geotrópico negativo, sendo endofílica (adaptada a viver em espaços interiores), monotrópica (todos os estádios de desenvolvimento alimentam-se das mesmas espécies de hospedeiros), heteroxênica (têm necessidade por três hospedeiros após a muda) e o período de alimentação depende de cada estágio (BARROS-BATTESTI *et al*., 2006; BECHARA *et al.*, 1995; DANTAS-TORRES, 2010) (Figura 1A).

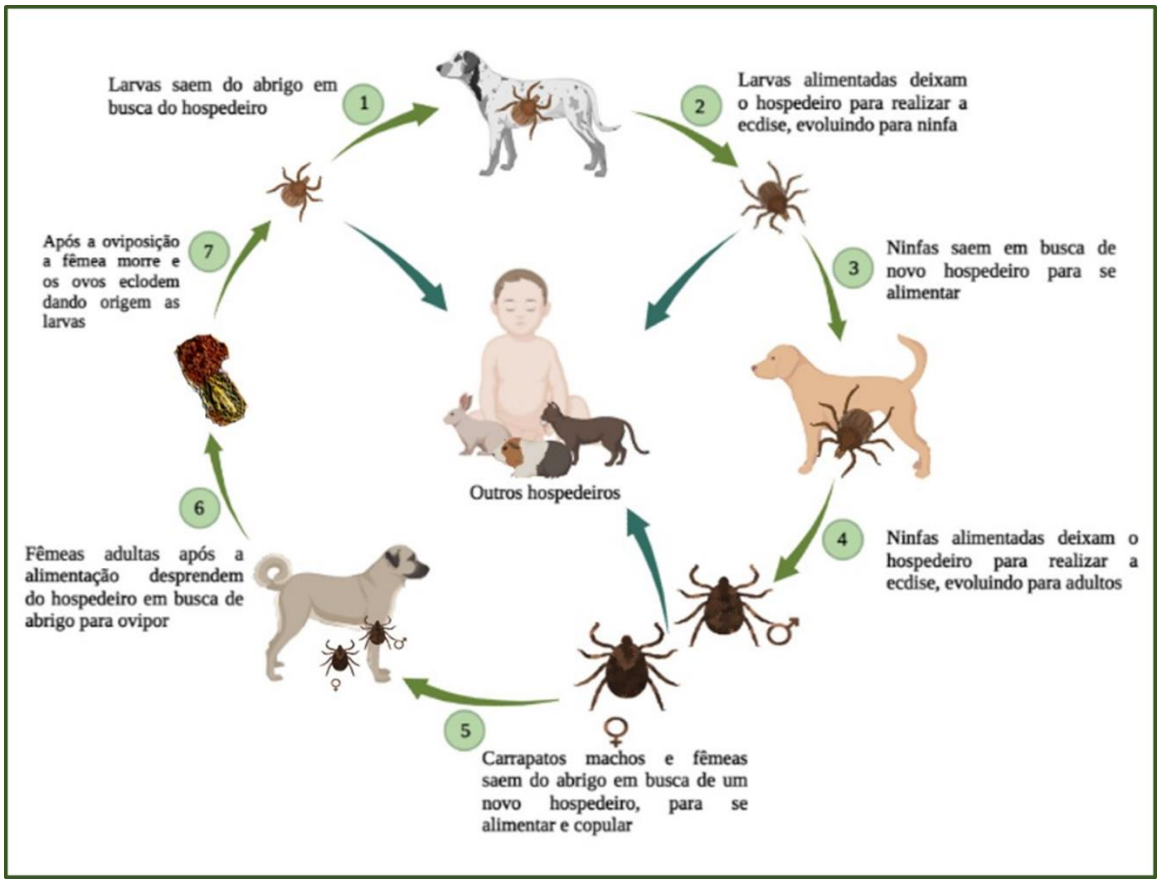

Figura 1. Representação esquemática do ciclo de vida de *R. sanguineus s.l.* Criado com BioRender.com

As larvas e ninfas ficam escondidas nas frestas e buracos saem em busca do hospedeiro e, após a fixação, se alimentam durante um período de três a onze dias, até terminar o repasto sanguíneo. As larvas são pequenas (0,54 mm de comprimento; 0,39 mm de largura) e têm apenas três pernas. As ninfas têm quatro pares de pernas, bastante semelhantes aos adultos, porém menores (1,14 mm a 1,3 mm de comprimento; 0,57 mm a 0,66 mm de largura) e sexualmente imaturas, com ausência da abertura genital. Após a

alimentação, esses estágios se desprendem do hospedeiro e saem em busca de um novo abrigo para realizar a ecdise (DANTAS-TORRES, 2008; ESTRADA-PEÑA; SANCHEZ, 1988).

As fêmeas se alimentam durante um período de cinco a dez dias e os machos até por quinze dias. Antes da alimentação, as fêmeas são bastante semelhantes aos machos em tamanho e cor, exceto pelo escudo incompleto (COOLEY, 1946; DANTAS-TORRES, 2008). Assim como as larvas e ninfas, as fêmeas desprendem-se do hospedeiro em busca de abrigo; no caso delas, para depositar os seus ovos, em média 4.000 ovos, morrendo após a postura (KOCH, 1982) (Fig. 1B). Diferentemente, os machos podem realizar alimentação em mais de um cão, consumindo sangue para o processo de espermatogênese (GRAY *et al.*, 2013; LITTLE *et al*., 2007).

Figura 2. Fêmea ingurgitada de *Rhipicephalus sanguineus* s.l. (A) em processo de ovoposição (B).

Os carrapatos podem ser encontrados por toda a superfície corporal, tendo preferência pelas zonas ventrais e zonas com pele fina, como face, orelhas, axilas e regiões interdigital, inguinal e perianal. Os adultos possuem preferência por locais de difícil acesso ao hospedeiro, como as orelhas; já as larvas e ninfas são encontradas na região ventral e membros inferiores (DANTAS-TORRES; OTRANTO, 2011).

2.1.2 Importância médica e médica-veterinária

Os cães são os principais hospedeiros do carrapato *R. sanguineus* s.l., e possivelmente responsáveis pela manutenção de grandes populações de carrapatos (ESCCAP, 2022; MAURELLI *et al.*, 2018). Apesar de *R. sanguineus* s.l. possuir monotropismo pelo cão, pode parasitar outras espécies de mamíferos, incluindo o ser humano (DANTAS-TORRES, 2010; DANTAS-TORRES *et al*., 2006; RODRÍGUEZ-

VIVAS *et al*., 2016). Esse evento está intimamente relacionado com a linhagem do carrapato, uma vez que o parasitismo em humanos por *R. sanguineus* s.l. é mais comum na Europa do que em países da América do Sul (GUGLIELMONE *et al.*, 2006; PAROLA *et al.*, 2008). Todavia, isso também pode estar relacionado com a explosão populacional de *R. sanguineus* s.l., causando altos níveis de infestação ambiental e, consequentemente, facilitando o contato docarrapato com hospedeiros não preferenciais (DANTAS-TORRES, 2008).

O parasitismo por *R. sanguineus* s.l. é uma causa comum de desordens para a saúde do hospedeiro, não só pelo prurido, mas também pelo sofrimento do animal e pelo potencial zoonótico de alguns destes (DANTAS-TORRES, 2010; PALMAS *et al*., 2001). De fato, as doenças transmitidas por carrapatos, são causadas principalmente por *Anaplasma platys*, *Babesia canis vogeli*, *Babesia gibsoni*, *Ehrlichia canis* e *Rickettsia* spp., e as doenças provocadas por esses patógenos apresentam elevadas morbidade e mortalidade em cães em todo o mundo (DANTAS-TORRES, 2010, 2008; SOLANO-GALLEGO; BANETH, 2011; TROTTA *et al.*, 2012).

A realidade é que as doenças transmitidas por carrapatos são comuns, acometendo não somente os cães, mas também outras espécies, incluindo o homem (DANTAS-TORRES; CHOMEL; OTRANTO, 2012; DURON *et al.*, 2015b; PAROLA *et al.*, 2008). Para evitar os danos provocados pelos carrapatos à saúde humana e animal, é necessário que se estabeleça o controle e a prevenção desses artrópodes. Todavia esse é um trabalho árduo, diante do desenvolvimento da resistência pelos carrapatos e a consequente diminuição da eficácia de moléculas carrapaticidas (BORGES et al., 2007; BECKER *et al.*, 2019; KLAFKE *et al.*, 2017

2.1.3 Controle do carrapato do cão

O método mais comum de controle de carrapatos em cães é a aplicação de carrapaticidas (VALE *et al*., 2022; BORGES *et al*., 2011). As moléculas carrapaticidas atuam no sistema nervoso do carrapato, com mecanismos de ações diversos. Assim, os organofosforados (OP) e os carbamatos são inibidores da acetilcolinesterase, os piretróides sintéticos (PS) são moduladores dos canais de sódio, amidinas são agonistas de octopamina, os neonicotinóides são agonistas da acetilcolina, as lactonas macrocíclicas (LM) ativam os canais de cloro, os organoclorados e fenilpirazóis são antagonistas dos canais de cloro controlados pelo ácido gama-

aminobutírico (GABA) e as isoxazolinas bloqueiam os canais de cloro dependentes de ligante tanto dos receptores de GABA como de glutamato (ABBAS *et al.*, 2018; OZOE *et al.*, 2010; ZHAO; GAMMIE, 2014).

Os carrapaticidas comumente utilizados são eficazes para eliminar infestações por carrapatos e prevenir reinfestações durante um certo período (DE LA FUENTE, 2018). Porém, o uso prolongado dessas moléculas seleciona populações de *R. sanguineus* s.l. resistentes às diferentes bases químicas (BECKER *et al.*, 2019; KLAFKE *et al.*, 2017). Segundo o banco de dados "Arthropod Pesticide Resistance" (APRD) (MOTA-SANCHEZ; WISE, 2020), populações de *R. sanguineus* s.l. foram consideradas resistentes a 5 grupos de moléculas, tais como amitraz, BHC/ciclodienos, DDT, organofosforados e permetrina.

O primeiro relato de resistência de *R. sanguineus* s.l. ocorreu em 1954: ao Dieldrim, do grupo dos organoclorados, atualmente proibidos em diversos países devido a sua alta toxicidade (BRASIL, 2009; BROWN; PAL, 1958; WHITEHEAD, 2005). Bioensaios realizados no Brasil também já detectaram cepas de *R. sanguineus* s.l. resistentes a cipermetrina, deltametrina e coumafós (BORGES, *et al.*, 2007). Já para *R. sanguineus* s.s. do Rio Grande do Sul, foi detectada resistência a deltametrina e fipronil (BECKER *et al.*, 2019).

A resistência aos carrapaticidas é um tema bastante relevante, principalmente no que diz respeito às dificuldades e aos gastos envolvidos no desenvolvimento de novos moléculas (ABBAS *et al.*, 2018). Para uma boa compreensão sobre o tema, é importante descrever o conceito sobre resistência, que é a seleção de um traço (ou traços) hereditário(s) específico(s) em uma população de artrópodes, responsável pela sobrevivência de uma população a uma dose de acaricida. A Organização Mundial de Saúde (OMS) encontrou três formas de classificar a resistência, como a identificação dos genes subjacentes que conferem o traço de resistência herdada, mensuração de susceptibilidade quando submetido à dose padrão e resistência levando a falha no controle (COLES; DRYDEN, 2014)

Os mecanismos envolvidos no desenvolvimento da resistência podem ser relacionados à sensibilidade metabólica, comportamental e de penetração cuticular do sítio alvo (FERRARI, 1996; WHO, 1970). Um exemplo do mecanismo de resistência da via metabólica foi compreendido através de uma cepa de *R. sanguineus* s.l. que apresentou esterases aumentadas quando em contato com piretróides. Portanto, é imprescindível que o médico veterinário possua o conhecimento técnico das classes

químicas e dos mecanismos de ação, recomendando a rotação de moléculas com diferentes mecanismos de ação, com o intuito de evitar a seleção de carrapatos resistentes (COLES; DRYDEN, 2014; IRAC, 2012). Entretanto, poucos estudos no Brasil tiveram a intenção de conhecer como os veterinários clínicos indicam o tratamento antiparasitário.

Diante dos casos de resistência e a dificuldade no desenvolvimento de novas moléculas, a maioria das pesquisas ao longo da última década vem enfatizando que o uso de moléculas de origem vegetal pode ser uma opção promissora para o controle de carrapatos (ARAÚJO *et al.*, 2016; CRUZ *et al.*, 2013; NEVES *et al.*, 2011; PEIXOTO *et al.*, 2015). As plantas aromáticas que produzem óleos essenciais (OEs) possuem componentes aromáticos proporcionadores de odor, sabor ou aroma distintivo de uma planta. Os OEs são subprodutos do metabolismo das plantas e proporcionam várias funções para as plantas, como atração ou repelência de insetos e defesa contra patógenos (KOUL *et al*., 2008).

Nessa perspectiva dos OEs, diversas pesquisas foram realizadas e comprovaram que as moléculas provenientes de OEs possuem ação carrapaticida (ARAÚJO *et al.*, 2016; MONTEIRO *et al.*, 2009; NOVATO *et al.*, 2015). Os OEs apresentam principalmente componentes pertencentes à classe dos terpenos, incluindo os monoterpenos e sesquiterpenos; o segundo formado por moléculas aromáticas e alifáticas, conhecidas como fenilpropanóides (BAKKALI *et al.*, 2008). Outra vantagem em relação a essas moléculas diz respeito a sua associação de monoterpenos com moléculas sintéticas capazes de reduzir a concentração de moléculas sintéticas, o que pode aumentar a penetrabilidade do composto sintético no carrapato (TAVARES *et al.*, 2022).

2.2 Uso do Carvacrol para o controle de carrapatos

Carvacrol (2-metil-5-(1-metiletil)-fenol) é um monoterpeno, isômero do timol, produzido por diversas plantas aromáticas das famílias Lamiaceae e Verbaneceae, incluindo *Lippia triplinervis* (LAGE *et al*., 2013), *Lippia. gracilis* (CRUZ *et al.*, 2013), *Origanum onites* (COSKUN *et al.*, 2008), e *Origanum. bilgeri* (KOC *et al.*, 2013) (Fig. 3). Essa molécula apresenta forte odor e, por isso, é utilizada como aromatizante e conservante natural na alimentação humana. Além da utilização do carvacrol como ingrediente alimentar, diversas pesquisas já foram realizadas para avaliar a atividade

biológica, farmacológica e toxicológica do carvacrol (SUNTRES; COCCIMIGLIO; ALIPOUR 2015).

Figura 3. Estrutura molecular do Carvacrol a partir do *Origanum onites.*

Pesquisas recentes têm revelado a atividade do carvacrol sobre diversos artrópodes (GLAVAN *et al.*, 2020; LI *et al.*, 2021), principalmente como carrapaticida, apresentando essa atividade em diferentes espécies e fases de vida, como *Amblyomma sculptum, Rhipicephalus microplus, R. sanguineus* s.l., *Ixodes scapularis* e *Amblyomma americanum* (CARDOSO *et al.*, 2020; DOLAN *et al.*, 2009; LIMA DE SOUZA *et al.*, 2019; VALE *et al.*, 2021). O mecanismo de ação do carvacrol ainda não foi elucidado, no entanto alguns estudos propõem que o carvacrol é um inibidor da acetilcolinesterase (AChE) (CARDOSO *et al.*, 2020). A AChE é uma enzima responsável pela hidrólise da acetilcolina e pode ser usada como marcador da função colinérgica (GAUTHIER; GRÜNEWALD, 2012).

Outras pesquisas visam a entender como o carvacrol age sobre a morfologia de *R. sanguineus* s.l. Uma dessas pesquisas, realizada por Lima de Souza *et al.* (2019), observou a ocorrência de alterações morfológicas na forma dos oócitos (de arredondados a irregulares) e nas vesículas germinativas de fêmeas semi-ingurgitadas tratadas com baixas doses de carvacrol (20 µL/mL). Buscando minimizar mais ainda a toxicidade, o acetilado do carvacrol também foi estudado sobre nematódeos gastrintestinais, e avaliada a toxicidade em camundongos, o que demonstrou baixa toxicidade (ANDRE *et al.*, 2016). Ainda em carrapatos, Oliveira *et al.* (2020) avaliaram a atividade carrapaticida e as alterações provocadas por doses subletais de acetilcarvacrol, obtendo como resultados alterações no tegumento e na reprodução de fêmeas de *R. sanguineus* s.l.

Apesar dos estudos sobre a atividade carrapaticida do carvacrol, até o momento as pesquisas estão concentradas em estudos *in vitro*, avaliando apenas o contato direto

do carrapato com o terpeno. Assim, pouco se sabe sobre a atividade do carvacrol *in vivo*, bem como seu efeito residual e outras formas de administração.

3. OBJETIVOS

3.1. Objetivo Geral

Avaliar quais as recomendações sobre o controle de parasitos de cães no Brasil, os efeitos do carvacrol e as alterações histológicas no tegumento de *R. sanguineus* s.l.

3.2. Objetivos Específicos

- Obter informações sobre os métodos recomendados por veterinários do Brasil no controle de parasitos em cães e sobre a percepção da ineficácia de alguns medicamentos.
- Fornecer informações para melhor adaptar e atualizar as diretrizes às condições no Brasil.
- Determinar a CL50 (concentração letal média) do carvacrol *in vitro* para larvas e ninfas de *R. sanguineus* s.l.
- Desenvolver técnica de bioensaio *in vivo* para o modelo animal camundongo Swiss para avaliar o efeito carrapaticida do carvacrol administrado via oral.
- Avaliar alterações histológicas em que o carvacrol é capaz de provocar no tegumento de ninfas de *R. sanguineus* s.l. alimentadas em camundongos tratados por via oral.

4. RESULTADOS

Os resultados obtidos na presente tese estão sendo apresentados sob a forma de capítulos, através de dois artigos científicos nos status de publicado e em preparação para submissão em periódicos internacionais especializados na área:

Capítulo 1

Status: Publicado

Vale, Tássia Lopes do et al. Practices employed by veterinary practitioners for controlling canine gastrointestinal helminths and ectoparasites. 2021, v. 30, n. 4.

Periódico: *Revista Brasileira de Parasitologia Veterinária* [online]. ISSN 1984-2961. https://doi.org/10.1590/S1984-29612021079

Fator de Impacto: 1.4. Qualis: A3.

Capítulo 2

Status: A ser submetido

Vale, Tássia Lopes do et al. Efeito carrapaticida *in vitro* e *in vivo* do carvacrol sobre *Rhipicephalus sanguineus* sensu lato e suas alterações no tegumento do carrapato.

Periódico: *Veterinary Parasitology*.

Fator de Impacto: 2.82. Qualis: A1.

6. REFERÊNCIAS BIBLIOGRÁFICAS

ABBAS, A.; ABBAS, R. Z.; MASOOD, S.; IQBAL, Z.; KHAN, M. K.; SALEEMI, M. K.; RAZA, M. A.; MAHMOOD, M. S.; KHAN, J. A.; SINDHU, Z. U. D. Acaricidal and insecticidal effects of essential oils against ectoparasites of veterinary importance. **Boletin Latinoamericano y del Caribe de Plantas Medicinales y Aromaticas**, vol. 17, nº 5, p. 441–452, 2018.

ABINPET. Associação Brasileira da Indútria de produtos de origem animal. 2019. **Mercado Pet Brasileiro**. Disponível em: http://abinpet.org.br/. Acessado on: 2 maio 2020.

AHANTARIG, A.; TRINACHARTVANIT, W.; BAIMAI, V.; GRUBHOFFER, L. Hard ticks and their bacterial endosymbionts (or would be pathogens). **Folia Microbiologica**, vol. 58, nº 5, p. 419–428, set. 2013. https://doi.org/10.1007/s12223-013-0222-1.

ALHO, A. M.; LIMA, C.; COLELLA, V.; CARVALHO, L. M. de; OTRANTO, D. Awareness of zoonotic diseases and parasite control practices: a survey of dog and cat owners in Qatar. **Parasites {&} Vectors**, vol. 11, p. 1–7, 2018. https://doi.org/https://doi.org/10.1186/s13071-018-2720-0.

ANDRE, W. P. P.; RIBEIRO, W. L. C.; CAVALCANTE, G. S.; SANTOS, J. M. L. dos; MACEDO, I. T. F.; PAULA, H. C. B. de; DE FREITAS, R. M.; DE MORAIS, S. M.; MELO, J. V. de; BEVILAQUA, C. M. L. Comparative efficacy and toxic effects of carvacryl acetate and carvacrol on sheep gastrointestinal nematodes and mice. **Veterinary Parasitology**, vol. 218, p. 52–58, 15 mar. 2016. https://doi.org/10.1016/j.vetpar.2016.01.001.

ARAÚJO, L. X.; NOVATO, T. P. L.; ZERINGOTA, V.; MATURANO, R.; MELO, D.; DA SILVA, B. C.; DAEMON, E.; DE CARVALHO, M. G.; MONTEIRO, C. M. O. Synergism of thymol, carvacrol and eugenol in larvae of the cattle tick, Rhipicephalus microplus, and brown dog tick, Rhipicephalus sanguineus. **Medical and Veterinary Entomology**, vol. 30, nº 4, p. 377–382, 1 dez. 2016. https://doi.org/10.1111/mve.12181.

BAKKALI, F.; AVERBECK, S.; AVERBECK, D.; IDAOMAR, M. Biological effects of essential oils - A review. **Food and Chemical Toxicology**, vol. 46, nº 2, p. 446–475, 2008. https://doi.org/10.1016/j.fct.2007.09.106.

BARROS-BATTESTI, D. M.; ARZUA, M.; BECHARA, G. H. Carrapatos de Importância Médico-Veterinária da Região Neotropical: Um guia ilustrado para identificação de espécies. **Butantan**, p. 223, 2006. Disponível em: https://www.scienceopen.com/document?vid=8523b141-4c7f-4f54-b133-503b08b1349f.

BEATI, L.; KEIRANS; JAMES E. Analisys of the systematic relationships among ticks of the genera Rhipicephalus and Boophilus (Acari: Ixodidae) based on mitochondrial 12S ribosomal DNA gene sequences and morphological characters. **Source: Journal of Parasitology**, vol. 87, nº 1, p. 32–48, 2001. DOI 10.1645/0022. Disponível em: https://doi.org/10.1645/0022-.

BECHARA, G. H.; PABLO, M.; SZABÓ, J.; FERREIRA, B. **Rhipicephalus sanguineus tick in Brazil: Feeding and reproductive aspects under laboratorial conditions**. [*S. l.*: *s. n.*], 1995. Disponível em: https://www.researchgate.net/publication/284679334.

BECKER, S.; WEBSTER, A.; DOYLE, R. L.; MARTINS, J. R.; RECK, J.; KLAFKE, G. M. Resistance to deltamethrin, fipronil and ivermectin in the brown dog tick, Rhipicephalus sanguineus sensu stricto, Latreille (Acari: Ixodidae). **Ticks and Tick-borne Diseases**, vol. 10, nº 5, p. 1046–1050, 2019. DOI 10.1016/j.ttbdis.2019.05.015. Disponível em: https://doi.org/10.1016/j.ttbdis.2019.05.015.

BORGES, L. M. F.; SOARES, F. S.; FONSECA, I. N.; CHAVES, V. V.; LOULY, C. C. B. Resistência acaricida em larvas de Rhipicephalus sanguineus (Acari Ixodidae) de Goiânia-GO, Brasil. **Revista de Patologia Tropical**, vol. 36, nº 1, p. 85–98, 2007.

BORGES, M. F. L.; SOUSA, L. A. D.; BARBOSA, C. S. Perspectives for the use of plant extracts to control the cattle tick Rhipicephalus (Boophilus) microplus. **Rev. Bras. Parasitol. Vet**, vol. 20, nº 2, p. 89–96, 2011. DOI https://doi.org/10.1590/S1984-29612011000200001. Disponível em: www.cbpv.com.br/rbpv.

BOWSER, N. H.; ANDERSON, N. E. Dogs (Canis familiaris) as sentinels for human infectious disease and application to Canadian populations: A systematic review. **Veterinary Sciences**, vol. 5, nº 4, 2018. https://doi.org/10.3390/vetsci5040083.

BRASIL, M. D. J.-M. M. D. S.-M. M. D. D. E. C. E. –MDIC; A.-G. D. U.-A. **LEI Nº 11.936, DE 14 DE MAIO DE 2009. Proíbe a fabricação, a importação, a exportação, a manutenção em estoque, a comercialização e o uso de diclorodifeniltricloretano (DDT) e dá outras providências. Diário Oficial da União Brasília, DF**. [*S. l.*: *s. n.*], 2009.

BROWN, A. W. A.; PAL, R. **Insecticide Resistance in Arthropods World Health Organization**. Monograph Ser. Geneva: [*s. n.*], 1958. vol. 38,

CARDOSO, A. dos S.; SANTOS, E. G. G.; LIMA, A. da S.; TEMEYER, K. B.; PÉREZ DE LEÓN, A. A.; COSTA, L. M.; SOARES, A. M. dos S. Terpenes on Rhipicephalus (Boophilus) microplus: Acaricidal activity and acetylcholinesterase inhibition. **Veterinary Parasitology**, vol. 280, 1 abr. 2020. https://doi.org/10.1016/j.vetpar.2020.109090.

CHOMEL, B. B. Zoonoses in the Bedroom. **Emerging Infectious Diseases**, vol. 17, nº 2, p. 167–172, 2011. https://doi.org/10.3201/eid1702101070.

COIMBRA-DORES, M. J.; NUNES, T.; DIAS, D.; ROSA, F. Rhipicephalus sanguineus (Acari: Ixodidae) species complex: morphometric and ultrastructural analyses. **Experimental and Applied Acarology**, vol. 70, nº 4, p. 455–468, 1 dez. 2016. https://doi.org/10.1007/s10493-016-0095-5.

COLES, T. B.; DRYDEN, M. W. **Insecticide/acaricide resistance in fleas and ticks infesting dogs and cats**. [*S. l.*: *s. n.*], 2014. Disponível em: http://www.parasitesandvectors.com/content/7/1/8.

COOLEY, R. A. **The genera Boophilus, Rhipicephalus, and Haemaphysalis (Ixodidae) of the new world**. [*S. l.*]: US Government Printing Office, 1946. vol. 187, .

COSKUN, S.; GIRISGIN, O.; KÜRKCÜOGLU, M.; MALYER, H.; GIRISGIN, A. O.; KIRIMER, N.; BASER, K. H. Acaricidal efficacy of Origanum onites L. essential oil against Rhipicephalus turanicus (Ixodidae). **Parasitology Research**, vol. 103, nº 2, p. 259–261, 2008. https://doi.org/10.1007/s00436-008-0956-x.

CRUZ, E. M. de O.; COSTA-JUNIOR, L. M.; PINTO, J. A. O.; SANTOS, D. de A.; ARAUJO, S. A. de; ARRIGONI-BLANK, M. de F.; BACCI, L.; ALVES, P. B.; CAVALCANTI, S. C. de H.; BLANK, A. F. Acaricidal activity of Lippia gracilis essential oil and its major constituents on the tick Rhipicephalus (Boophilus) microplus. **Veterinary Parasitology**, vol. 195, nº 1–2, p. 198–202, 1 jul. 2013. https://doi.org/10.1016/j.vetpar.2012.12.046.

DANTAS-TORRES, F. Biology and ecology of the brown dog tick, Rhipicephalus sanguineus. **Parasites and Vectors**, vol. 3, nº 1, p. 1–11, 2010a. https://doi.org/10.1186/1756-3305-3-26.

DANTAS-TORRES, F. The brown dog tick, Rhipicephalus sanguineus (Latreille, 1806) (Acari: Ixodidae): From taxonomy to control. **Veterinary Parasitology**, vol. 152, nº 3–4, p. 173–185, 2008. https://doi.org/10.1016/j.vetpar.2007.12.030.

DANTAS-TORRES, F.; CHOMEL, B. B.; OTRANTO, D. Ticks and tick-borne diseases: A One Health perspective. **Trends in Parasitology**, vol. 28, nº 10, p. 437–446, 2012. DOI 10.1016/j.pt.2012.07.003. Disponível em: http://dx.doi.org/10.1016/j.pt.2012.07.003.

DANTAS-TORRES, F.; FIGUEREDO, L. A.; BRANDÃO-FILHO, S. P. Rhipicephalus sanguineus (Acari: Ixodidae), the brown dog tick, parasitizing humans in Brazil. **Revista da Sociedade Brasileira de Medicina Tropical**, vol. 39, nº 1, p. 64–67, 2006. https://doi.org/10.1590/S0037-86822006000100012.

DANTAS-TORRES, F.; OTRANTO, D. Further thoughts on the taxonomy and vector role of Rhipicephalus sanguineus group ticks. **Veterinary Parasitology**, vol. 208, nº 1–2, p. 9–13, 28 fev. 2015. https://doi.org/10.1016/j.vetpar.2014.12.014.

DANTAS-TORRES, F.; OTRANTO, D. Rhipicephalus sanguineus on dogs: Relationships between attachment sites and tick developmental stages. **Experimental and Applied Acarology**, vol. 53, nº 4, p. 389–397, 2011. https://doi.org/10.1007/s10493-010-9406-4.

DE LA FUENTE, J. Controlling ticks and tick-borne diseases…looking forward. **Ticks and Tick-borne Diseases**, vol. 9, nº 5, p. 1354–1357, 1 jul. 2018. https://doi.org/10.1016/j.ttbdis.2018.04.001.

DE OLIVEIRA, M. V. S.; KONIG, I. F. M.; REIS, A. C.; SILVA, L.; PECONICK, A. P.; THOMASI, S. S.; LIMA-DE-SOUZA, J. R.; CAMARGO-MATHIAS, M. I.; REMEDIO, R. N. Sublethal concentrations of acetylcarvacrol affect reproduction and integument morphology in the brown dog tick Rhipicephalus sanguineus sensu lato

(Acari: Ixodidae). **Experimental and Applied Acarology**, vol. 82, nº 2, p. 265–279, 1 out. 2020. https://doi.org/10.1007/s10493-020-00538-7.

DE OLIVEIRA MONTEIRO, C. M.; DAEMON, E.; APARECIDO CLEMENTE, M.; DOS SANTOS ROSA, L.; MATURANO, R. Acaricidal efficacy of thymol on engorged nymphs and females of Rhipicephalus sanguineus (Latreille, 1808) (Acari: Ixodidae). **Parasitology Research**, vol. 105, nº 4, p. 1093–1097, 2009. https://doi.org/10.1007/s00436-009-1530-x.

DEMMA, L. J.,; TRAEGER, M. S. ,; NICHOLSON, W. L. ,; PADDOCK, C. D. ,; BLAU, D. M. ,; EREMEEVA, M. E. ,; DASCH, D. A. ,; LEVIN, M. L. ,; JOSEPH SINGLETON, Jr. ,; ZAKI, S. R. ,; CHEEK, J. E. ,; SWERDLOW, D. L. ,; MCQUISTON, J. H. Rocky Mountain Spotted Fever from an Unexpected Tick Vector in Arizona. **New England Journal of Medicine**, vol. 353, nº 6, p. 587–594, 2005. Disponível em: www.nejm.org.

DOLAN, M. C.; JORDAN, R. A.; SCHULZE, T. L.; SCHULZE, C. J.; MANNING, M. C.; RUFFOLO, D.; SCHMIDT, J. P.; PIESMAN, J.; KARCHESY, J. J. **Ability of Two Natural Products, Nootkatone and Carvacrol, to Suppress Ixodes scapularis and Amblyomma americanum (Acari: Ixodidae) in a Lyme Disease Endemic Area of New Jersey**. [*S. l.*: *s. n.*], 2009. Disponível em: https://academic.oup.com/jee/article/102/6/2316/2199345.

DURON, O.; SIDI-BOUMEDINE, K.; ROUSSET, E.; MOUTAILLER, S.; JOURDAIN, E. The Importance of Ticks in Q Fever Transmission: What Has (and Has Not) Been Demonstrated? **Trends in Parasitology**, vol. 31, nº 11, p. 536–552, 1 nov. 2015a. https://doi.org/10.1016/j.pt.2015.06.014.

EISEN RJ, KUGELER KJ, EISEN L, BEARD CB, P. CD. Tick-Borne Zoonoses in the United States: Persistent and Emerging Threats to Human Health. **ILAR Journal**, vol. 58, nº 3, p. 319–335, 2018. https://doi.org/10.1093/ilar/ilx005.Tick-Borne.

ESCCAP. European Scientific Counsel Companion Animal Parasites (ESCCAP). Guideline 03 - control of ectoparasites in dogs and cats. 2022. Disponível em: https://www.esccap.org/uploads/docs/4ce0ad9k_0720_ESCCAP_GL3__English_v17_1p.pdf. Acessado on: 29 jun. 2022.

ESTRADA-PEÑA, A.; SANCHEZ, C. Morfologīa comparada de Rhipicephalus sanguineus y R. **Rev. Ibér. Parasitol.**, vol. 48, nº 1, p. 51–62, 1988.

FAZA, A. P.; PINTO, I. S. B.; FONSECA, I.; ANTUNES, G. R.; MONTEIRO, C. M. de O.; DAEMON, E.; MUNIZ, M. de S.; MARTINS, M. F.; FURLONG, J.; PRATA, M. C. de A. A new approach to characterization of the resistance of populations of Rhipicephalus microplus (Acari: Ixodidae) to organophosphate and pyrethroid in the state of Minas Gerais, Brazil. **Experimental Parasitology**, vol. 134, nº 4, p. 519–523, ago. 2013. https://doi.org/10.1016/j.exppara.2013.04.006.

FERRARI, J. Insecticide resistance. *In*: BEATY, B. J.; MARQUARDT, W. C.; NIWOT, C. O. (orgs.). **The Biology of Disease Vectors**. Colorado: University Press Colorado, 1996. p. 512–529.

GAUTHIER, M.; GRÜNEWALD, B. Neurotransmitter Systems in the Honey Bee Brain: Functions in Learning and Memory. **Honeybee Neurobiology and Behavior**. [*S. l.*]: Springer Netherlands, 2012. p. 155–169. https://doi.org/10.1007/978-94-007-2099-2_13.

GLAVAN, G.; NOVAK, S.; BOŽIČ, J.; JEMEC KOKALJ, A. Comparison of sublethal effects of natural acaricides carvacrol and thymol on honeybees. **Pesticide Biochemistry and Physiology**, vol. 166, 1 jun. 2020. https://doi.org/10.1016/j.pestbp.2020.104567.

GRAY, J.; DANTAS-TORRES, F.; ESTRADA-PEÑA, A.; LEVIN, M. Systematics and ecology of the brown dog tick, Rhipicephalus sanguineus. **Ticks and Tick-borne Diseases**, vol. 4, nº 3, p. 171–180, 2013. https://doi.org/10.1016/j.ttbdis.2012.12.003.

GUGLIELMONE, A. A.; BEATI, L.; BARROS-BATTESTI, D. M.; LABRUNA, M. B.; NAVA, S.; VENZAL, J. M.; MANGOLD, A. J.; SZABÓ, M. P. J.; MARTINS, J. R.; GONZÁLEZ-ACUÑA, D.; ESTRADA-PEÑA, A. Ticks (Ixodidae) on humans in South America. **Experimental and Applied Acarology**, vol. 40, nº 2, p. 83–100, out. 2006. https://doi.org/10.1007/s10493-006-9027-0.

GUTIERREZ, J. A.; ZHAO, X.; KEMPER, C. J.; PLUMMER, P. R.; BAUER, S. M.; HUTCHENS, D. E.; SMITH, C. K.; WHITE, W. H. **An In Vivo Rodent Model for Identifying and Characterizing Acaricides**. [*S. l.*: *s. n.*], 2006. Disponível em: https://academic.oup.com/jme/article/43/3/526/879885.

IRAC. **Insecticide Resistance Action Committee (IRAC): MoA Classification Scheme Prepared by: IRAC International MoA Working Group Approved by: IRAC Executive IRAC MoA Classification Contents**. [*S. l.*: *s. n.*], 2012. Disponível em: www.irac-online.orgorenquiries@irac-online.orgwww.irac-online.orgorenquiries@irac-online.orgFurtherinformationisavailableat:www.irac-online.orgorenquiries@irac-online.org. Acessado on: 21 ago. 2022.

KLAFKE, G.; WEBSTER, A.; DALL AGNOL, B.; PRADEL, E.; SILVA, J.; DE LA CANAL, L. H.; BECKER, M.; OSÓRIO, M. F.; MANSSON, M.; BARRETO, R.; SCHEFFER, R.; SOUZA, U. A.; CORASSINI, V. B.; DOS SANTOS, J.; RECK, J.; MARTINS, J. R. Multiple resistance to acaricides in field populations of Rhipicephalus microplus from Rio Grande do Sul state, Southern Brazil. **Ticks and Tick-borne Diseases**, vol. 8, nº 1, p. 73–80, 1 jan. 2017. https://doi.org/10.1016/j.ttbdis.2016.09.019.

KOC, S.; OZ, E.; CINBILGEL, I.; AYDIN, L.; CETIN, H. Acaricidal activity of Origanum bilgeri P.H. Davis (Lamiaceae) essential oil and its major component, carvacrol against adults Rhipicephalus turanicus (Acari: Ixodidae). **Veterinary Parasitology**, vol. 193, nº 1–3, p. 316–319, 2013. https://doi.org/10.1016/j.vetpar.2012.11.010.

KOCH, H. G. Oviposition of the Brown Dog Tick (Acari: Ixodidae) in the Laboratory 1. **Ann. Entomol. Soc. Am.**, vol. 75, p. 583–586, 1982. Disponível em: https://academic.oup.com/aesa/article/75/5/583/63731.

KOUL, O.; WALIA, S.; DHALIWAL, G. S.; Biopestic. Essential Oils as Green Pesticides: Potential and Constraints. **Biopestic. Int.**, vol. 4, nº 1, p. 63–84, 2008.

LABRUNA, M. Biologia-ecologia de Rhipicephalus sanguineus (Acari: Ixodidae). **Revista Brasileira de Parasitologia Veterinária**, vol. 13, p. 123–124, 2004. .

LI, M.; LIU, S.; YIN, Z.; BERNIGAUD, C.; GUILLOT, J.; FANG, F. Activity of terpenes derived from essential oils against Sarcoptes scabiei eggs. **Parasites and Vectors**, vol. 14, nº 1, 1 dez. 2021. https://doi.org/10.1186/s13071-021-05094-6.

LIMA DE SOUZA, J. R.; OLIVEIRA, P. R. de; ANHOLETO, L. A.; ARNOSTI, A.; DAEMON, E.; REMEDIO, R. N.; CAMARGO-MATHIAS, M. I. Effects of carvacrol on oocyte development in semi-engorged Rhipicephalus sanguineus sensu lato females ticks (Acari: Ixodidae). **Micron**, vol. 116, p. 66–72, 1 jan. 2019. https://doi.org/10.1016/j.micron.2018.09.015.

LITTLE, S. E.; HOSTETLER, J.; KOCAN, K. M. Movement of Rhipicephalus sanguineus adults between co-housed dogs during active feeding. **Veterinary Parasitology**, vol. 150, nº 1–2, p. 139–145, 30 nov. 2007. https://doi.org/10.1016/j.vetpar.2007.08.029.

MAURELLI, M. P.; PEPE, P.; COLOMBO, L.; ARMSTRONG, R.; BATTISTI, E.; MORGOGLIONE, M. E.; COUNTURIS, D.; RINALDI, L.; CRINGOLI, G.; FERROGLIO, E.; ZANET, S. A national survey of Ixodidae ticks on privately owned dogs in Italy. **Parasites and Vectors**, vol. 11, nº 1, 16 jul. 2018. https://doi.org/10.1186/s13071-018-2994-2.

MONTEIRO, C. M.O., DAEMON, E., APARECIDO CLEMENTE, M., DOS SANTOS ROSA, L., & MATURANO, R. (2009). Acaricidal efficacy of thymol on engorged nymphs and females of *Rhipicephalus sanguineus* (Latreille, 1808)(Acari: Ixodidae). *Parasitology research*, *105*, 1093-1097. https://doi.org/10.1007/s00436-009-1530-x

MORAES-FILHO, J.; MARCILI, A.; NIERI-BASTOS, F. A.; RICHTZENHAIN, L. J.; LABRUNA, M. B. Genetic analysis of ticks belonging to the Rhipicephalus sanguineus group in Latin America. **Acta Tropica**, vol. 117, nº 1, p. 51–55, jan. 2011. https://doi.org/10.1016/j.actatropica.2010.09.006.

MOTA-SANCHEZ, D.; WISE, J. C. Arthropod Pesticide Resistance Database (ARPD). 23 jun. 2020. **Michigan State University**. Disponível em: http://www.pesticideresistance.org/. Acessado on: 22 jun. 2022.

NAVA, S.; BEATI, L.; VENZAL, J. M.; LABRUNA, M. B.; SZABÓ, M. P. J.; PETNEY, T.; SARACHO-BOTTERO, M. N.; TARRAGONA, E. L.; DANTAS-TORRES, F.; SILVA, M. M. S.; MANGOLD, A. J.; GUGLIELMONE, A. A.; ESTRADA-PEÑA, A. Rhipicephalus sanguineus (Latreille, 1806): Neotype designation, morphological re-description of all parasitic stages and molecular characterization. **Ticks and Tick-borne Diseases**, vol. 9, nº 6, p. 1573–1585, 2018. DOI 10.1016/j.ttbdis.2018.08.001. Disponível em: https://doi.org/10.1016/j.ttbdis.2018.08.001.

NAVA, S.; ESTRADA-PEÑA, A.; PETNEY, T.; BEATI, L.; LABRUNA, M. B.; SZABÓ, M. P. J.; VENZAL, J. M.; MASTROPAOLO, M.; MANGOLD, A. J.; GUGLIELMONE, A. A. The taxonomic status of Rhipicephalus sanguineus (Latreille, 1806). **Veterinary Parasitology**, vol. 208, nº 1–2, p. 2–8, 2015. https://doi.org/10.1016/j.vetpar.2014.12.021.

NEVES, I. D. A.; DA CAMARA, C. A. G. ,; DE OLIVIERA, J. C. S. ,; DE ALMEIDA, A. v. Acaricidal Activity and Essential Oil Composition of Petiveria alliacea L. from Pernambuco (Northeast Brazil). **Journal of Essential Oil Research**, vol. 23, nº 1, p. 23–26, 2011. DOI 10.1080/10412905.2011.9700426. Disponível em: https://doi.org/10.1080/10412905.2011.9700426.

NOVATO, T. P. L.; ARAÚJO, L. X.; DE MONTEIRO, C. M. O.; MATURANO, R.; SENRA, T. de O. S.; DA SILVA MATOS, R.; GOMES, G. A.; DE CARVALHO, M. G.; DAEMON, E. Evaluation of the combined effect of thymol, carvacrol and (E)-cinnamaldehyde on Amblyomma sculptum (Acari: Ixodidae) and Dermacentor nitens (Acari: Ixodidae) larvae. **Veterinary Parasitology**, vol. 212, nº 3–4, p. 331–335, 15 set. 2015. https://doi.org/10.1016/j.vetpar.2015.08.021.

NWANADE, C.F., WANG, M., WANG, T., YU, Z., LIU, J., 2020. Botanical acaricides and repellents in tick control: current status and future directions. Exp. Appl. Acarol. 81, 1–35. https://doi.org/10.1007/s10493-020-00489-z.

OVERGAAUW, P. A. M.; VINKE, C. M.; VAN HAGEN, M. A. E.; LIPMAN, L. J. A. A one health perspective on the human-companion animal relationship with emphasis on zoonotic aspects. **International Journal of Environmental Research and Public Health**, vol. 17, nº 11, 1 jun. 2020. https://doi.org/10.3390/ijerph17113789.

OZOE, Y.; ASAHI, M.; OZOE, F.; NAKAHIRA, K.; MITA, T. The antiparasitic isoxazoline A1443 is a potent blocker of insect ligand-gated chloride channels. **Biochemical and Biophysical Research Communications**, vol. 391, nº 1, p. 744–749, 1 jan. 2010. https://doi.org/10.1016/j.bbrc.2009.11.131.

PALMAS, C.; BORTOLETTI, G.; CONCHEDDA, M.; CONTINI, C.; GABRIELE, F.; ECCA, A. R. Study on immunobiology in ectoparasites of public health interest: Rhipicephalus sanguineus. **Parassitologia**, vol. 43 Suppl 1, p. 29—35, dez. 2001. Disponível em: http://europepmc.org/abstract/MED/12078475.

PAROLA, P.; SOCOLOVSCHI, C.; JEANJEAN, L.; BITAM, I.; FOURNIER, P. E.; SOTTO, A.; LABAUGE, P.; RAOULT, D. Warmer weather linked to tick attack and emergence of severe Rickettsioses. **PLoS Neglected Tropical Diseases**, vol. 2, nº 11, 2008. https://doi.org/10.1371/journal.pntd.0000338.

PEGRAM, R. G.; CLIFFORD, C. M.; WALKER, J. B.; KEIRANS, J. E. Clarification of the Rhipicephalus sanguineus group (Acari, Ixodoidea, Ixodidae). I. R. sulcatus Neumann, 1908 and R. turanicus Pomerantsev, 1936. **Syst Parasitol**, vol. 10, p. 3–26, 1987. https://doi.org/https://doi.org/10.1007/BF00009099.

PEIXOTO, M. G.; COSTA-JÚNIOR, L. M.; BLANK, A. F.; LIMA, A. da S.; MENEZES, T. S. A.; SANTOS, D. de A.; ALVES, P. B.; CAVALCANTI, S. C. de H.; BACCI, L.; ARRIGONI-BLANK, M. de F. Acaricidal activity of essential oils from

Lippia alba genotypes and its major components carvone, limonene, and citral against Rhipicephalus microplus. **Veterinary Parasitology**, vol. 210, nº 1–2, p. 118–122, 30 maio 2015. https://doi.org/10.1016/j.vetpar.2015.03.010.

REMEDIO, R. N.; NUNES, P. H.; CAMARGO-MATHIAS, M. I. The extensible integument of Rhipicephalus sanguineus female ticks in different feeding stages: A morphological approach. **Acta Zoologica**, v. 96, n. 3, p. 319–327, 1 jul. 2015.

RODRÍGUEZ-VIVAS, R. I.; APANASKEVICH, D. A.; OJEDA-CHI, M. M.; TRINIDAD-MARTÍNEZ, I.; REYES-NOVELO, E.; ESTEVE-GASSENT, M. D.; PÉREZ DE LEÓN, A. A. Ticks collected from humans, domestic animals, and wildlife in Yucatan, Mexico. **Veterinary Parasitology**, vol. 215, p. 106–113, 15 jan. 2016. https://doi.org/10.1016/j.vetpar.2015.11.010.

SANCHES, G. S.; ÉVORA, P. M.; MANGOLD, A. J.; JITTAPALAPONG, S.; RODRIGUEZ-MALLON, A.; GUZMÁN, P. E. E.; BECHARA, G. H.; CAMARGO-MATHIAS, M. I. Molecular, biological, and morphometric comparisons between different geographical populations of Rhipicephalus sanguineus sensu lato (Acari: Ixodidae). **Veterinary Parasitology**, vol. 215, p. 78–87, 2016. DOI 10.1016/j.vetpar.2015.11.007. Disponível em: http://dx.doi.org/10.1016/j.vetpar.2015.11.007.

SILVA, A. B., DUARTE, M. M., DA COSTA CAVALCANTE, R., DE OLIVEIRA, S. V., VIZZONI, V. F., DE LIMA DURÉ, A. Í., ... & GAZETA, G. S. (2017). *Rickettsia rickettsii* infecting *Rhipicephalus sanguineus* sensu lato (Latreille 1806), in high altitude atlantic forest fragments, Ceará state, Brazil. **Acta Tropica**, *173*, 30-33.

ŠLAPETA, J.; CHANDRA, S.; HALLIDAY, B. The "tropical lineage" of the brown dog tick Rhipicephalus sanguineus sensu lato identified as Rhipicephalus linnaei (Audouin, 1826). **International Journal for Parasitology**, vol. 51, nº 6, p. 431–436, 1 maio 2021. https://doi.org/10.1016/j.ijpara.2021.02.001.

SOLANO-GALLEGO, L.; BANETH, G. Babesiosis in dogs and cats-Expanding parasitological and clinical spectra. **Veterinary Parasitology**, vol. 181, nº 1, p. 48–60, 2011. DOI 10.1016/j.vetpar.2011.04.023. Disponível em: http://dx.doi.org/10.1016/j.vetpar.2011.04.023.

SOUSA, A. I. P.; CASTRO, G. J. S.; TAVARES, C. P.; VALE, T. L. do; COSTA-JUNIOR, L. M.; SOARES, A. M. S. In vitro assessment of the acaricidal activity of a carvacrol shampoo on tick larvae. **Experimental Parasitology**, vol. 242, 1 nov. 2022. https://doi.org/10.1016/j.exppara.2022.108364.

SUNTRES, Z. E.; COCCIMIGLIO, J.; ALIPOUR, M. The Bioactivity and Toxicological Actions of Carvacrol. **Critical Reviews in Food Science and Nutrition**, vol. 55, nº 3, p. 304–318, 23 fev. 2015. https://doi.org/10.1080/10408398.2011.653458.

TAVARES, C. P.; SABADIN, G. A.; SOUSA, I. C.; GOMES, M. N.; SOARES, A. M. S.; MONTEIRO, C. M. O.; VAZ, I. S.; COSTA-JUNIOR, L. M. Effects of carvacrol and thymol on the antioxidant and detoxifying enzymes of Rhipicephalus microplus (Acari: Ixodidae). **Ticks and Tick-borne Diseases**, vol. 13, nº 3, 1 maio 2022. https://doi.org/10.1016/j.ttbdis.2022.101929.

TROTTA, M.; NICETTO, M.; FOGLIAZZA, A.; MONTARSI, F.; CALDIN, M.; FURLANELLO, T.; SOLANO-GALLEGO, L. Detection of Leishmania infantum, Babesia canis, and rickettsiae in ticks removed from dogs living in Italy. **Ticks and Tick-borne Diseases**, vol. 3, nº 5–6, p. 294–297, 2012. DOI 10.1016/j.ttbdis.2012.10.031. Disponível em: http://dx.doi.org/10.1016/j.ttbdis.2012.10.031.

VALE, L.; DE PAULA, L. G. F.; VIEIRA, M. S.; ALVES, S. das G. A.; JUNIOR, N. R. de M.; GOMES, M. D. F.; TEIXEIRA, W. F. P.; RIZZO, P. V.; FREITAS, F. M. C.; FERREIRA, L. L.; LOPES, W. D. Z.; MONTEIRO, C. Binary combinations of thymol, carvacrol and eugenol for Amblyomma sculptum control: Evaluation of in vitro synergism and effectiveness under semi-field conditions. **Ticks and Tick-borne Diseases**, vol. 12, nº 6, 1 nov. 2021. https://doi.org/10.1016/j.ttbdis.2021.101816.

VILELA, V. L. R.; FEITOSA, T. F.; BEZERRA, R. A.; KLAFKE, G. M.; RIET-CORREA, F. Multiple acaricide-resistant Rhipicephalus microplus in the semi-arid region of Paraíba State, Brazil. **Ticks and Tick-borne Diseases**, vol. 11, nº 4, 2020. https://doi.org/10.1016/j.ttbdis.2020.101413.

WALKER, J. B. (Jane B.; KEIRANS, J. E.; HORAK, I. **The genus Rhipicephalus (Acardi, Ixodidae) : a guide to the brown ticks of the world**. [*S. l.*]: Cambridge University Press, 2000.

WHITEHEAD, R. **The UK Pesticide Guide 2005**. Wallingford, Oxford: Cabi, 2005. vol. 24, .

WHO. **World Health Organization: Insecticide resistance and vector control. Seventeenth Report of the WHO Expert Committee on Insecticides**. [*S. l.*: *s. n.*], 1970.

ZHAO, C.; GAMMIE, S. C. Glutamate, GABA, and glutamine are synchronously upregulated in the mouse lateral septum during the postpartum period. **Brain Research**, vol. 1591, nº 1, p. 53–62, 2014. https://doi.org/10.1016/j.brainres.2014.10.023.

Capítulo 1

Practices employed by veterinary practitioners for controlling canine gastrointestinal helminths and ectoparasites

---

Neste capítulo consta o artigo publicado no periódico *Braz J Vet Parasitol* Qualis A3; Fator de Impacto: 1.4.
2021; 30(4): e007021 | https://doi.org/10.1590/S1984-29612021079

Revista Brasileira de Parasitologia Veterinária
Brazilian Journal of Veterinary Parasitology
ISSN 1984-2961 (Electronic)
www.cbpv.org.br/rbpv

Original Article

Practices employed by veterinary practitioners for controlling canine gastrointestinal helminths and ectoparasites

Práticas realizadas por veterinários para controle de helmintos gastrintestinais e ectoparasitos de cães

Tássia Lopes do Vale[1]; Isabella Chaves Sousa[2]; Caio Pavão Tavares[1]; Naylene Carvalho Silva[1]; Hermes Ribeiro Luz[2]; Matheus Nobate Gomes[3]; Neil Sargison[4]; Livio Martins Costa-Junior[1]*

[1]Programa de Pós-graduação em Ciências da Saúde, Universidade Federal do Maranhão – UFMA, São Luís, MA, Brasil
[2]Programa de Pós-graduação em Biotecnologia, Universidade Federal do Maranhão – UFMA, São Luís, MA, Brasil
[3]Graduação em Ciências Biológicas, Universidade Federal do Maranhão – UFMA, São Luís, MA, Brasil
[4]University of Edinburgh, Royal (Dick) School of Veterinary Studies, Roslin, Midlothian, EH25 9RG, United Kingdom

How to cite: do Vale TL, Sousa IC, Tavares CP, Silva NC, Luz HR, Gomes MN, et al. Practices employed by veterinary practitioners for controlling canine gastrointestinal helminths and ectoparasites. *Braz J Vet Parasitol* 2021; 30(4): e007021. https://doi.org/10.1590/ S1984-29612021079

Abstract

The present study attempted to evaluate the practical experience and methods employed by Brazilian veterinary practitioners for control of parasites. Twenty-one questions were asked of 403 veterinary practitioners based in different climatic zones with reference to parasite epidemiology from the country. Administration of a combination of drugs at three-month intervals was the most common regime recommended for prophylaxis against gastrointestinal helminths, with a single treatment repeated after 15 days. Routine prophylaxis against dog ectoparasites was recommended by 82.4% veterinary practitioners, and 46.6% changed the drug compound used. Monthly prophylaxic treatments for ectoparasites, using systemic, topical and/or collar-impregnated drugs, was recommended by 21.5% veterinary practitioners. Side-effects of ectoparasiticide-impregnated collars were suspected by 58% of the veterinary practitioners. Isoxazolines were the most frequently used chemical group to treat ectoparasites in dogs. Poor efficacy of fipronil in controlling ticks was suspected by 79.5% of the veterinary practitioners. The isoxazolines and combination of anthelmintic compounds are the most common drugs to prevent or treat ectoparasites and gastrointestinal nematodes, respectively. The suspect of the inefficacy of antiparasitic drugs is shared among the veterinary practitioners from part of Brazil. Guidelines are needed, specifically for the control of gastrointestinal helminths and ectoparasites in Brazilian dogs.

Keywords: Gastrointestinal nematodes, ectoparasites, dogs, antiparasitic drugs, control strategies.

Resumo

O presente estudo avaliou os métodos de controle empregados por médicos veterinários clínicos para o controle de parasitos de cães no Brasil. Vinte e uma perguntas foram feitas a 403 veterinários de diferentes regiões do país. O uso de associações de compostos ativos em intervalos de três meses foi o mais recomendado para profilaxia de helmintos gastrointestinais, repetido após 15 dias. A profilaxia de rotina contra ectoparasitos foi recomendada por 82,4% dos veterinários, e 46,6% mudam rotineiramente o composto indicado. Tratamentos profiláticos mensais para ectoparasitos, com produtos sistêmicos, tópicos e / ou impregnados com colar, foram recomendados por 21,5% dos veterinários. Os efeitos colaterais das coleiras impregnadas com ectoparasiticidas foram relatados por 58% dos médicos veterinários. As isoxazolinas foram o grupo químico mais utilizado para tratar ectoparasitos em cães. A baixa eficácia do fipronil no controle de carrapatos foi suspeitada por 79,5% dos médicos veterinários. As isoxazolinas e a associação de compostos anti-helmínticos são os medicamentos mais comuns para prevenir ou tratar ectoparasitos e nematoides gastrointestinais, respectivamente. A suspeita da ineficácia dos antiparasitários é compartilhada entre os médicos veterinários de algumas regiões do Brasil. Orientações são necessárias, especificamente para o controle de helmintos e ectoparasitos gastrointestinais em cães no Brasil.

Palavras-chave: Nematódeos gastrointestinais, ectoparasitos, cães, antiparasitários, estratégias de controle.

Received April 15, 2021. Accepted August 27, 2021.

*Corresponding author: Livio Martins Costa-Junior. E-mail: livio.martins@ufma.br

Braz J Vet Parasitol 2021; 30(4): e007021 | https://doi.org/10.1590/S1984-29612021079

Introduction

Parasites are important global causes of infectious disease in dogs, in particular in warm and humid tropical environments that provide optimal conditions for survival and development of free-living stages of a variety of parasitic arthropods, protozoa and helminths, many associated with dogs (Dantas-Torres et al., 2020). Brazil is the largest tropical country in the world, occupying a highly diverse land area; and has approximately 55.1 million dogs (ABINPET, 2019; IBGE, 2019). These conditions are ideal for the completion of parasite life cycles and facilitate disease transmission.

Several common canine gastrointestinal helminth (in particular, *Toxocara canis* and *Ancylostoma* spp.) and arthropods (in particular, ticks, phlebotomine sand flies and mosquitoes) parasites are responsible for zoonotic diseases in Brazil (Dantas-Torres & Otranto, 2014). Effective and sustainable gastrointestinal helminth and arthropod parasite control is, therefore, a fundamental priority to ensure good states of welfare in dogs and reduce the potential for zoonotic transmission (Chomel & Sun, 2011). Recognised best practice should involve both management, such as addressing the sanitary conditions of the environment to interrupt parasite life cycles, and use of antiparasitic drugs strategically targeted to account for factors involved in the parasites' life histories (Alho et al., 2018). However, there is a global

tendency among dog owners and veterinary practitioners to rely on the simpler option of regular and non-targeted, one-fits-all antiparasitic drug treatments.

Guidelines have been elaborated for the treatment and control of companion animal parasites in north America, Europe and Tropical regions under the auspices of the Companion Animal Parasite Council (CAPC) founded in 2002 (CAPC, 2019), the European Scientific Council Companion Animal Parasites (ESCCAP) founded in 2005 (ESCCAP, 2019), and the Tropical Council for Companion Animal Parasites (TroCCAP) founded in 2015 (Traub et al., 2015) (TroCCAP, 2019). These guidelines were updated in 2020, with the notable addition of highlighting the need to disseminate information to veterinary practitioners in tropical regions (Dantas-Torres et al., 2020).

Faced with the emergence of antiparasitic drug resistance (Furtado et al., 2014; Becker et al., 2019), Brazilian veterinary practitioners have an important role in ensuring the sustainable control of the large diversity of gastrointestinal helminths and arthropods that affect dogs. The present study, therefore, aimed to evaluate the practical experience and methods employed by veterinary practitioners for parasite control in dogs in Brazil, using a structured questionnaire format. This included questions that explored perceptions of drug efficacy and specific side-effects. Critical knowledge gaps relating to the practicalities of diagnosis, treatment, and control of parasitic diseases of dogs are identified with reference to better informing Brazilian veterinary practitioners.

Materials and Methods

Study population

There are 124,253 registered veterinary practitioners in Brazil, albeit the true number of these working with companion animals is unknown (CFMV, 2019). In this context was used the formula to infinite population described by Miot (2011) was to calculate a requirement to question 384 veterinary practitioners in order to generate representative responses with a 95% confidence level (the true percentage of the population who would pick an answer, $Z\alpha/2 = 1.96$) and a margin of error (referred to here as a confidence interval) of 5% ($E = 0.05$). The δ was considered as 0.5.

$$n = [(Z\alpha\ \delta / 2 \times) \div E]^2 \qquad (1)$$

A questionnaire survey was developed to explore theoretical knowledge and understanding of canine parasitology as it pertains to situations that are routinely encountered in veterinary practices. The sample collect was by convenience in two different moments. Firstly, the questionnaire was delivered online to veterinary practitioners working with companion animals, and publicised through social media and personal mailing lists of the Brazilian authors. In addition, veterinary practitioners who participated in the Brazilian Conference of Small Animals Clinic (BCSAC) in 2018 were invited to fill out the questionnaire in printed form. Because of the type of sampling and mainly because of the BCSAC audience, the sample studied may not represent the entire population of Brazilian veterinary practitioners. This methodological bias must be considered in this study. The respondents could freely not answer any questions, or give up on completion at any stage. Duplicate responses were excluded, along with those from veterinary practitioners without a professional registration number, or from another country. All responders freely consented to their participation in the survey and

personal data were not exposed. All personal data were handled only by two members of the research team, further ensuring total data protection and anonymity. All data were handled according to Brazilian data protection law current at the time of the survey.

Questionnaire structure

The questionnaire was designed first to obtain data pertaining to inclusivity, and to gauge the respondents' attitudes towards parasite control. This was followed by 13 closed, six open and two mixed questions that were subdivided into two sections according canine gastrointestinal helminth or arthropod parasites (Supplementary material). Parasitic protozoa and *Dirofilaria immitis* were not the focus of the present study to avoid potential confusion with the diagnosis and management of gastrointestinal helminth and tick-borne parasites. Specific risk factors such as host age, hygiene and environmental conditions were also not analysed in the present study.

Questions in the gastrointestinal helminth section explored knowledge and attitudes towards coproparasitological examination, principles of antiparasitic drug use, anthelmintic resistance mitigation, and perceptions of drug efficacy. Questions in the arthropod section explored knowledge of and attitudes towards common ectoparasite infestations, treatment and prophylaxis regimes using systemic, topical and/or impregnated collar formulations, drug side-effects, drug resistance or tolerance mitigation strategies, and suspicion of drug inefficacy. More than one response was possible for questions about common ectoparasites and antiparasitic drug use, and suspicion of inefficacy. The questionnaire was piloted and refined with seven veterinarians, and the final version is shown in the supplementary material.

Data analysis

Five hundred and thirty-two responses were received. One duplicate entry, 123 responses from unregistered veterinary practitioners (veterinary students, or failure to provide a professional registration number), and five responses from non-Brazilian veterinarians were removed, leaving a total of 403 responses from veterinary practitioners based across Brazil (5% more than the calculated target). A comparison of the number of responders and the distribution of the Brazilian population (IBGE, 2012) of each geographic region was performed using Chi-square to demonstrate that the sample was representative of the country. The responses were coded and transformed into spreadsheets using Epi-Info® software 7.2.2.6 (Centres for Disease Control and Prevention; USA). Drugs that were described by label name were identified using the SINDAN website (SINDAN, 2019) and entered according to the active compounds. The final data were summarised into percentages, and Chi-square and Fisher's exact tests were used to perform pairwise comparisons between categories of the same independent variable proportions. A p value < 0.05 was considered as statistically significant. Analyses were performed using GraphPad Prism 7.0 (Graphpad Software, Inc., San Diego, CA, USA).

Distribution maps of responders and of suspected fipronil resistance in each state and Brazilian biomes were created using the program QGis 3.14. The shapes of Brazilian biomes and states were obtained from the Brazilian Institute of Geography and Statistics (IBGE) database (https://portaldemapas.ibge.gov.br/portal.php#homepage) (IBGE, 2019).

Results

The 403 respondents were veterinary practitioners working in 149 municipalities (from the total of 5,568 municipalities of Brazil) from 24 of the 27 Brazilian states, distributed proportionally according to Brazilian population across each political region (Figure 1A). Of the six official biomes present in Brazil, no responses were received from Pantanal (Figure 1A), which represents only 1.8% of the total Brazilian territory area (IBGE, 2019). Analysis of the data by biome was not the purpose of the present study and was not performed; nevertheless the description of responses from different Brazilian biomes was important to demonstrate that the data were

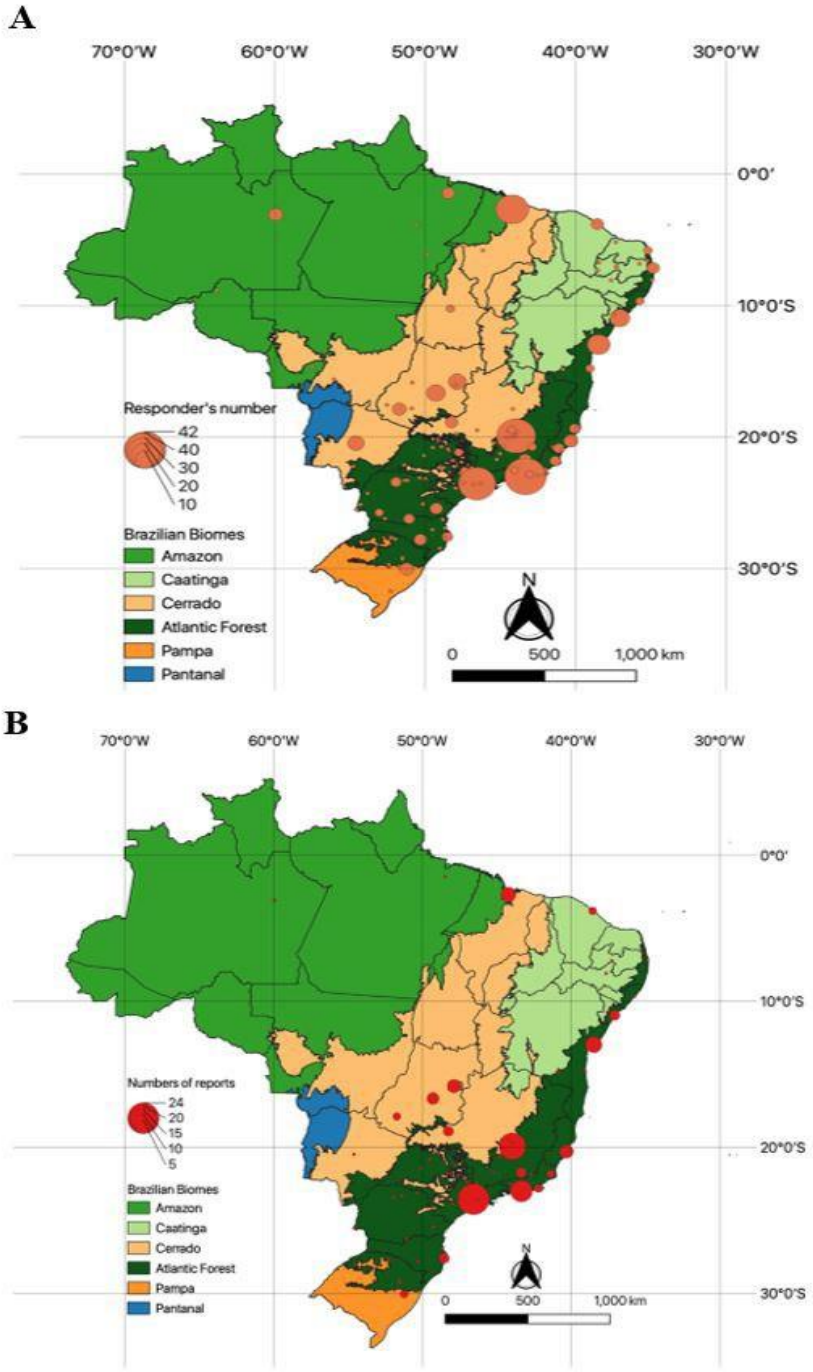

Figure 1. Distribution of the number of responders (A) and reports of inefficacy suspicious of fipronil against ectoparasites (B) in different states and biomes of Brazil.

representative. Two hundred and eighty-eight (71.4%) of these respondents stated (from a veterinary practitioner perspective) that dog owners consider parasite control to be important.

Gastrointestinal helminth control practices

The drugs used by Brazilian veterinary practitioners for treatment or prophylaxis of canine gastrointestinal helminths are shown in Table 1. Each drug was considered as one response and multiple responses were accepted giving rise to 495 responses describing recommended drugs. The multiple responses were not considered as concomitant treatments. Drug combinations were deduced from commercial brands. 32.3% of the responses recommended treatment with a single drug active (n=160 from benzimidazoles, pyrazinoisoquinoline, pyrimidine and macrocyclic lactone chemical groups) and 62.2% recommended the use of drugs with combinations of compounds (n=308). A combination of praziquantel plus pyrantel plus febantel was most commonly used with 25.3% recommendation by Brazilian veterinary practitioners. Treatments with a single active, such as praziquantel (7.9%), ivermectin (5.7%) and albendazole (5.1%) were recommended by some veterinary practitioners.

Eighty-three respondents (20.6%) perceived anthelmintic inefficacy, and a further five respondents who reported suspected inefficacy of two drugs. Inefficacy was suspected for benzimidazole drugs (28.4%), combinations of drugs (23.9%) and, macrocyclic lactones (12.5%) (Table 1). Despite the large range of anthelmintic compounds available in Brazil, only 57.0% of the respondents routinely changed the drug that they recommended.

Table 1. The percentages and number of responses (in bracket) showing the chemical groups and drug compounds recommended by Brazilian veterinarians for treatment and prophylaxis of canine gastrointestinal helminths and suspicion of inefficacy.

Chemical group / compound	Recommended[1]	Suspect inefficacy[2]
Benzimidazoles	**14.3 (71)^{B}**	**28.4 (25)^{A}**
Albendazole	5.1 (25)^{c,d}	8.0 (7)^{a}
Fenbendazole	2.2 (11)^{e}	8.0 (7)^{a}
Febantel	4.2 (21)^{d,e}	2.3 (2)^{b}
Mebendazole	2.8 (14)^{e}	10.2 (9)^{a,b}
Pyrazinoisoquinoline	**7.9 (39)^{C}**	**9.1 (8)^{C}**
Praziquantel	7.9 (39)^{c}	9.1 (8)^{a,b}
Pyrimidine	**2.2 (11)^{D}**	**10.2 (9)^{C}**
Pyrantel	2.2 (11)^{e}	10.2 (9)^{a,b}
Macrocyclic lactone	**7.9 (39)^{C}**	**12.5 (11)^{B,C}**
Ivermectin	5.7 (28)^{c,d}	10.2 (9)^{a,b}
Milbemycin oxime	2.2 (11)^{e}	2.3 (2)^{b}
Combinations	**62.2 (308)^{A}**	**23.9 (21)^{A,B}**

Praziquantel+Febantel	4.0 (20)[d,e]	-
Praziquantel+Febendazole	2.2 (11)[e]	-
Praziquantel+Pyrantel	7.9 (39)[c]	8.0 (7)[a,b]
Praziquantel+Pyrantel+Febantel	25.3 (125)[a]	9.1 (8)[a,b]
Praziquantel+Pyrantel+Fenbendazole	15.6 (77)[b]	3.4 (3)[b]
Praziquantel+Pyrantel+Febantel+Ivermectin	7.3 (36)[c]	3.4 (3)[b]
Others	**5.5 (27)[c,d,C]**	**15.9 (14)[a,B,C]**

[1] Recommendations based on a total of 495 responses (multiple responses were allowed); [2]Suspicion of inefficacy based on a total of 88 responses (multiple responses were allowed). In bold the total number of responses by chemical group or combination. The different letters signify statistical differences with p <0.05, being lower letters among the compounds and capital letters among the chemical group or combination.

The regimes used by interviewed veterinary practitioners for the prophylaxis and treatment of canine gastrointestinal helminths are shown in Table 2. More respondents (46.1%) recommended administration of prophylactic treatments at 3-month intervals. More respondents (60.7%) recommended repeating drug administration after 15 days for the treatment of gastrointestinal nematode infections. Less the half (43.5%) of veterinary practitioners requested laboratory examination to confirm parasite species infection before the treatment (Table 2). Those veterinary practitioners who requested laboratory confirmation, did so once (15.9%), or twice a year (21.1%).

Table 2. The percentages and number (n) of responses (in bracket) of Brazilian veterinarian regimes of recommendations to control canine gastrointestinal helminths.

Regimes	% (n)
Prophylaxis of gastrointestinal helminth	
Every month	18.8 (76)[b]
Every 3-months	46.1 (186)[a]
Every 6-months	21.8 (88)[b]
Other (irregular intervals)	13.1 (53)[c]
Treatment of gastrointestinal helminth	
Treatment and repeat after 15 days	60.7 (245)[a]
Treatment for three consecutive days	18.8 (76)[b]
Treatment for three consecutive days and repeat after 15 days	8.9 (36)[c]
Other (irregular intervals)	10.9 (44)[c]
Not answer	0.5 (2)[d]
Request laboratory examination	
No	52.6 (212)[a]
Every 3-months	6.0 (24)[c]
Every 6-months	21.1 (85)[b]

Once a year	15.9 (64)[b]
Other (irregular intervals)	0.5 (2)[d]
Not answer	4.0 (16)[c]

The different letters signify statistical differences with p <0.05 among different action for the same regime.

Control practices against ectoparasites

The most frequently reported canine ectoparasites were ticks followed by fleas, mites and lice (Figure 2). The drugs used for treatment or prophylaxis of canine ectoparasitic infestations are shown in Table 3. Each drug was considered as one response and multiple responses were accepted, resulting in 750 responses for recommended drugs. Isoxazolines were the most frequently recommended ectoparasiticide drug active (60.7%), followed by fipronil (14.5%).

Two hundred and five respondents suspected of poor efficacy. Fipronil inefficacy was suspected by 163 respondents (79.5%) in the 19 Brazilian states with highest densities of dogs and highest numbers of respondents (Figure 1B). Suspicion of inefficacy of the recently launched isoxazoline compounds was reported by seven respondents (3.4%) (Table 3).

Prophylaxis against ectoparasitic infestations was recommend by 333 (82.7%) of the Brazilian veterinary practitioners. The regimes used by Brazilian veterinary practitioners for the prophylaxis of canine ectoparasites are shown in Table 4. A minimum of monthly systemic, topical and/or drug impregnated collar treatments was recommended by 87 respondents (21.5%), followed by three-monthly treatments recommended by 83 (20.6%). However, 93 respondents (23.0%) recommended treatments at irregular times.

One hundred and eighty-eight respondents (46.6%) frequently changed the drug products (but not necessarily the actives) used. Skin irritation caused by acaricide/insecticide impregnated collars was reported by 234 respondents (58%). Three hundred and seventy-two of the veterinary practitioners (92.3%) recommended environmental hygiene management alongside prophylactic or therapeutic drug use for canine ectoparasites.

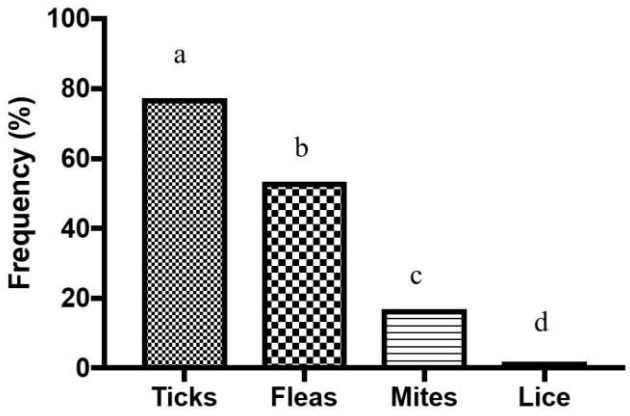

Figure 2. Frequency of the most common ectoparasites in dogs reported by veterinary practitioners from Brazil. Multiple responses were allowed. The different letters signify statistical differences with p <0.05.

Table 3. The percentages and number of responses (in bracket) of the chemical group (in bold) and drug compounds recommended and suspect inefficacy used by Brazilian veterinarians to treat canine ectoparasites.

Chemical group / compound	Recommended[1]	Suspect inefficacy[2]
Phenylpyrazol	**14.5 (109)^{B}**	**79.5 (163)^{A}**
Fipronil	14.5 (109)^{c}	79.5 (163)^{a}
Isoxazolines	**60.7 (455)^{A}**	**3.4 (7)^{C}**
Afoxolaner	14.0 (105)^{c}	3.4 (7)^{c}
Fluralaner	26.0 (195)^{a}	-
Sarolaner	20.7 (155)^{b}	-
Pyrethroids	**4.3 (32)^{D}**	**6.3 (13)^{B,C}**
Deltamethrin	2.8 (21)^{f,g}	2.9 (6)^{c}
Permethrin	1.5 (11)^{h}	3.4 (7)^{c}
Macrocyclic lactone	**6.1 (46)^{D}**	**-**
Ivermectin	1.3 (10)^{h}	-
Selamectin	4.8 (36)^{e}	-
Combinations	**5.5 (41)^{D}**	**-**
Imidacloprid, flumethrin	3.6 (27)^{e,f}	-
Imidacloprid, methoprene	1.9 (14)^{g,h}	-
Others	**8.9 (67)^{d,C}**	**10.7 (22)^{b,B}**

Multiple responses were allowed with 750 (^{1}) responses to recommend, and 205 (^{2}) responses suspect of inefficacy. In bold the total number of responses by chemical group or combination. The different letters signify statistical differences with $p < 0.05$, being lower letters among the compounds and capital letters among the chemical group or combination.

Table 4. The percentages and number (n) of responses (in bracket) of regimes used by Brazilian veterinarians for prophylaxis of canine ectoparasites.

Regimes	% (n)
Prophylaxis of ectoparasites	
Every month	21.5 (87)^{a,b}
Every 3-months	20.6 (83)^{a,b}
Every 6-months	17.3 (70)^{b}
Other (irregular intervals)	23.0 (93)^{a}
Not answer	17.3 (70)^{b}

The different letters signify statistical differences with $p < 0.05$.

Discussion

The climatic conditions in tropical areas are favourable to the development of environmental parasite stages, resulting in high levels of infectious challenge (Robinson et al., 1989; Epe, 2009). In Brazil, dogs from different regions are hosts for a large number of pathogenic flea, louse, mite, tick and fly ectoparasites, and tapeworm and roundworm gastrointestinal endoparasites (Dantas-Torres & Otranto, 2014). Effective control regimes specific to Brazilian dogs are essential to ensure satisfactory states of animal welfare (Despommier, 2003; Epe, 2009). The value of antiparasitic drugs is threatened by the

emergence of resistance or tolerance; hence recommendations must account for responsible and potentially sustainable therapeutic and prophylactic drug use. In this report, we describe the first wide-scale survey of Brazilian veterinary practitioners' practices and attitudes towards canine parasite management.

Dog owners have an important role to play, as they are responsible for putting recommendations into practice. According to 71.4% of the veterinarians surveyed, dog owners are concerned about the impact of highly visible ectoparasites (Costa-Junior et al., 2012; Heukelbach et al., 2012) and gastrointestinal helminths on their animals. Similarly, half of the pet owners from a study in Canadá had concern for their pet's parasites, and the veterinarians are the primary sources of owner education (Evason et al., 2021).

Veterinary practitioners have an important role to play in educating pet owners with different financial and educational backgrounds (Pereira et al., 2016) about parasites, which present animal welfare and public health risks (Strube et al., 2019). However, it is imperative to add owner's education in a routine of veterinary practice to reduce the risk of pet parasitism and zoonoses (Palmer et al., 2010; Baneth et al., 2012). On the other hand, veterinarian's education and scientific updating are also necessary to improve the knowledge and consequently the control of pet parasitism and zoonoses (Overgaauw & Boersema, 1996).

It has been recommended that all dogs should be tested for gastrointestinal parasites at least once every three months and after administration of anthelmintic drugs (TroCCAP, 2019), to account for continuous reinfection (Little et al., 2009) as prevails in Brazil. Despite this recommendation, 52.6%, of the Brazilian veterinary practitioners did not request coproparasitological examination and the majority simply recommended fixed interval treatments. In other countries, the proportion of veterinary practitioners that recommend the treatment without a coprological diagnostic increased in the last years (Kornblatt & Schantz, 1980; Harvey et al., 1991; Stull et al., 2007). The coprological diagnosis of gastrointestinal parasites of dogs in Brazil is predominantly based on relatively inexpensive sedimentation or floatation methods. Better understanding is needed concerning why these are not widely adopted, for example due to potential inaccessibility or perceptions of poor sensitivity.

Prophylaxis for gastrointestinal helminths was performed every 3-months by 46% of the veterinary practitioners and 61% recommended repeat treatments after 15 days (Table 4). This repetition is necessary because most of the products recommended have reduced action against immature or migratory stages, for example pyrantel only targets intestine stages while benzimidazoles have short half-lives (Plumb, 2018). The high rate of non-targeted drug administration by Brazilian veterinary practitioners, could potentially result in a high selection pressure for anthelmintic resistance (Shalaby, 2013), especially in tropical and subtropical regions that have a favorable conditions for the development of gastrointestinal parasites (Klimpel et al., 2010; Dantas-Torres & Otranto, 2014; Alho et al., 2018). Dogs owners from Belgium, Netherlands, and Finland had lower anthelmintic treatment frequency than in the present study (Pullola et al., 2006; Lempereur et al., 2020), which can be justified by epidemiology and risk factors of the nematodes in these temperate countries. Nevertheless, the number of dogs deworm in the UK is similar to Brazil, demonstrating that there is also an owner behavioral aspect (Pennelegion et al., 2020).

Regional specific knowledge of the epidemiology of parasites is necessary to inform appropriate drug treatment, reducing the selection pressure for resistance. Simply increasing the frequency of treatment incurs increased costs and potentially increases the selection pressure for resistance. Resistant strains of *Ancylostoma caninum* and *Dipylidium caninum*

have been reported in the USA (Jesudoss Chelladurai et al., 2018; Jimenez-Castro et al., 2019; Kitchen et al., 2019). A mutation in a β-tubulin gene has been reported linked benzimidazole resistance in *A. caninum* from Brazil (Furtado et al., 2014). It is, therefore, necessary to monitor to the effectiveness of anthelmintic drugs used in the management of canine gastrointestinal helminths in order to respond to emergence of resistance (Kopp et al., 2007).

Anthelmintic drug treatments of puppies for the control of maternally transmitted nematodes should start at the age of two weeks old, and be repeated every 15 days until the age of eight weeks (TroCCAP, 2019). Thereafter, treatments should be monthly in areas with high prevalence of gastrointestinal helminths (TroCCAP, 2019). For these strategies, 62.2% of the participating veterinary practitioners recommended the use of anthelmintic drug combination products, in particular involving praziquantel plus pyrantel plus a benzimidazole drug (Table 1), which is similar to that used in veterinary practice in other countries (Matos et al., 2015). Combinations of broad spectrum anthelmintics are required to treat the polyparasitism that it commonly encountered in Brazilian kennel dogs. This strategy is also potentially helpful in achieving improved, additive efficacy, decreased toxicity, and reduced development of drug resistance (Bartram et al., 2012; Rinaldi et al., 2015; Lanusse et al., 2018).

According to the veterinary practitioners' perspective, ticks, followed by fleas, mites and lice are the most important ectoparasites of dogs in Brazil (Figure 2). In the same way, *Rhipicephalus sanguineus* sensu lato, *Ctenocephalides canis* and *Ctenocephalides felis felis* are considered the most common canine ectoparasites worldwide, albeit the prevalence and importance of infestation differ according to the geographic region of dogs (Costa-Junior et al., 2012; Ebrahimzade et al., 2016).

Regular use of ectoparasiticides is widely recommended to protect dogs against ectoparasites and the pathogens that they transmit (CAPC, 2019). 82.7% of the respondents recommend the use of ectoparasiticide products (impregnated collars, pour-ons, spot-ons or tablets) as a prophylactic measure, with 21.5% recommending administration every month and 20.6% recommending three-monthly treatments.

Brazil is an endemic area for canine visceral leishmaniasis and dogs serve as a source of infection for phlebotomine sand flies (Rocha et al., 2020). The use of insecticide impregnated dog collars, which have both an anti-feeding and insecticidal activity is considered to be a useful tool for canine control of leishmaniasis (David et al., 2001). In addition to being used as an insecticide against sand flies, these collars are also recommended for tick and flea control (Silva et al., 2018). The dog collars constantly release the active chemical; and long half-life residues may be responsible for the selection of resistance in ticks (Beirão et al., 2009). 58% of the veterinary practitioner respondents had noticed skin irritation in dogs as a side-effect to collars.

The present study addresses the frequency of application or administration of acaricides in dogs in Brazil. The most frequently recommended drugs for the control of ectoparasites were fluralaner (26%), sarolaner (20.7%), afoxolaner (14%), and fipronil (14.5%). Slightly more than half of respondents recommend the rotation of these principles. Interestingly, all of the drugs mentioned in the control of ectoparasites, with the exception of fipronil, are the latest to be released on the market. Frequent use of ectoparasiticides may exert a selection pressure for resistance or tolerance in tick populations (Rodriguez-Vivas et al., 2017; Becker et al., 2019).

Several studies have shown resistance of *R. sanguineus* s.l. to permethrin in the USA (Eiden et al., 2016), cypermethrin and coumaphos in Brazil (Borges et al., 2007), amitraz in Panama (Miller et al., 2001), and ivermectin in Mexico (Rodriguez-Vivas et al., 2017). In Brazil, the first

report of *R. sanguineus* s.l. resistance to fipronil was recently published (Becker et al., 2019), supporting the veterinary practitioners' perceptions of inefficacy of in this study (Table 3). The suspicion of inefficacy of recently introduced isoxazolines is noteworthy.

Almost all of the veterinary practitioner respondents recommend environmental management for the control of ectoparasites in dogs in Brazil. The knowledge that ticks and fleas are non-permanent parasites makes it necessary to consider the management of environmental stages (Dantas-Torres & Otranto, 2014). Despite the variety of available products with proven efficacy against canine ectoparasites, there are no guidelines for the control of ectoparasites in the environment that take into account the different regions of Brazil. It is necessary to remind veterinary practitioners that the chemical control of other arthropods that co-infect dogs is more complex, and that the misuse of these compounds can cause environmental pollution and toxicity to humans and other organisms (Dantas-Torres & Otranto, 2014; Paz et al., 2008).

This study shows the wide use of antiparasitic products and treatment regimens for parasite control in dogs in Brazil. The isoxazoline and combination of anthelmintic compounds are the most common drugs to prevent or treat ectoparasites and gastrointestinal nematodes, respectively. The suspect of the inefficacy of antiparasitic drugs is shared among the veterinary practitioners from Brazil. It is necessary to improve awareness of the use of antiparasitic products that require the attention of veterinary practitioners and industry to applied effective and sustainable parasite control strategies.

Acknowledgements

The authors acknowledge the financial support received from CNPq (Brazilian National Council for Scientific and Technological Development), FAPEMA (Maranhão State Research Foundation) and FINEP (Funding Authority for Studies and Projects) (PRONEM 01773/14 and IECT (Science and Technology Institute of Maranhão) Biotechnology). This study was financed in part by CAPES, Finance Code 001. The authors wish to tanks CNPq to awarding a fellowship to L.M. Costa-Júnior and scholarship to M.N.G and thank FAPEMA and CAPES (Brazilian Federal Agency for support and evaluation of graduate education) for the scholarships to T.L.V, I.C.S, C.P.T, and N.C.S. We also thank COMAC from SINDAN for market data support and Vicente F. Pinheiro-Neto for the questionnaire collection.

References

Alho AM, Lima C, Colella V, Madeira de Carvalho L, Otranto D, Cardoso L. Awareness of zoonotic diseases and parasite control practices : a survey of dog and cat owners in Qatar. *Parasit Vectors* 2018; 11(1): 133. http://dx.doi.org/10.1186/s13071-018-27200. PMid:29554937.

Associação Brasileira da Indútria de Produtos de Origem Animal – ABINPET. *Mercado Pet Brasileiro* [online]. 2019 [cited 2020 Mar 5]. Available from: http://abinpet.org.br

Baneth G, Bourdeau P, Bourdoiseau G, Bowman D, Breitschwerdt E, Capelli G, et al. Vector-borne diseases--constant challenge for practicing veterinarians: recommendations from the CVBD World Forum. *Parasit Vectors* 2012; 5(1): 55. http://dx.doi. org/10.1186/1756-3305-5-55. PMid:22433172.

Bartram DJ, Leathwick DM, Taylor MA, Geurden T, Maeder SJ. The role of combination anthelmintic formulations in the sustainable control of sheep nematodes. *Vet Parasitol* 2012; 186(3-4): 151-158. http://dx.doi.org/10.1016/j.vetpar.2011.11.030. PMid:22245073.

Becker S, Webster A, Doyle RL, Martins JR, Reck J, Klafke GM. Resistance to deltamethrin, fipronil and ivermectin in the brown dog tick, *Rhipicephalus sanguineus* sensu stricto, Latreille (Acari: ixodidae). *Ticks Tick Borne Dis* 2019; 10(5): 1046-1050. http://dx.doi. org/10.1016/j.ttbdis.2019.05.015. PMid:31175029.

Beirão BCB, Rosinelli ADS, Da Silva LCS, Santos GDGC, Ruthes LD, Molento MB. Protocolos utilizados em consultórios, clínicas e hospitais veterinários para o controle de endo e ectoparasitas em pequenos animais em Curitiba. *Arch Vet Sci* 2009; 14(4): 221-227. http://dx.doi.org/10.5380/avs.v14i4.13435.

Borges LMF, Soares SF, Fonseca IN, Chaves VV, Louly CCB. Resistência acaricida em larvas de *Rhipicephalus sanguineus* (Acari: Ixodidae) de Goiânia-GO, Brasil. *Rev Patol Trop* 2007; 36(1): 87-95.

Chomel BB, Sun B. Zoonoses in the Bedroom. *Emerg Infect Dis* 2011; 17(2): 167-172. http://dx.doi.org/10.3201/eid1702.101070. PMid:21291584.

Companion Animal Parasite Council – CAPC. [online]. 2019 [cited 2019 Nov 25]. Available from: https://capcvet.org/guidelines/ hookworms/

Conselho Federal de Medicina Veterinária – CFMV. [online]. 2019 [cited 2020 Aug 25]. Available from: http://portal.cfmv.gov.br/ noticia/index/id/5827/secao/6

Costa-Junior LM, Rembeck K, Mendonça FLM, Azevedo SC, Passos LMF, Ribeiro MFB. Occurrence of ectoparasites on dogs in rural regions of the state of Minas Gerais, Brazil. *Rev Bras Parasitol Vet* 2012; 21(3): 237-242. http://dx.doi.org/10.1590/S198429612012000300011. PMid:23070433.

Dantas-Torres F, Ketzis J, Mihalca AD, Baneth G, Otranto D, Tort GP, et al. TroCCAP recommendations for the diagnosis, prevention and treatment of parasitic infections in dogs and cats in the tropics. *Vet Parasitol* 2020; 283: 109167. http://dx.doi.org/10.1016/j. vetpar.2020.109167. PMid:32580071.

Dantas-Torres F, Otranto D. Dogs, cats, parasites, and humans in Brazil: opening the black box. *Parasit Vectors* 2014; 7(1): 22. http://dx.doi.org/10.1186/1756-3305-7-22. PMid:24423244.

David JR, Stamm LM, Bezerra HS, Souza RN, Killick-Kendrick R, Lima JWO. Deltamethrin-impregnated dog collars have a potent anti-feeding and insecticidal effect on *Lutzomyia longipalpis* and *Lutzomyia migonei. Mem Inst Oswaldo Cruz* 2001; 96(6): 839-847. http://dx.doi.org/10.1590/S0074-02762001000600018. PMid:11562713.

Despommier D. Toxocariasis: clinical aspects, epidemiology, medical ecology, and molecular aspects. *Clin Microbiol Rev* 2003; 16(2): 265-272. http://dx.doi.org/10.1128/CMR.16.2.265-272.2003. PMid:12692098.

Ebrahimzade E, Fattahi R, Ahoo MB. Ectoparasites of stray dogs in Mazandaran, Gilan and Qazvin Provinces, north and center of Iran. *J Arthropod Borne Dis* 2016; 10(3): 364-369. PMid:27308294.

Eiden AL, Kaufman PE, Allan SA, Oi F. Establishing the discriminating concentration for permethrin and fipronil resistance in *Rhipicephalus sanguineus* (Latreille) (Acari : Ixodidae), the brown dog tick. *Pest Manag Sci* 2016; 72(7): 1390-1395. http://dx.doi. org/10.1002/ps.4165. PMid:26449370.

Epe C. Intestinal Nematodes: biology and control. *Vet Clin North Am Small Anim Pract* 2009; 39(6): 1091-1107, vi-vii. http://dx.doi. org/10.1016/j.cvsm.2009.07.002. PMid:19932365.

European Scientific Counsel Companion Animal Parasites – ESCCAP. [online]. 2019 [cited 2019 Nov 3]. Available from: https:// www.esccap.org/

Evason M, McGrath M, Stull J. Companion animal preventive care at a veterinary teaching hospital - Knowledge, attitudes, and practices of clients. *Can Vet J* 2021; 62(5): 484-490. PMid:33967287.

Furtado LFV, Bello ACPP, dos Santos HA, Carvalho MRS, Rabelo ÉML. First identification of the F200Y SNP in the β-tubulin gene linked to benzimidazole resistance in *Ancylostoma caninum*. *Vet Parasitol* 2014; 206(3-4): 313-316. https://doi.org/10.1016/j.vetpar.2014.10.021.

Harvey JB, Roberts JM, Schantz PM. Survey of veterinarians' recom mendations for treatment and control of intestinal parasites in dogs: public health implications. *J Am Vet Med Assoc* 1991; 199(6): 702-707. PMid:1955360.

Heukelbach J, Frank R, Ariza L, de Sousa Lopes I, de Assis E Silva A, Borges AC, et al. High prevalence of intestinal infections and ectoparasites in dogs, Minas Gerais State (Southeast Brazil). *Parasitol Res* 2012; 111(5): 1913-1921. http://dx.doi.org/10.1007/ s00436-012-3037-0. PMid:22825284.

Instituto Brasileiro de Geografia e Estatística – IBGE. *Censo Brasileiro de 2010*. Rio de Janeiro: IBGE; 2012.

Instituto Brasileiro de Geografia e Estatística – IBGE. *Biomas e sistema costeiro-marinho do Brasil: compatível com a escala 1:250 000*. Rio de Janeiro: IBGE; 2019.

Jesudoss Chelladurai J, Kifleyohannes T, Scott J, Brewer MT. Praziquantel Resistance in the Zoonotic Cestode *Dipylidium caninum. Am J Trop Med Hyg* 2018; 99(5): 1201-1205. http://dx.doi.org/10.4269/ajtmh.18-0533. PMid:30226153.

Jimenez Castro PD, Howell SB, Schaefer JJ, Avramenko RW, Gilleard JS, Kaplan RM. Multiple drug resistance in the canine hookworm *Ancylostoma caninum*: an emerging threat? *Parasit Vectors* 2019; 12(1): 576. http://dx.doi.org/10.1186/s13071-0193828-6. PMid:31818311.

Kitchen S, Ratnappan R, Han S, Leasure C, Grill E, Iqbal Z, et al. Isolation and characterization of a naturally occurring multidrugresistant strain of the canine hookworm, *Ancylostoma caninum. Int J Parasitol* 2019; 49(5): 397-406. http://dx.doi.org/10.1016/j. ijpara.2018.12.004. PMid:30771359.

Klimpel S, Heukelbach J, Pothmann D, Rückert S. Gastrointestinal and ectoparasites from urban stray dogs in Fortaleza (Brazil): high infection risk for humans? *Parasitol Res* 2010; 107(3): 713-719. http://dx.doi.org/10.1007/s00436-010-1926-7. PMid:20532563.

Kopp SR, Kotze AC, McCarthy JS, Coleman GT. High-level pyrantel resistance in the hookworm *Ancylostoma caninum. Vet Parasitol* 2007; 143(3-4): 299-304. http://dx.doi.org/10.1016/j.vetpar.2006.08.036. PMid:17011128.

Kornblatt AN, Schantz PM. Veterinary and public health considerations in canine roundworm control: a survey of practicing veterinarians. *J Am Vet Med Assoc* 1980; 177(12): 1212-1215. PMid:7440322.

Lanusse C, Canton C, Virkel G, Alvarez L, Costa-Junior L, Lifschitz A. Strategies to optimize the efficacy of anthelmintic drugs in ruminants. *Trends Parasitol* 2018; 34(8): 664-682. http://dx.doi.org/10.1016/j.pt.2018.05.005. PMid:29960843.

Lempereur L, Nijsse R, Losson B, Marechal F, De Volder A, Schoormans A, et al. Coprological survey of endoparasite infections in owned dogs and owners' perceptions of endoparasite control in Belgium and the Netherlands. *Vet Parasitol Reg Stud Reports* 2020; 22: 100450. http://dx.doi.org/10.1016/j.vprsr.2020.100450. PMid:33308762.

Little SE, Johnson EM, Lewis D, Jaklitsch RP, Payton ME, Blagburn BL, et al. Prevalence of intestinal parasites in pet dogs in the United States. *Vet Parasitol* 2009; 166(1-2): 144-152. http://dx.doi.org/10.1016/j.vetpar.2009.07.044. PMid:19716659.

Matos M, Alho AM, Owen SP, Nunes T, Madeira de Carvalho L. Parasite control practices and public perception of parasitic diseases: A survey of dog and cat owners. *Prev Vet Med* 2015; 122(1-2): 174-180. http://dx.doi.org/10.1016/j.prevetmed.2015.09.006. PMid:26404913.

Miller RJ, George JE, Guerrero F, Carpenter L, Welch JB. Characterization of Acaricide Resistance in *Rhipicephalus sanguineus* (Latreille) (Acari: Ixodidae) Collected from the Corozal Army Veterinary Quarantine Center, Panama. *J Med Entomol* 2001; 38(2): 298-302. http://dx.doi.org/10.1603/0022-2585-38.2.298. PMid:11296838.

Miot HA. Sample size in clinical and experimental trials. *J Vasc Bras* 2011; 10(4): 275-279. http://dx.doi.org/10.1590/S167754492011000400001. PMid:30787944.

Overgaauw PA, Boersema JH. Assessment of an educational campaign by practicing veterinarians in The Netherlands on human and animal Toxocara infections. *Tijdschr Diergeneeskd* 1996; 121(21): 615-618. PMid:8966710.

Palmer CS, Robertson ID, Traub RJ, Rees R, Andrew Thompson RC. Intestinal parasites of dogs and cats in Australia: the veterinarian's perspective and pet owner awareness. *Vet J* 2010; 183(3): 358-361. http://dx.doi.org/10.1016/j.tvjl.2008.12.007.

Paz GF, Leite RC, de Oliveira PR. Controle de *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae) no canil da Escola de Veterinária da UFMG, Belo Horizonte, Minas Gerais, Brasil. *Rev Bras Parasitol Vet* 2008; 17(1): 41-44. http://dx.doi.org/10.1590/ S1984-29612008000100009. PMid:18554440.

Pennelegion C, Drake J, Wiseman S, Wright I. Survey of UK pet owners quantifying internal parasite infection risk and deworming recommendation implications. *Parasit Vectors* 2020; 13(1): 218. http://dx.doi.org/10.1186/s13071-020-04086-2. PMid:32336273.

Pereira A, Martins Â, Brancal H, Vilhena H, Silva P, Pimenta P, et al. Parasitic zoonoses associated with dogs and cats: A survey of Portuguese pet owners' awareness and deworming practices. *Parasit Vectors* 2016; 9(1): 245. http://dx.doi.org/10.1186/s13071016-1533-2. PMid:27160667.

Plumb DC. *Plumb's Veterinary Drug Handbook: Desk*. 9th ed. Hoboken, NJ, USA: John Wiley & Sons; 2018.

Pullola T, Vierimaa J, Saari S, Virtala AM, Nikander S, Sukura A. Canine intestinal helminths in Finland: prevalence, risk factors and endoparasite control practices. *Vet Parasitol* 2006; 140(3-4): 321-326. http://dx.doi.org/10.1016/j.vetpar.2006.04.009. PMid:16650936.

Rinaldi L, Pennacchio S, Musella V, Maurelli MP, La Torre F, Cringoli G. Helminth control in kennels: is the combination of milbemycin oxime and praziquantel a right choice? *Parasit Vectors* 2015; 8(1): 30. http://dx.doi.org/10.1186/s13071-015-0647-2. PMid:25595418.

Robinson RD, Thompson DL, Lindo JF. A survey of intestinal helminths of well-cared-for dogs in Jamaica, and their potential public health significance. *J Helminthol* 1989; 63(1): 32-38. http://dx.doi.org/10.1017/S0022149X00008695. PMid:2723384.

Rocha MF, Michalsky ÉM, de Oliveira Lara-Silva F, Valadão JL, França-Silva JC, Pinheiro LC, et al. Dogs with divergent serology for visceral leishmaniasis as sources of *Leishmania* infection for *Lutzomyia longipalpis* phlebotomine sand flies – an observational study in an endemic area in Brazil. *PLoS Negl Trop Dis* 2020; 14(2): e0008079. http://dx.doi.org/10.1371/journal.pntd.0008079. PMid:32078634.

Rodriguez-Vivas RI, Ojeda-Chi MM, Trinidad-Martinez I, Pérez de León AA. First documentation of ivermectin resistance in
Rhipicephalus sanguineus sensu lato (Acari: ixodidae). *Vet Parasitol* 2017; 233: 9-13. http://dx.doi.org/10.1016/j.vetpar.2016.11.015. PMid:28043394.

Shalaby HA. Anthelmintics resistance; how to overcome it? *Iran J Parasitol* 2013; 8(1): 18-32. PMid:23682256.

Silva RAE, Andrade AJ, Quint BB, Raffoul GES, Werneck GL, Rangel EF, et al. Effectiveness of dog collars impregnated with 4% deltamethrin in controlling visceral leishmaniasis in *Lutzomyia longipalpis* (Diptera: Psychodidade: Phlebotominae) populations. *Mem Inst Oswaldo Cruz* 2018; 113(5): e170377. http://dx.doi.org/10.1590/0074-02760170377. PMid:29590235.

Sindicato Nacional da Indústria de Produtos para Saúde Animal – SINDAN. *Compêndio de produtos veterinários* [online]. 2019 [cited 2019 Nov 25]. Available from: https://sistemas.sindan.org.br/cpvs/pesquisar.aspx

Strube C, Neubert A, Springer A, Von Samson-Himmelstjerna G. Survey of German pet owners quantifying endoparasitic infection risk and implications for deworming recommendations. *Parasit Vectors* 2019; 12(1): 203. http://dx.doi.org/10.1186/s13071-0193410-2. PMid:31053079.

Stull JW, Carr AP, Chomel BB, Berghaus RD, Hird DW. Small animal deworming protocols, client education, and veterinarian perception of zoonotic parasites in western Canada. *Can Vet J* 2007; 48(3): 269-276. http://dx.doi.org/10.4141/cjas68-037. PMid:17436903.

Traub RJ, Irwin P, Dantas-Torres F, Tort GP, Labarthe NV, Inpankaew T, et al. Toward the formation of a Companion Animal Parasite Council for the Tropics (CAPCT). *Parasit Vectors* 2015; 8(1): 271. http://dx.doi.org/10.1186/s13071-015-0884-4. PMid:25963851.

Tropical Council for Companion Animal Parasites – TroCCAP. *Guidelines for the diagnosis, treatment and control of canine endoparasites in the tropics* [online]. 2019 [cited 2020 Aug 21]. Available from: https://www.troccap.com/2017press/wp-content/uploads/2019/05/TroCCAP_Canine_Endo_Guidelines_English_Ver2.pdf

Capítulo 2

Efeito carrapaticida *in vitro* e *in vivo* do carvacrol sobre *Rhipicephalus sanguineus* sensu lato e suas alterações no tegumento do carrapato

---

Neste capítulo consta o artigo a ser submetido no periódico *Veterinary Parasitology* Qualis A1; Fator de Impacto: 2.2.

Resumo

Rhipicephalus sanguineus sensu lato (s.l.), carrapato marrom do cão, é um ectoparasito que exige a adoção de medidas de controle por transmitir vários patógenos aos cães. O controle é realizado com moléculas carrapaticidas sintéticas e, mais recentemente, vêm se destacando a busca de moléculas extraídas de plantas, como os monoterpenos. Carvacrol é um monoterpeno, encontrado em óleos essenciais de diversas espécies vegetais como orégano, manjericão e que já foi sintetizado em laboratório, sendo vendido comercialmente. Diversos estudos já demonstraram a atividade carrapaticida do carvacrol em formulações tópicas. Adicionalmente, existem lacunas sobre a ação do carvacrol na cutícula de carrapatos. O presente estudo teve como objetivo avaliar a atividade carrapaticida do carvacrol *in vitro* através do ensaio de imersão de larvas e ninfas e *in vivo* pela administração oral em camundongos, e ainda obter informações acerca dos efeitos dessa molécula sobre o tegumento das ninfas alimentadas nesse hospedeiro tratado. A atividade carrapaticida *in vitro* do carvacrol em larvas e ninfas foi realizada através do ensaio de imersão usando nas concentrações 5,0; 3,5; 2,45; 1,71; 1,20; 0,84; 0,58; 0,41; 0,28 e 0,20 mg/mL diluído em etanol 95% PA-triton 0,02%. Para encontrar a concentração letal média CL_{50} as larvas e ninfas foram contadas em vivas e mortas 24h após a imersão. E para avaliação da administração oral, os camundongos Swiss foram infestados artificialmente com 25 ninfas de *R. sanguineus* s.l. Os animais foram alocados em três grupos: controle negativo (Sorbitol), carvacrol (60 mg/kg) e controle positivo (Lotilaner 20 mg/kg/). Para determinar o efeito carrapaticida *in vivo*, foi realizada a contagem das ninfas mortas 48h após o tratamento e 48h após a segunda infestação. Posteriormente, os carrapatos sobreviventes da segunda infestação e que se alimentaram foram dissecados para coleta do tegumento e análise histológica. Os resultados obtidos no ensaio *in vitro* demonstraram que carvacrol apresentou uma CL_{50} de 1,2 mg/mL no teste de imersão de larvas e 1,82mg/mL para ninfas. Já a mortalidade dos carrapatos infestados nos camundongos foi de 17% para o grupo carvacrol na primeira infestação e 100% para o controle positivo ($p<0,05$). Na segunda infestação, não houve diferença significativa entre os grupos ($p>0,05$). Apesar desses resultados de eficiência, as ninfas que se alimentaram dos animais tratados com carvacrol apresentavam agregação de grânulos de proteínas entre as camadas epitelial e subcuticular, o que mostra a ação desse monoterpeno no ciclo de vida do carrapato. O estudo demonstrou pela primeira vez que o carvacrol e o lotilaner administrados através da via oral causam alterações no tegumento de *Rhipicephalus sanguineus* s.l., podendo interferir no desenvolvimento deste carrapato. E o carvacrol pode ser utilizado para o desenvolvimento de produtos com ação carrapaticida.

Palavras-chave: carrapato, carvacrol, terpenos, carrapato marrom e camundongos.

Abstract

Rhipicephalus sanguineus sensu lato (s.l.), brown dog tick, is an ectoparasite that requires the adoption of control measures for transmitting several pathogens to dogs. Control is carried out with synthetic acaricide molecules and, more recently, the search for molecules extracted from plants, such as monoterpenes, has been highlighted. Carvacrol is a monoterpene, found in essential oils of several plant species such as oregano, basil and which has already been synthesized in the laboratory and sold commercially. Several studies have already demonstrated the acaricide activity of carvacrol in topical formulations. Additionally, there are gaps regarding the action of carvacrol on the cuticle of ticks. The present study aimed to evaluate the acaricidal activity of carvacrol *in vitro* through the immersion test of larvae and nymphs and *in vivo* by oral administration in mice, and to obtain information about the effects of this molecule on the tegument of nymphs fed on this treated host. The *in vitro* acaricidal activity of carvacrol on larvae and nymphs was performed through the immersion test using concentrations of 5.0; 3.5; 2.45; 1.71; 1.20; 0.84; 0.58; 0.41; 0.28 and 0.20 mg/mL diluted in 95% ethanol PA-0.02% triton. To find the average lethal LC_{50} concentration, larvae, and nymphs were counted alive and dead 24h after immersion. And for evaluation of oral administration, Swiss mice were artificially infested with 25 nymphs of *R. sanguineus* s.l. The animals were divided into three groups: negative control (Sorbitol), carvacrol (60 mg/kg), and positive control (Lotilaner 20 mg/kg/). To determine the acaricidal effect *in vivo*, dead nymphs were counted 48 hours after treatment and Day +9. Subsequently, ticks that survived the second infestation and that had fed were dissected for tegument collection and histological analysis. The results obtained in the *in vitro* assay showed that carvacrol presented a LC_{50} of 1.2 mg/mL in the immersion test for larvae and 1.82 mg/mL for nymphs. Mortality of infested ticks in mice was 17% for the carvacrol group in the first infestation and 100% for the positive control ($p<0.05$). Second infestation, there was no significant difference between groups ($p>0.05$). Despite these efficiency results, the nymphs that fed on animals treated with carvacrol showed aggregation of protein granules between the epithelial and subcuticular layers, which shows the action of this monoterpene in the tick's life cycle. The study demonstrated for the first time that carvacrol and lotilaner administered orally cause changes in the tegument of *Rhipicephalus sanguineus* s.l., which may interfere with the development of this tick. Carvacrol can be used for the development of products with acaricide action.

Keywords: tick, carvacrol, terpenes, brown tick, and mice.

Introdução

Rhipicephalus sanguineus sensu lato (s.l.) (Acari: Ixodidae) é o carrapato mais comum encontrado em cães de áreas urbanas, sendo o principal responsável pela transmissão de agentes patogênicos que ocasionam doenças graves para esses animais, causadas por patógenos como *Babesia canis, Babesia vogeli, Hepatozoon canis, Mycoplasma haemocanis, Erlichia canis, Rickettsia rickettsii* e *Rickettsia coronorii* (Dantas-Torres et al., 2006; Mentz et al., 2016; Silva et al., 2017; Trotta et al., 2012). Diante de todos os desafios que os cães enfrentam em relação à manutenção da saúde, o controle dos carrapatos é fundamental, sendo os produtos sintéticos os mais utilizados para este fim, embora muitas vezes sejam empregados de forma errônea (Chomel, 2011). Este fato tem selecionado indivíduos de *R. sanguineus* s.l. resistentes, o que foi sugerido e comprovado no Brasil e em outros países (Vale et al., 2021; Miller et al., 2001; Becker et al., 2019; Eiden et al., 2015; Rodriguez-Vivas et al., 2017).

A existência de carrapatos resistentes às diferentes classes químicas e a demora para surgirem novas moléculas são um grande desafio no controle, de modo que são necessárias constantes pesquisas (Faza et al., 2013; Kiss et al., 2012). O uso de moléculas de origem vegetal é uma opção promissora, uma vez que já foi demonstrada a atividade carrapaticida em condições de laboratório e de campo, em pesquisas com *R. sanguineus* s.l. (Coelho et al., 2020; Monteiro et al., 2021; Lima de Souza et al., 2019; Delmonte et al., 2017; Novato et al., 2015; Sousa et al., 2022; Vale et al., 2021).

O carvacrol é um monoterpeno volátil, encontrado em óleos essenciais de espécies da família Lamiaceae, incluindo os gêneros *Thymus*, *Origanum*, *Satureja* e *Coridothymus*, e que possui atividade carrapaticida para diferentes espécies de carrapatos (Lima et al., 2017; Vale et al., 2021; Tavares et al., 2022), incluíndo R. sanguinues (Oliveira et al., 2020; Konig et al., 2021; Lima de Souza et al., 2019; Sousa et al., 2022). Este terpeno é classificado como seguro (GRAS) pela Food and Drug Administration (FDA) para uso como conservante e ingrediente aromatizante de alimentos (FDA, 2013; Suntres et al., 2015). As pesquisas desenvolvidas com o carvacrol estão concentradas em ensaios *in vitro* e na administração tópica (Dantas et al., 2021; Lima et al., 2019; Sousa et al., 2022) demonstrando a eficácia e provocando alterações no tegumento e várias outras estruturas de *R. sanguineus* s.l (Lima de Souza et al., 2019; Konig et al., 2021). Entretanto, existe a necessidade de conhecer os efeitos

do carvacrol em diferentes vias de administração, pois a resposta pode variar devido às propriedades farmacocinéticas e farmacodinâmicas dessa molécula (Xavier et al., 2022).

O objetivo do presente estudo foi avaliar a atividade carrapaticida *in vitro* do carvacrol, padronizar um modelo de estudo *in vivo* para avaliar a atividade através da administração oral em camundongos Swiss infestados artificialmente com ninfas de *R. sanguineus* s.l. e investigar as possíveis alterações morfológicas no tegumento do carrapato após o tratamento.

2. Materiais e Métodos

2.1 Carrapatos

As larvas e ninfas de *R. sanguineus* s.l. foram obtidas de uma colônia estabelecida a partir de teleóginas coletadas de um cão do município de São José de Ribamar - Maranhão, mantida em coelhos *Oryctolagus cuniculus* Linnaeus, 1758, a partir de câmaras de alimentação fixadas no dorso dos coelhos com cola comercial não tóxica (Bechara *et al.*, 1995). Para a realização dos bioensaios, foram utilizadas larvas e ninfas com idade entre 15 e 25 dias pós-ecdise. Esta pesquisa foi aprovada pelo Comitê de Ética do uso de Animais – CEUA, registrada com o nº 23115.005443/2017-51.

2.2 Compostos

Carvacrol (5-isopropyl-2-methylphenol; CAS 499-75-2,) e o D-Sorbitol (2S,3R,4R,5R)-Hexane-1,2,3,4,5,6-hexol: CAS S1876) foram adquiridos da Sigma-Aldrich (São Paulo, SP, Brazil), ambos com 99% de pureza. Para os bioensaios *in vitro* com larvas e ninfas não alimentadas, foram testadas até a máxima concentração de 5 mg/mL, usando etanol P.A como solvente da solução estoque e etanol-triton 0,02% com diluição seriada a 70%. Para o bioensaio *in vivo* por meio da administração oral, o carvacrol foi adicionado ao sorbitol para evitar desconforto e irritação à mucosa do animal.

2.3 Bioensaios *in vitro*

O teste de imersão larval (TIL) foi realizado de acordo com Klafke *et al.* (2006). Para esse teste, aproximadamente 100 larvas não alimentadas de *R. sanguineus* s.l. foram imersas em um microtubo do tipo eppendorf contendo 1 mL de carvacrol nas concentrações de 5,0; 3,5; 2,45; 1,71; 1,20; 0,84; 0,58; 0,41; 0,28; and 0,20 mg/mL

diluído em etanol 95% PA-triton 0,02%. A água destilada e etanol 95% PA-triton 0,02% foram usados como controle negativo. Após o período de imersão, as larvas foram colocadas no centro de uma folha de papel de filtro medindo 8,5 cm × 7,5 cm, dobrada ao meio e vedada. Os testes foram realizados em triplicatas para cada concentração. Os pacotes foram colocados em uma câmara climatizada a 27 ± 1°C e UR> 80 ± 10 (SOLAB® SL-200, São Paulo, Brazil) por 24h. Finalizado esse período, foi realizada a contagem de larvas vivas e mortas.

No teste de imersão de ninfas não alimentadas, foram utilizados os procedimentos e concentrações conforme o descrito anteriormente. Entretanto, foram usadas 10 ninfas não alimentadas em cada microtubo do tipo eppendorf contendo 1 mL de solução. Todos os testes foram feitos em triplicata, o que totalizou 30 ninfas por concentração, conforme adaptação de Monteiro et al. (2009).

2.4 Bioensaio *in vivo*

2.4.1 Animais

Camundongos Swiss, saudáveis, machos, com idade de 6-8 semanas, sem exposição prévia a infestação com carrapatos, foram utilizados como hospedeiros. Os animais foram alojados em gaiolas coletivas de polipropileno, forradas com maravalha, e alimentados com ração comercial e água filtrada em bebedouros, *ad libitum*. Todos os animais dos diferentes grupos foram alojados na mesma sala, com temperatura entre 22 e 24ºC, umidade relativa (UR%) entre 60 e 80% e luz 12:12h/fotociclo escuro. O comportamento dos animais foi avaliado diariamente e, ao final do experimento, os animais foram eutanasiados.

2.4.2 Tratamento Oral

Foram utilizados 15 camundongos Swiss como hospedeiros experimentais de ninfas em jejum de *R. sanguineus* s.l., segundo adaptação da metodologia descrita por Bechara *et al*. (1995). Para isso, câmaras de alimentação confeccionadas com tubos de polipropileno de 2,0 mL foram cortadas em aproximadamente 1,5 cm do topo e então fixadas sobre o dorso dos camundongos com cola não tóxica (Dia -2) (Figura 1). Para este procedimento, os animais foram sedados com uma associação de Xilazina (5mg/kg) e Quetamina (90mg/kg) e ainda receberam reposição de fluidos (NaCl 0,09%).

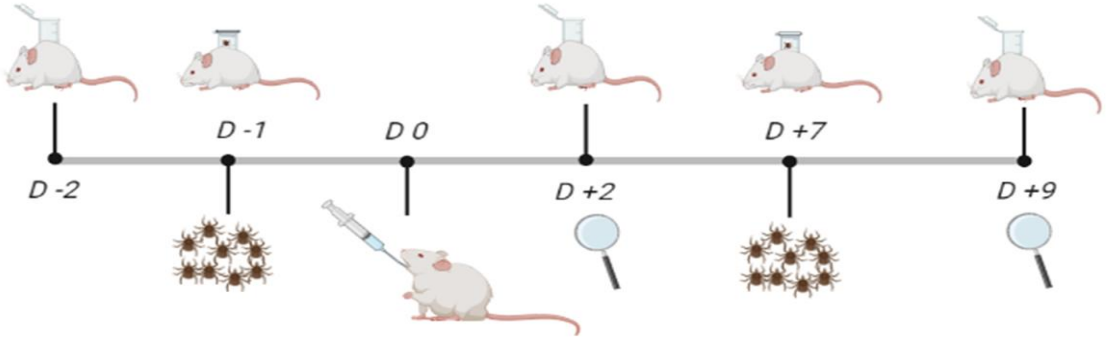

Figura 1. Delineamento experimental da infestação de *Rhipicephalus sanguineus* sensu lato em camundongos Swiss. Criado com BioRender.com

No Dia -1, 25 ninfas não alimentadas foram colocadas dentro de cada câmara de alimentação, e, após 24 horas (Dia 0), os camundongos foram divididos em três grupos com cinco animais: Grupo controle negativo (Neg), para o qual foi administrado, por via oral, 0,1 mL de sorbitol; grupo carvacrol (Carv), para o qual foram administrados, por via oral, 60 mg/kg de carvacrol em cada animal; e grupo controle positivo (Pos), que recebeu, por via oral, 20 mg/kg de Lotilaner por animal. Todos os animais foram pesados individualmente para realizar os cálculos das dosagens. Os grupos então foram tratados por via oral com auxílio de uma agulha de gavagem para garantir a precisão da dosagem. Cada camundongo foi observado por 2 horas após administração para observar alguma alguma reação adversa.

Os animais foram contidos e os carrapatos contados com o auxílio de uma lupa após 48 horas (Dia +2) do tratamento. Os animais foram novamente reinfestados no Dia +7 pós-tratamento e a contagem realizada no Dia +9, visando a avaliar a eficácia da persistência do tratamento (Figura 2). Durante a contagem dos carrapatos, somente os animais com a câmara fechada e sem aberturas foram considerados para avaliar a atividade carrapaticida.

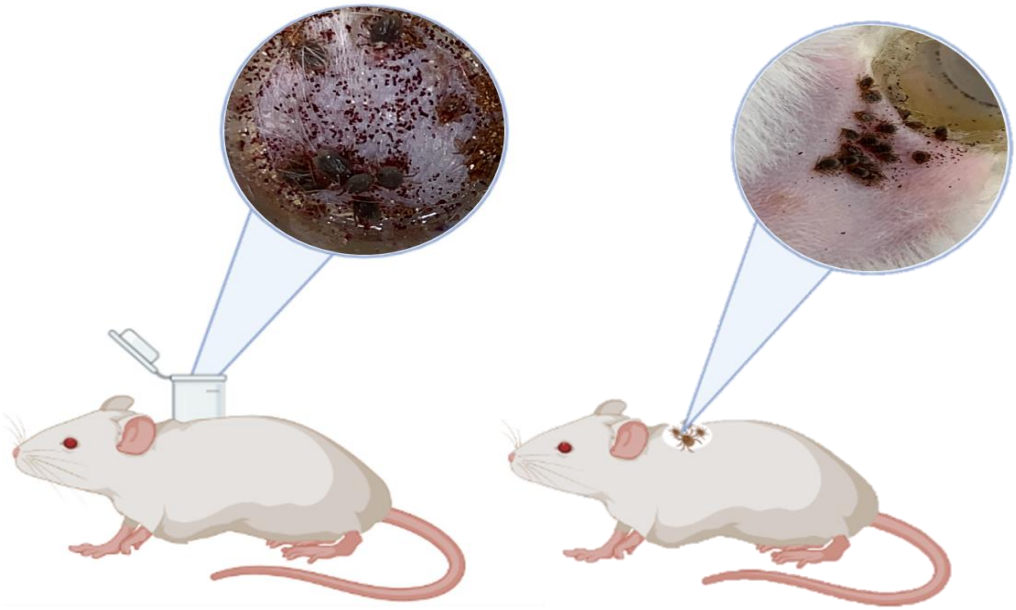

Figura 2. Infestação de *Rhipicephalus sanguineus* sensu lato em camundongos Swiss. Criado com Biorender.com

2.5. Análise histológica dos tegumentos das ninfas

A análise histológica foi realizada a partir dos tegumentos das ninfas alimentadas nos camundongos Swiss (Figura 1). Os carrapatos tratados foram dissecados em solução fisiológica a 0,06% para remoção do escudo dorsal e depois fixados em paraformaldeído a 4%, durante 30 dias. Depois, as amostras foram imersas em solução tampão fosfato (PBS) por 24h, desidratadas em sequência de etanol a 70, 80, 90 e 95% (1 hora cada banho), incorporados em Leica historesina por 7 dias e incluídas em moldes de plástico de polietileno (6 x 8 mm) previamente preenchidos com Leica polimerizada. Após a polimerização da resina, os blocos foram seccionados com 3 µm em micrótomo (LEICA RM 2255, Leica®). Os cortes foram corados com hematoxilina e eosina aquosa de Harris, e examinados e documentados com microscópio de campo claro com câmera acoplada (Olympus BX51/Olympus SC30).

2.6. Análise estatística

Nos bioensaios *in vitro*, calculou-se a concentração letal (LC_{50}) do carvacrol usando a regressão não linear. A eficiência carrapaticida no ensaio *in vivo* foi calculada a partir do percentual de redução da média aritmética dos carrapatos vivos do grupo tratado (GT) comparado à média aritmética do grupo controle (GC) usando a fórmula [(GC-GT) / GC]x100 (Marchiondo, 2007). A normalidade dos resultados da eficiência carrapaticida foi testada pelo teste de Shapiro-Wilk e posteriormente analisada por

Kruskal-Wallis, seguido pelo teste Dunns. Todas as análises estatísticas foram realizadas utilizando o software GraphPad Prism 8.0 (GraphPad Inc., CA, EUA) adotando nível de significância de 5%.

3. Resultados

3.1 Bioensaios *in vitro*

O carvacrol mostrou atividade carrapaticida *in vitro* com CL_{50} de 1,2 e 1,8 mg/mL sobre larvas e ninfas de *R. sanguineus*, respectivamente (Figura 3).

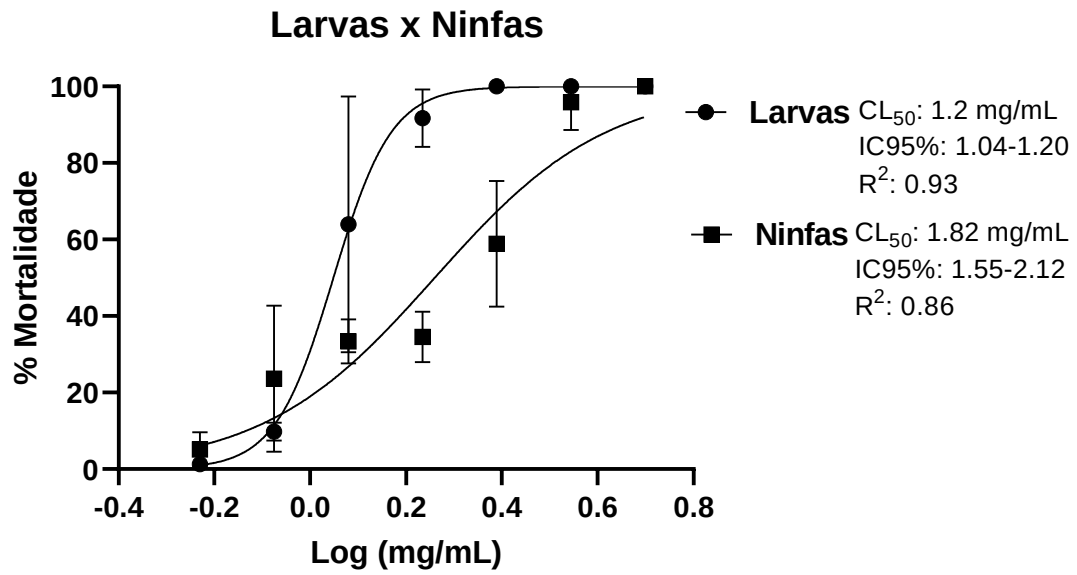

Figura 3. Eficácia do carvacrol sobre larvas e ninfas de *Rhipicephalus sanguineu* sensu lato. Cada ponto representa a média+- desvio padrão das três replicatas biológicas. CL_{50}: Concentração letal média (mg/mL) para 50% dos indivíduos; IC: intervalo de confiança de 95%; R^2: Coeficiente de Correlação de Regressão.

3.2 Bioensaio *in vivo*

Apesar da atividade carrapaticida no teste *in vitro* de contato, quando administrado oralmente, o carvacrol apresentou uma eficácia de 17%. Já no controle postivo (lotilaner), obteve-se uma atividade carrapaticida de 100% e houve uma diferença estatística em relação aos demais tratamentos no Dia +2 ($p<0,05$). No dia +9 pós-tratamento, houve uma redução da eficácia no lotilaner, igualando-se ao controle

negativo (Figura 4). Não foram observados efeitos adversos relacionados aos tratamentos.

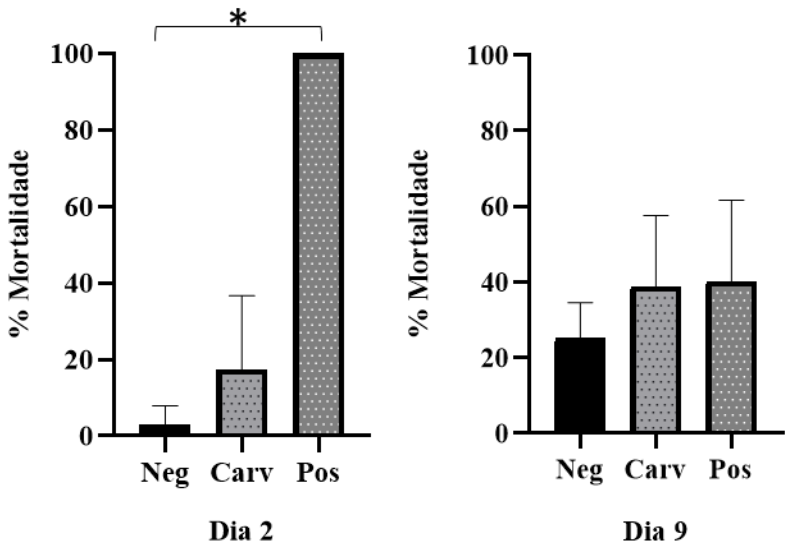

Figura 4. Percentual de mortalidade de *Rhipicephalus sanguineus* sensu lato infestados artificialmente em camundongos Swiss tratados por via oral: Neg (Controle negativo: sorbitol), Carv (Carvacrol: 60mg/kg) e Pos (Controle positivo: lotilaner 20mg/kg). Diferenças estatísticas foram encontradas entre os tratamentos através do teste Kruskal-Wallis, seguido por Dunn (*: $p<0.05$).

3.3 Análise histológica dos tegumentos das ninfas

O tegumento das ninfas do grupo controle negativo apresentou epitélio formado por uma camada de células pequenas e com formato variando de achatado a arredondado com seu citoplasma levemente acidófilo,núcleos esféricos e fortemente corados pela hematoxilina (Figura 5A).

As ninfas do grupo tratado com carvacrol (60 mg/kg) e coletadas após nove dias de tratamento apresentaram epicutícula, procutícula (exocutícula e endocutícula) semelhantes àquelas encontradas nos carrapatos do grupo controle negativo. Entretanto, a camada epitelial foi fortemente danificada. Em muitas regiões do tegumento das ninfas tratadas com carvacrol, ocorreram a destruição do epitélio e o surgimento de áreas desorganizadas. Quando presente, esse epitélio foi formado por uma camada de células bem pequenas e sobrepostas com núcleo arredados (Figura 5B).

O tegumento das ninfas oriundas dos animais tratados com lotilaner (20 mg/kg) apresentou alterações decorrentes da ação do composto sintético utilizado no grupo positivo, principalmente na região epidérmica e na cutícula (Figura 5C). A cutícula apresentou a epicutícula mais fina e menos corada em comparação ao controle negativo.

Ademais, a procutícula estava maior e menos corada também quando em relação ao controle negativo. Além disso, essa camada não apresentava mais subdivisões, formando uma camada única. Foi observada uma intensa desorganização das células epiteliais, exibindo alguns pontos de interrupção. Em algumas regiões, o epitélio aparece deformado e, em outras, ocorre o desaparecimento completo das células do tegumento das ninfas tratadas com lotilaner. Também se observaram vacúolos pequenos e arredondados que seguem em direção ao interior do corpo desses carrapatos (Figura 5C).

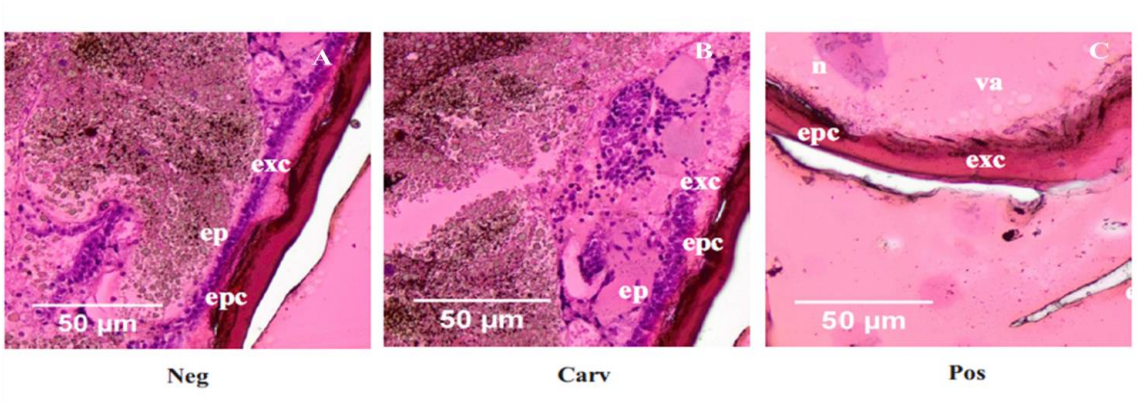

Figura 5. Cortes histológicos dos tegumentos das ninfas de *Rhipicephalus sanguineus* sensu lato corados com hematoxilina e eosina (HE). Grupo controle negativo (sorbitol) (A). Carvacrol 60 mg/kg (B) e Lotilaner 20 mg/kg (C). Ep – epitélio; epc – epicutícula; exc – procutícula; n – núcleo; va – vacúolos.'

4. Discussão

O presente estudo caracteriza-se como o primeiro teste da atividade carrapaticida do carvacrol através da administração oral, com importantes resultados da ação sobre o tegumento dos carrapatos oriundos de animais tratados. Diversos estudos já relataram a atividade carrapaticida de moléculas oriundas de óleos essenciais, como o carvacrol, o qual foi o foco do presente estudo (Coelho et al., 2020, Monteiro et al., 2021, Cardoso et al., 2020), além de levantarem hipóteses de mecanismos de ação na fisiologia de carrapatos (Cardoso et al., 2020; Tavares et al., 2022). Entretanto, nenhum avaliou formulação oral ou mesmo observou a cutícula dos carrapatos oriundos de animais tratados. O modelo experimental com camundongos Swiss infestados com *R. sanguineus* s.l. mostrou-se viável para a realização de bioensaios para avaliar a atividade carrapaticida.

A atividade carrapaticida do carvacrol já foi comprovada em diferentes espécies de carrapatos, bem como em diferentes estágios (Araújo et al.; 2016; Lima de Souza et al., 2019; 2022). Recentemente, foi comprovada a toxicidade do carvacrol em

Rhipicephalus microplus quando aumentou a atividade de enzimas de estresse oxidativos e desintoxicantes (Tavares et al., 2022). A atividade carrapaticida *in vitro* encontrada no presente estudo em baixas concentrações em estágios de larvas e ninfas reforça o potencial do carvacrol como molécula carrapaticida.

Apesar de o carvacrol ter apresentado uma atividade carrapaticida em ambos os estágios, as larvas demonstraram uma suscetibilidade maior em relação às ninfas (Figura 3). Isso ocorre devido às diferenças morfológicas e fisiológicas dos Ixodidae durante os estágios de larvas e ninfas. Como todos os artrópodes, os carrapatos são cobertos por um exoesqueleto que reveste o corpo e é responsável pela resistência estrutural, protegendo contra a perda de água e ainda sendo fonte de energia (Sonenshine & Roe, 2013).

O tegumento da larva favorece a ação dos compostos xenobióticos, já que é mais fácil permear o tegumento dos estágios mais imaturos do carrapato em comparação aos estágios mais avançados, devido ao fato da espessura da cutícula da larva ser mais fina (Sonenshine & Roe, 2014). Resultados semelhantes a esta pesquisa foram observados com timol (isômero do carvacrol) incorporado em formulações para uso tópico, mostrando que as larvas possuem uma susceptibilidade maior em relação aos demais estágios (Delmonte et al., 2017; Coelho et al., 2020; Monteiro et al., 2021).

Adicionalmente sabe-se que a via de administração pode alterar completamente a eficácia de qualquer medicamento. Dentro do cenário dos carrapaticidas de administração oral, damos destaque aos compostos da família das isoxazolinas (Afoxolaner, Fluralaner, Sarolaner e Lotilaner) uma classe de carrapaticida atualmente bastante utilizada para cães e gatos, atuando na inibição dos canais de cloreto controlados por glutamato e ácido gama-aminobutírico (GABA) em invertebrados (Zhou et al., 2022). Nesse contexto, buscamos entender o mecanismo de ação e administração oral do carvacrol e comparamos com o Lotilaner (controle positivo).

A administração oral de carvacrol mostrou-se eficaz no tratamento de infecções por *Campylobacter jejuni* e *Schistosoma mansoni* em camundongos, em doses que variaram de 100 e 200 mg/kg, respectivamente, demonstrando o efeito biológico dessa molécula, mesmo sendo administrado por via oral (Mousavi et al., 2020; Xavier et al., 2022). Logo, ao considerar a escassez de informações sobre os efeitos carrapaticida do carvacrol *in vivo*, buscou-se avaliar essa ação em camundongos Swiss infestados artificialmente.

Camundongos selvagens como *Peromyscus* que são hospedeiros de *Ixodes scapularis* já foram utilizados para avaliar a atividade carrapaticida de diversas moléculas sintéticas, como na administração de iscas contendo fipronil e fluralaner (Gutierrez et al., 2006; Poché et al., 2020). Nesse contexto, o camundongo Swiss foi escolhido como um modelo experimental para avaliar a atividade carrapaticida do carvacrol. Esse modelo se mostrou viável, pois as ninfas de *R. sanguineus* s.l. fixaram-se e realizaram a alimentação. Apesar do sucesso do modelo experimental, o carvacrol apresentou uma baixa atividade carrapaticida mesmo após 48 horas do tratamento, enquanto o lotilaner apresentou 100%. Esses resultados podem estar relacionados à distribuição do carvacrol nos tecidos do animal, principalmente a pele, o que pode ter retardado os efeitos do carvacrol e uma bioacumulação significativa para induzir à mortalidade nos carrapatos quando administrado por via oral (Aqil et al., 2007; Cal, 2006; Dancik et al., 2015; Kao et al., 1988). Além do mais, a disponibilidade do carvacrol para gerar mortalidade nas primeiras horas pode ter sido diminuída em função do metabolismo de primeira passagem e sua ligação a proteínas plasmáticas. Essa proposição justifica também o efeito residual constatado para o carvacrol, semelhante ao desencadeado pelas isoxazolinas (Pfister & Armstrong, 2016), principalmente ao se considerarem as propriedades físico-químicas do carvacrol, como por exemplo um log de P de 3,6 e uma alta de taxa de ligação a proteínas (Bansal et al., 2022; Oliveira, 2020). É importante salientar que ocorreu um aumento da mortalidade do controle negativo ao longo do período de observação, possivelmente em função do desenvolvimento de resistência do hospedeiro após a primeira infestação (Bechara et al., 1995) (Fig. 3).

Com a finalidade de avaliar se o carvacrol poderia estar atuando sobre o carrapato e o induzindo a alterações morfológicas, mesmo com baixa eficiência de mortalidade visual, decidiu-se então investigar possíveis alterações morfológicas no tegumento do carrapato. Essa estrutura foi escolhida porque o tegumento forma o exoesqueleto do carrapato e é uma estrutura importante para mudança de fase (ecdise) e de proteção eficiente para evitar a transposição de produtos, impedindo a penetração de agentes químicos nos ectoparasitas (Lima de Souza et al., 2022).

Um composto que altere a morfologia dessa estrutura pode impedir o desenvolvimento do carrapato, bem como de seu ciclo de vida, contribuindo assim para o seu controle. Apesar de estudos anteriores demonstrarem o potencial de modificação morfofisiológica do carvacrol e do acetilcarvacrol em tecidos de *R. sanguineus* s.l.

(Lima de Souza et al., 2019; Oliveira et al., 2020), este é o primeiro estudo a detalhar essas alterações após a administração oral do carvacrol. Adicionalmente, este é o primeiro estudo de observação das lesões no tegumento em carrapatos após tratamento com a isoxazolina lotilaner.

Apesar da baixa atividade carrapaticida do carvacrol e o lotilaner no Dia +9 após o tratamento, alterações histológicas dos tegumentos foram observadas no grupo exposto ao carvacrol (60 mg/mL), pois a camada epitelial foi fortemente danificada. Em muitas regiões dos tegumentos das ninfas tratadas com carvacrol, ocorreu a destruição do epitélio e surgimento de áreas desorganizadas. Quando presente, esse epitélio foi formado por uma camada de células bem pequenas e sobrepostas com núcleos arredondados. Isso pode ser justificado pela ação do carvacrol, que penetra no interior das células epiteliais, danificando-as. Essas células prejudicadas não poderão sintetizar e armazenar os componentes que constituem a cutícula. Resultados semelhantes foram observados por Lima de Souza *et al*. (2022) em fêmeas semi-ingurgitadas de *R. sanguineus* s.l. expostas ao carvacrol aplicado topicamente. Os resultados sugerem que o carvacrol administrado por via oral, mesmo não ocasionando mortalidade imediata, apresenta grande potencial carrapaticida ao provocar efeitos deletérios aos carrapatos, com alterações no tegumento de ninfas similares ao estudo mencionado anteriormente.

As ninfas do grupo tratado com lotilaner (20 mg/kg) exibiram uma alteração significativa na morfologia tegumentar: epicutícula mais finas e menos coradas e procutícula muito mais espessa, menos corada e sem subdivisões. Além disso, o epitélio é intensamente desorganizado e lesionado. Em algumas regiões, ele aparece deformado e, em outras, ocorre o desaparecimento completo dessas células, além de vacúolos pequenos e arredondados que seguem em direção ao interior do corpo desses carrapatos. Possivelmente, isso sugere não somente que a morfologia mudou, mas também que a fisiologia foi afetada, pois células danificadas não são capazes de exercer suas funções totalmente ou parcialmente. Resultado análogo também foi observado por Lima de Souza et al. (2017) e Abreu et al. (2020) com fêmeas semi-ingurgitadas e ingurgitadas de *R. sanguineus* expostas ao óleo de neem (*Azadirachta indica* A. Juss) e água ozonizada. Tais estudos relataram que esse tipo de comportamento celular ocorre devido à toxicidade ocasionada por moléculas com potencial carrapaticida capazes de induzir as células a secretar componentes cuticulares mais rapidamente e em maior quantidade, na tentativa de minimizar os danos causados pela substância tóxica dentro do organismo.

5. Conclusão

O camundongo Swiss é um modelo eficiente para avaliação da atividade carrapaticida contra ninfas de *R. sanguineus* s.l. Além disso, o carvacrol é uma molécula com atividade carrapaticida *in vitro* e *in vivo* sobre *R. sanguineus* s.l., capaz de provocar alterações nos tegumentos das ninfas quando administrado por via oral em camundongos, mesmo depois de sofrer os processos de biotransformação e distribuição. Assim, esse terpeno pode ser utilizado no desenvolvimento de novas formulações para o controle de carrapatos. No entanto, é necessário o desenvolvimento de uma formulação que aumente sua absorção e distribuição para administração por via oral. Esta pesquisa demonstra o potencial dessa molécula que pode ser utilizada por via oral para o controle de carrapatos.

REFERÊNCIAS

Abreu, M.R. de, Delalibera Junior, I., Pereira, N.R.C., Camargo-Mathias, M.I., 2020. Exposure of Ticks Rhipicephalus sanguineus sensu lato (Acari:Ixodidae) to Ozonated Water: Integument Response. Ozone Sci Eng 42, 507–516. https://doi.org/10.1080/01919512.2020.1727310

Ahantarig, A., Trinachartvanit, W., Baimai, V., Grubhoffer, L., 2013. Hard ticks and their bacterial endosymbionts (or would be pathogens). Folia Microbiol (Praha). https://doi.org/10.1007/s12223-013-0222-1

Aqil, M., Ahad, A., Sultana, Y., Ali, A., 2007. Status of terpenes as skin penetration enhancers. Drug Discov Today 12, 1061–1067. https://doi.org/10.1016/j.drudis.2007.09.001

Araújo, L.X., Novato, T.P.L., Zeringota, V., Maturano, R., Melo, D., da Silva, B.C., Daemon, E., de Carvalho, M.G., Monteiro, C.M.O., 2016. Synergism of thymol, carvacrol and eugenol in larvae of the cattle tick, Rhipicephalus microplus, and brown dog tick, Rhipicephalus sanguineus. Med Vet Entomol 30, 377–382. https://doi.org/10.1111/mve.12181

Bansal, A., Kaushik, V., Sharma, N.R., 2022. Synthesis and in silico anti-metastatic evaluation of carvacrol derivative, 2-hydroxy-6-isopropyl-3-methylbenzalehyde. Mater Today Proc 57, 739–747. https://doi.org/10.1016/j.matpr.2022.02.219

Bechara, G.H., Szabó, M.P.J., Ferreira, B.R., Garcia, M. v, 1995. R. sanguineus tick in Brazil - feeding and reproductive aspects under lab conditions. Revista Brasileira de Parasitologia Veterinária 4, 61–66.

Becker, S., Webster, A., Doyle, R.L., Martins, J.R., Reck, J., Klafke, G.M., 2019. Resistance to deltamethrin, fipronil and ivermectin in the brown dog tick, Rhipicephalus sanguineus sensu stricto, Latreille (Acari: Ixodidae). Ticks Tick Borne Dis 10, 1046–1050. https://doi.org/10.1016/j.ttbdis.2019.05.015

Cal, K., 2006. Skin penetration of terpenes from essential oils and topical vehicles. Planta Med 72, 311–316. https://doi.org/10.1055/s-2005-916230

Cardoso, A. dos S., Santos, E.G.G., Lima, A. da S., Temeyer, K.B., Pérez de León, A.A., Costa, L.M., Soares, A.M. dos S., 2020. Terpenes on Rhipicephalus (Boophilus) microplus: Acaricidal activity and acetylcholinesterase inhibition. Vet Parasitol 280. https://doi.org/10.1016/j.vetpar.2020.109090

Chomel, B.B., 2011. Zoonoses in the Bedroom. Emerg Infect Dis 17, 167–172. https://doi.org/10.3201/eid1702101070

Coelho, L., de Paula, L.G.F., Alves, S. das G.A., Sampaio, A.L.N., Bezerra, G.P., Vilela, F.M.P., Matos, R. da S., Zeringóta, V., Borges, L.M.F., Monteiro, C., 2020. Combination of thymol and eugenol for the control of Rhipicephalus sanguineus sensu lato: Evaluation of synergism on immature stages and formulation development. Vet Parasitol 277. https://doi.org/10.1016/j.vetpar.2019.108989

da Silva Lima, A., Landulfo, G.A., Costa-Junior, L.M., 2019. Repellent Effects of Encapsulated Carvacrol on the Rhipicephalus (Boophilus) microplus (Acari: Ixodidae). J Med Entomol 56, 881–885. https://doi.org/10.1093/jme/tjy240

Dancik, Y., Bigliardi, P.L., Bigliardi-Qi, M., 2015. What happens in the skin? Integrating skin permeation kinetics into studies of developmental and reproductive toxicity following topical exposure. Reproductive Toxicology 58, 252–281. https://doi.org/10.1016/j.reprotox.2015.10.001

Dantas, A.G.B., de Souza, R.L., de Almeida, A.R., Xavier Júnior, F.H., Pitta, M.G. da R., Rêgo, M.J.B. de M., Oliveira, E.E., 2021. Development, characterization, and immunomodulatory evaluation of carvacrol-loaded nanoemulsion. Molecules 26. https://doi.org/10.3390/molecules26133899

Dantas-Torres, F., 2010. Biology and ecology of the brown dog tick, Rhipicephalus sanguineus. Parasit Vectors 3, 1–11. https://doi.org/10.1186/1756-3305-3-26

Dantas-Torres, F., 2008. The brown dog tick, Rhipicephalus sanguineus (Latreille, 1806) (Acari: Ixodidae): From taxonomy to control. Vet Parasitol 152, 173–185. https://doi.org/10.1016/j.vetpar.2007.12.030

Dantas-Torres, F., Figueredo, L.A., Brandão-Filho, S.P., 2006. Rhipicephalus sanguineus (Acari: Ixodidae), the brown dog tick, parasitizing humans in Brazil. Rev Soc Bras Med Trop 39, 64–67. https://doi.org/10.1590/S0037-86822006000100012

de Oliveira Monteiro, C.M., Daemon, E., Aparecido Clemente, M., dos Santos Rosa, L., Maturano, R., 2009. Acaricidal efficacy of thymol on engorged nymphs and females of Rhipicephalus sanguineus (Latreille, 1808) (Acari: Ixodidae). Parasitol Res 105, 1093–1097. https://doi.org/10.1007/s00436-009-1530-x

de Oliveira, M.V.S., Konig, I.F.M., Reis, A.C., Silva, L., Peconick, A.P., Thomasi, S.S., Lima-De-Souza, J.R., Camargo-Mathias, M.I., Remedio, R.N., 2020. Sublethal concentrations of acetylcarvacrol affect reproduction and integument morphology in the brown dog tick Rhipicephalus sanguineus sensu lato (Acari: Ixodidae). Exp Appl Acarol 82, 265–279. https://doi.org/10.1007/s10493-020-00538-7

Delmonte, C., Cruz, P.B., Zeringóta, V., de Mello, V., Ferreira, F., Amaral, M. da P.H., Daemon, E., 2017. Evaluation of the acaricidal activity of thymol incorporated in two formulations for topical use against immature stages of Rhipicephalus sanguineus sensu lato (Latreille, 1806) (Acari: Ixodidae). Parasitol Res 116, 2957–2964. https://doi.org/10.1007/s00436-017-5604-x

Demma, L.J., Traeger, M.S., Nicholson, W.L., Paddock, C.D., Blau, D.M., Eremeeva, M.E., Dasch, D.A., Levin, M.L., Joseph Singleton, Jr., Zaki, S.R., Cheek, J.E., Swerdlow, D.L.,, McQuiston, J.H., 2005. Rocky Mountain Spotted Fever from an Unexpected Tick Vector in Arizona. New England Journal of Medicine 353, 587–594.

Duron, O., Sidi-Boumedine, K., Rousset, E., Moutailler, S., Jourdain, E., 2015. The Importance of Ticks in Q Fever Transmission: What Has (and Has Not) Been Demonstrated? Trends Parasitol. https://doi.org/10.1016/j.pt.2015.06.014

Eiden, A.L., Kaufman, P.E., Oi, F.M., 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. https://doi.org/10.1093/jme/tjv005

Faza, A.P., Pinto, I.S.B., Fonseca, I., Antunes, G.R., Monteiro, C.M. de O., Daemon, E., Muniz, M. de S., Martins, M.F., Furlong, J., Prata, M.C. de A., 2013. A new approach to characterization of the resistance of populations of Rhipicephalus microplus (Acari: Ixodidae) to organophosphate and pyrethroid in the state of Minas Gerais, Brazil. Exp Parasitol 134, 519–523. https://doi.org/10.1016/j.exppara.2013.04.006

FDA, 2013. Everything added to food in United States (EAFUS) and carvacrol [WWW Document]. URL https://www.fda.gov. (accessed 10.15.22).

Gutierrez, J.A., Zhao, X., Kemper, C.J., Plummer, P.R., Bauer, S.M., Hutchens, D.E., Smith, C.K., White, W.H., 2006. An In Vivo Rodent Model for Identifying and Characterizing Acaricides, J. Med. Entomol.

Isman, M.B., Akhtar, Y., 2007. Plant Natural Products as a Source for Developing Environmentally Acceptable Insecticides. In: Insecticides design using advanced technologies. Berlin, Heidelberg. https://doi.org/https://doi.org/10.1007/978-3-540-46907-0_10

Kao, J., Hall, J., Helman, G., 1988. In vitro percutaneous absorption in mouse skin: Influence of skin appendages. Toxicol Appl Pharmacol 94, 93–103. https://doi.org/10.1016/0041-008X(88)90340-7

Khanikor, B., Parida, P., Yadav, R.N.S., Bora, D., 2013. Comparative mode of action of some terpene compounds against octopamine receptor and acetyl cholinesterase of mosquito and human system by the help of homology modeling and docking studies. J Appl Pharm Sci 3, 6–12. https://doi.org/10.7324/JAPS.2013.30202

Kiss, T., Cadar, D., Spînu, M., 2012. Tick prevention at a crossroad: New and renewed solutions. Vet Parasitol. https://doi.org/10.1016/j.vetpar.2012.02.010

Konig, I.F.M., Reis, A.C., Gonçalves, R.R.P., Oliveira, M.V.S., Silva, C.M., Melo, D. de S., Peconick, A.P., Thomasi, S.S., Remedio, R.N., 2021. Repellent activity of acetylcarvacrol and its effects on salivary gland morphology in unfed Rhipicephalus sanguineus sensu lato ticks (Acari: Ixodidae). Ticks Tick Borne Dis 12. https://doi.org/10.1016/j.ttbdis.2021.101760

Lima, A. da S., Landulfo, G.A., Costa-Junior, L.M., 2019. Repellent Effects of Encapsulated Carvacrol on the Rhipicephalus (Boophilus) microplus (Acari: Ixodidae). J Med Entomol 56, 881–885. https://doi.org/10.1093/jme/tjy240

Lima de Souza, J.R., Oliveira, P.R. de, Anholeto, L.A., Arnosti, A., Daemon, E., Remedio, R.N., Camargo-Mathias, M.I., 2019. Effects of carvacrol on oocyte

development in semi-engorged Rhipicephalus sanguineus sensu lato females ticks (Acari: Ixodidae). Micron 116, 66–72. https://doi.org/10.1016/j.micron.2018.09.015

Lima de Souza, J.R., Remedio, R.N., Arnosti, A., de Abreu, R.M.M., Camargo-Mathias, M.I., 2017. The effects of neem oil (Azadirachta indica A. JUSS) enriched with different concentrations of azadirachtin on the integument of semi-engorged Rhipicephalus sanguineus sensu lato (Acari: Ixodidae) females. Microsc Res Tech 80, 838–844. https://doi.org/10.1002/jemt.22871

Lima de Souza, J.R., de Oliveira, P.R., Anholeto, L.A., Sodelli, L.F., Ferreira, A.R.F., Remedio, R.N., Camargo-Mathias, M.I., 2022a. The bioactive compound carvacrol as a potential acaricide: An assessment of its effects on the integument of female Rhipicephalus sanguineus sensu lato ticks. Microsc Res Tech 85, 1784–1790. https://doi.org/10.1002/jemt.24038

Mentz, M.B., Trombka, M., da Silva, G.L., Silva, C.E., 2016. Rhipicephalus sanguineus (Acari: Ixodidae) biting a human being in Porto Alegre City, Rio Grande do Sul, Brazil. Rev Inst Med Trop Sao Paulo 58. https://doi.org/10.1590/S1678-9946201658035

Miller, R. J., George, J. E., Guerrero, F., Carpenter, L., & Welch, J. B. (2001). Characterization of acaricide resistance in Rhipicephalus sanguineus (Latreille)(Acari: Ixodidae) collected from the Corozal army veterinary quarantine center, Panama. *Journal of Medical Entomology*, Volume 38, Issue 2, 1 March 2001, Pages 298–302, https://doi.org/10.1603/0022-2585-38.2.298

Monteiro, C.M.O., Daemon, E., Aparecido Clemente, M., Dos Santos Rosa, L., Maturano, R., 2009. Acaricidal efficacy of thymol on engorged nymphs and females of Rhipicephalus sanguineus (Latreille, 1808) (Acari: Ixodidae). Parasitol Res 105, 1093–1097. https://doi.org/10.1007/s00436-009-1530-x

Monteiro, C., Ferreira, L.L., de Paula, L.G.F., de Oliveira Filho, J.G., de Oliveira Silva, F., Muniz, E.R., Menezes, K.M.F., de Camargo, F.R., de Oliveira Nonato, R., Martins, D.B., Marreto, R.N., Borges, L.M.F., 2021. Thymol and eugenol microemulsion for *Rhiphicephalus sanguineus* sensu lato control: Formulation development, field efficacy, and safety on dogs. Vet Parasitol 296. https://doi.org/10.1016/j.vetpar.2021.109501

Mousavi, S., Schmidt, A.M., Escher, U., Kittler, S., Kehrenberg, C., Thunhorst, E., Bereswill, S., Heimesaat, M.M., 2020. Carvacrol ameliorates acute campylobacteriosis in a clinical murine infection model. Gut Pathog 12. https://doi.org/10.1186/s13099-019-0343-4

Novato, T.P.L., Araújo, L.X., de Monteiro, C.M.O., Maturano, R., Senra, T. de O.S., da Silva Matos, R., Gomes, G.A., de Carvalho, M.G., Daemon, E., 2015. Evaluation of the combined effect of thymol, carvacrol and (E)-cinnamaldehyde on Amblyomma sculptum (Acari: Ixodidae) and Dermacentor nitens (Acari: Ixodidae) larvae. Vet Parasitol 212, 331–335. https://doi.org/10.1016/j.vetpar.2015.08.021

Oliveira, A.S., 2020. Carvacrol: A Brief Analysis of Molecular Properties and Their Therapeutic Potential. American Journal of Medicinal Chemistry 1–5. https://doi.org/10.31487/j.ajmc.2020.01.10

Pfister, K., Armstrong, R., 2016. Systemically and cutaneously distributed ectoparasiticides: A review of the efficacy against ticks and fleas on dogs. Parasit Vectors. https://doi.org/10.1186/s13071-016-1719-7

Poché, D.M., Franckowiak, G., Clarke, T., Tseveenjav, B., Polyakova, L., Poché, R.M., 2020. Efficacy of a low dose fipronil bait against blacklegged tick (Ixodes scapularis) larvae feeding on white-footed mice (Peromyscus leucopus) under laboratory conditions. Parasit Vectors 13. https://doi.org/10.1186/s13071-020-04258-0Remedio, R.N., Nunes, P.H., Anholeto, L.A., Oliveira, P.R., Camargo-Mathias, M.I., 2015. Morphological effects of neem (Azadirachta indica A. Juss) seed oil with known azadirachtin concentrations on the oocytes of semi-engorged Rhipicephalus sanguineus ticks (Acari: Ixodidae). Parasitol Res 114, 431–444. https://doi.org/10.1007/s00436-014-4200-6

Rodriguez-Vivas, R.I., Ojeda-Chi, M.M., Trinidad-Martinez, I., Pérez de León, A.A., 2017a. First documentation of ivermectin resistance in Rhipicephalus sanguineus sensu lato (Acari: Ixodidae). Vet Parasitol 233, 9–13. https://doi.org/10.1016/j.vetpar.2016.11.015

Russel, W.M.S., Burch, R.L., 1959. The principles of humane experimental technique. London: Methuen.

Silva, A.B., M.M.D., Cavalcante, R.C., Oliveira, S.V., Vizzoni, V.F., Duré, A.I.L., Iani, F.C.M., Machado-Ferreira, E., & Gazêta, G.S. "*Rickettsia rickettsii* infecting *Rhipicephalus sanguineus* sensu lato (Latreille 1806), in High Altitude Atlantic Forest Fragments, Ceara State, Brazil." Acta Tropica 173 (2017): 30-33. https://doi.org/10.1016/j.actatropica.2017.05.018

Sonenshine, D.E., Roe, R.M., 2013. Biology of ticks volume 2. Oxford University Press.

Sousa, A.I.P., Castro, G.J.S., Tavares, C.P., Vale, T.L. do, Costa-Junior, L.M., Soares, A.M.S., 2022. In vitro assessment of the acaricidal activity of a carvacrol shampoo on tick larvae. Exp Parasitol 242. https://doi.org/10.1016/j.exppara.2022.108364

Suntres, Z.E., Coccimiglio, J., Alipour, M., 2015. The Bioactivity and Toxicological Actions of Carvacrol. Crit Rev Food Sci Nutr 55, 304–318. https://doi.org/10.1080/10408398.2011.653458

Tavares, C.P., Sabadin, G.A., Sousa, I.C., Gomes, M.N., Soares, A.M.S., Monteiro, C.M.O., Vaz, I.S., Costa-Junior, L.M., 2022. Effects of carvacrol and thymol on the antioxidant and detoxifying enzymes of Rhipicephalus microplus (Acari: Ixodidae). Ticks Tick Borne Dis 13. https://doi.org/10.1016/j.ttbdis.2022.101929

Tong, F., Coats, J.R., 2010. Effects of monoterpenoid insecticides on [3H]-TBOB binding in house fly GABA receptor and 36Cl- uptake in American cockroach

ventral nerve cord. Pestic Biochem Physiol 98, 317–324. https://doi.org/10.1016/j.pestbp.2010.07.003

Tong, F., Gross, A.D., Dolan, M.C., Coats, J.R., 2013. The phenolic monoterpenoid carvacrol inhibits the binding of nicotine to the housefly nicotinic acetylcholine receptor. Pest Manag Sci 69, 775–780. https://doi.org/10.1002/ps.3443

Trapp, S.M., Dagnone, A.S., Vidotto, O., Freire, R.L., Amude, A.M., de Morais, H.S.A., 2006. Seroepidemiology of canine babesiosis and ehrlichiosis in a hospital population. Vet Parasitol 140, 223–230. https://doi.org/10.1016/j.vetpar.2006.03.030

Trotta, M., Nicetto, M., Fogliazza, A., Montarsi, F., Caldin, M., Furlanello, T., Solano-Gallego, L., 2012. Detection of Leishmania infantum, Babesia canis, and rickettsiae in ticks removed from dogs living in Italy. Ticks Tick Borne Dis 3, 294–297. https://doi.org/10.1016/j.ttbdis.2012.10.031

Vale, T.L. do, Sousa, I.C., Tavares, C.P., Silva, N.C., Luz, H.R., Gomes, M.N., Sargison, N., Costa-Junior, L.M., 2021. Practices employed by veterinary practitioners for controlling canine gastrointestinal helminths and ectoparasites. Revista Brasileira de Parasitologia Veterinária 30. https://doi.org/10.1590/s1984-29612021079

Vale, L., de Paula, L.G.F., Vieira, M.S., Alves, S. das G.A., Junior, N.R. de M., Gomes, M.D.F., Teixeira, W.F.P., Rizzo, P.V., Freitas, F.M.C., Ferreira, L.L., Lopes, W.D.Z., Monteiro, C., 2021. Binary combinations of thymol, carvacrol and eugenol for Amblyomma sculptum control: Evaluation of in vitro synergism and effectiveness under semi-field conditions. Ticks Tick Borne Dis 12. https://doi.org/10.1016/j.ttbdis.2021.101816

Xavier, E.S., de Souza, R.L., Rodrigues, V.C., Melo, C.O., Roquini, D.B., Lemes, B.L., Wilairatana, P., Oliveira, E.E., de Moraes, J., 2022. Therapeutic Efficacy of Carvacrol-Loaded Nanoemulsion in a Mouse Model of Schistosomiasis. Front Pharmacol 13. https://doi.org/10.3389/fphar.2022.917363

Zhou, X., Hohman, A.E., Hsu, W.H., 2022. Current review of isoxazoline ectoparasiticides used in veterinary medicine. J Vet Pharmacol Ther. https://doi.org/10.1111/jvp.12959

CONSIDERAÇÕES FINAIS

Os resultados obtidos no presente estudo revelaram como é recomendado o controle de parasitos em cães no Brasil, mostrando as práticas realizadas para o controle de parasitos necessita de adequação às particularidades do Brasil. No segundo capítulo, foi demonstrado o efeito carrapaticida do carvacrol sobre larvas e o tegumento de ninfas da espécie *R. sanguineus* s.l. através de bioensaios *in vitro* e *in vivo* com camundongos Swiss que permitiram concluir que:

1) A concentração letal média do carvacrol (CL_{50}) para larvas e ninfas é diferente, requisitando uma maior concentração do terpeno para o estágio mais avançado de *R. sanguineus* s.l.
2) O carvacrol administrado por via oral agiu sobre o tegumento, ou seja, foram observadas alterações histológicas das ninfas que se alimentaram dos animais tratados com carvacrol (60 mg/mL), pois as células cúbicas da epiderme apresentavam uma possível agregação de grânulos de proteínas entre as camadas epitelial e subcuticular.

APÊNDICES – PRODUÇÕES DURANTE O DOUTORADO

https://doi.org/10.1186/s13071-023-05661-z

Parasites & Vectors

RESEARCH

Open Access

Efficacy of lotilaner against myiasis caused by *Cochliomyia hominivorax* (Diptera: Calliphoridae) in naturally infested dogs

Tássia Lopes do Vale[1], Alcyjara Rego Costa[2], Leandro Macedo Miranda[2], Geovane Ferreira Silva[3],
Naylene Carvalho Sales Silva[1], Tiago Barbalho Lima[2], Daniel Praseres Chaves[2], Heinz Sager[4], Pedro Veloso Facury Lasmar[5] and Livio Martins Costa–Junior[1*]

Abstract

Background The New World screwworm fly, *Cochliomyia hominivorax*, is widely distributed across South America. This parasitic insect is a significant cause of primary myiasis in animals, including dogs. There is an urgent need for a rapid and efficient treatment to improve the recovery of affected animals. In the present study we evaluated the potential of lotilaner for the treatment of myiasis caused by *C. hominivorax* larvae in naturally infested dogs. Lotilaner ™ belongs to the isoxazoline class of chemical compounds and is marketed as Credelio for use against ticks and fleas in dogs and cats.

Methods Eleven dogs with naturally acquired myiasis were enrolled in this study based on the severity of lesions and the number of identified larvae. All animals received a single oral administration of lotilaner at a minimum dose of 20.5 mg/kg body weight. After treatment, the number of expelled larvae, live or dead, was determined at 2, 6 and 24 h, and the larval expulsion rate, larvicidal effect and overall efficacy were calculated. After 24 h, the remaining larvae were removed, counted and identified. The lesions were cleaned, and palliative treatment was administered when necessary, according to the animal's health status.

Results All larvae were identified as *C. hominivorax*. The larval expulsion rate was 80.5% and 93.0% at 2 and 6 h posttreatment, respectively. Lotilaner showed an overall efficacy of 100% at 24 h post-treatment.

Conclusions Lotilaner demonstrated a rapid onset of action and a high efficacy against *C. hominivorax*. We therefore recommend lotilaner for the effective treatment of myiasis in dogs.

Keywords New World screwworm fly, Ectoparasites, Dogs, Myiasis, Control strategies

Background

Myiasis is a disease caused by the larval stages of several species of flies that feed on tissue from the hosts. However, in South America, including Brazil, the New World screwworm (NWS), *Cochliomyia hominivorax*

(Diptera: Calliphoridae), is the main species causing primary myiasis in humans and warm-blooded animals [1, 2]. Historically, the geographic distribution of NWS extended from the southern USA to the central region of Argentina [3]. In the late 1960s, the USA successfully

*Correspondence:
Livio Martins Costa–Junior
livio.martins@ufma.br
Full list of author information is available at the end of the article

launched a program aimed at eradication of this species by releasing sterile insects within its national borders, later expanding the program to Mexico and finally to continental Central America [4]. Panama currently represents the biological barrier, achieved by releasing sterile insects within its territory along the border with Colombia [5]. As a result of these eradication programs, the current distribution of this parasitic fly comprises the Caribbean and South American countries, with the exception of Chile. There is a proposal for an eradication program in Uruguay which, if implemented, will require constant epidemiological surveillance on the border with Brazil and Argentina [6].

To eradicate *C. hominivorax* and eliminate the need for epidemiological surveillance, it is necessary, in addition to the massive release of sterile insects, to diagnose and treat all cases of myiasis in a given area [4, 7]. In companion animals, NWS myiasis is a debilitating disease and can be fatal depending on the time to diagnosis and treatment, level of infestation and the site of infestation [8]. The threat of reinvasion of the NWS in areas where it has been eradicated increases proportionally with increasing international animal trade and the travel of pets and humans. Dogs are important hosts for NWS, and the diagnosis and treatment of myiasis are essential for effective control of this disease, as described for the outbreaks in Florida (USA) in 2016 [9]. Control measures are even more important in the context of the One Health perspective and the fact that NWS is not limited to domestic animals [10].

In this context, a rapid and efficient treatment is necessary to improve the recovery of affected animals and reduce the possibility of reinvasion in the areas where this parasitic fly has been eradicated. Various topical and systemic drugs are used to treat myiasis in dogs (e.g. macrocyclic lactones, nitenpyram and spinosad) [8, 11– 13]. More recently, other parasiticides, such as isoxazolines, have been described for the effective treatment of cutaneous myiasis [11, 14, 15].

The isoxazoline parasiticide lotilaner acts as a potent non-competitive antagonist of GABA-activated chloride channels from arthropods [16]. Lotilaner has been

registered for use in dogs and cats in a chewable tablet-formulation as Credelio™ (Elanco Animal Health, Indianapolis, IN, USA) and demonstrated efficacy against ticks [17–19], mange mite [20] and fleas [21, 22]. The efficacy of lotilaner against myiasis by the Old-World screwworm (*Chrysomya bezziana*) was recently shown in two cats from Malaysia [23]. Although the action of isoxazolines on *C. hominivorax* has been documented, the efficacy of lotilaner on myiasis caused by this parasite has not yet been shown. The objective of the present work was to verify the potential of lotilaner for the treatment of naturally acquired screwworm myiasis caused by *C. hominivorax* in dogs.

Methods

The experimental procedures were approved by the animal research ethics committee of the Federal University of Maranhão—UFMA (CIAEP: 02.0341.2019), Brazil, under protocol number 23115.005441/2017-62.

Experimental design

Eleven client-owned dogs (5 males, 6 females) that were naturally infested with *C. hominivorax* and with active myiasis were enrolled in the study. All larvae collected during the experiment were maintained in 70% ethanol and identified as *C. hominivorax,* according to Stojanovich et al. [24]. The age of the enrolled dogs ranged from 1.5 to 10.0 years and with body weight ranged between 3.3 and 25.0 kg. The dogs had not received any ectoparasiticide treatment in the 120 days immediately preceding the experiment. The myiasis lesions were distributed in different body areas: mammary (3 dogs), eye (3 dogs), scrotum (2 dogs), neck and thoracic and pelvic limbs (1 dog each).

After myiasis had been diagnosed, based on the obser-

vation of larvae in the wound, the dogs received lotilaner (Credelio™) in a single dose orally, following the manufacturer's recommended dose for control of fleas and ticks in dogs. The doses ranged from 23.9 to 40.9 mg/ kg body weight. After treatment, the dogs were kept in individual kennels with a removable tray (surface area of 0.3–0.8 m^2 according to body weight). The dogs were observed at 2 and 6 h post-treatment at which times expelled larvae were collected and quantified. At 24 h post-treatment, the remaining larvae were mechanically removed from the wound. Larvae without movement were considered to be dead. After the removal of the remaining larvae, the wounds were cleaned and an anti-

inflammatory (0.2 mg/kg of meloxicam, applied subcutaneously [SC]) and antibiotic (15.0 mg/kg of amoxicillin trihydrate, SC) treatment was administered before the dogs were sent home. The dogs were evaluated at home daily for general health conditions, and the wounds were cleaned by the owners until complete healing had been achieved. **Data analysis**

The evaluation of the efficacy of lotilaner against *C. hominivorax* was calculated based on the formulae described by Oliveira et al. [13]. The overall efficacy was calculated as: [(number of dead larvae expelled + number of live larvae expelled + number of dead larvae removed)/ total number of larvae] × 100. The larval expulsion efficacy was calculated at 2, 6 and 24 h post-treatment using the formula: [(number of dead larvae expelled + number of live larvae expelled)/total number of larvae] × 100. The larvicidal efficacy was calculated using the formula: [(number of dead larvae expelled + number of dead larvae removed)/total number of larvae] × 100.

Results

All larvae collected during the study were confirmed as *C. hominivorax*. No adverse effects related to lotilaner or any other treatment were observed throughout the study. Each dog had only a single wound with active myiasis and an average of 35 larvae (range: 1–100) (Fig. 1).

A rapid onset of activity was observed, with highest mean expulsion of larvae at 2 h (80.5%), which increased up to 93.0% at 24 h post-treatment (Fig. 2a). Expulsed larvae comprised both live and dead larvae (Fig. 1). Larvae that were removed from the wounds 24 h post-treatment were all dead, and the overall efficacy was 100% at that time point (Fig. 2c).

The mean larvicidal efficacy of lotilaner was rather low at 41.1% at 24 h post-treatment (Fig. 2b), which suggests that the efficacy of the treatment was mostly driven by larval expulsion. The combination of larva expulsion and the larvicidal effect results in a high cumulative efficacy of 80.5%, 93.0% and 100.0% at 2, 6, and 24 h post-treatment, respectively (Fig. 2c).

Discussion

Myiasis is an infestation of live tissue by the larvae of several species of flies [25]. Larvae of *C. hominivorax* are the main cause of primary myiasis, which represents one of the major skin diseases in dogs in areas where this fly species is present [1, 26]. The number of wounds and larvae in each dog enrolled in the present study was similar to that reported in earlier studies in other regions of Brazil [27]. The highest prevalence of myiasis in Brazil has been reported to be in adult dogs, with no gender predilection [27, 28]. These earlier reports corroborate the observations made on the dogs enrolled in the present study.

Topical insecticides in ointment or spray formulations were the main treatments for myiasis before effective systemic compounds became commercially available. Lotilaner belongs to the isoxazoline chemical class, and chemical compounds in this class are the most recommended for use in Brazil for the control of ectoparasites [29]. The oral administration of a drug has the advantage of reduced exposure risks for pet owners and the environment, compared to topical administration. At the same time, it is expected that used compounds used lead to fast elimination of the larvae. The rapid activity of lotilaner and high larval expulsion at 2 h post-treatment, as shown in the present study, can be explained by the early peaking of lotilaner concentration in the blood within 2 h after treatment [30]. This result is in agreement with those from earlier efficacy studies against fleas, where 64.0% of adult *Ctenocephalides felis* were eliminated 2 h after treatment [21]. Insecticidal compounds that have a rapid onset of efficacy are preferred options for treating primary myiasis in dogs as they allow quick restoration of quality of life

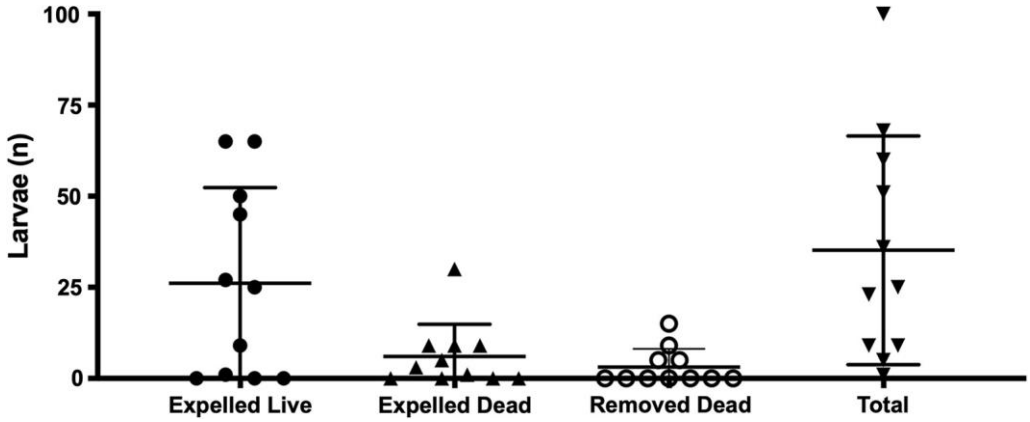

Fig. 1 Mean and standard deviation of the number of expelled, dead, removed and total number of larvae of *Cochliomyia hominivorax* per dog treated with a single oral dose of Credelio™ (lotilaner)

[15]. Therefore, a high and rapid expulsion of larvae is important, as it can promote faster healing of lesions and prevent animals from having to undergo procedures such as sedation and prolonged antibiotic therapy. Furthermore, the rapid expulsion of larvae from the host tissue can reduce the risk of foreign body infection and the need to debride the lesion to remove the larvae [23]. Given the lack of guidance for treating myiasis in dogs, the use of systemic molecules should be recommended due to the above-mentioned advantages [14, 15].

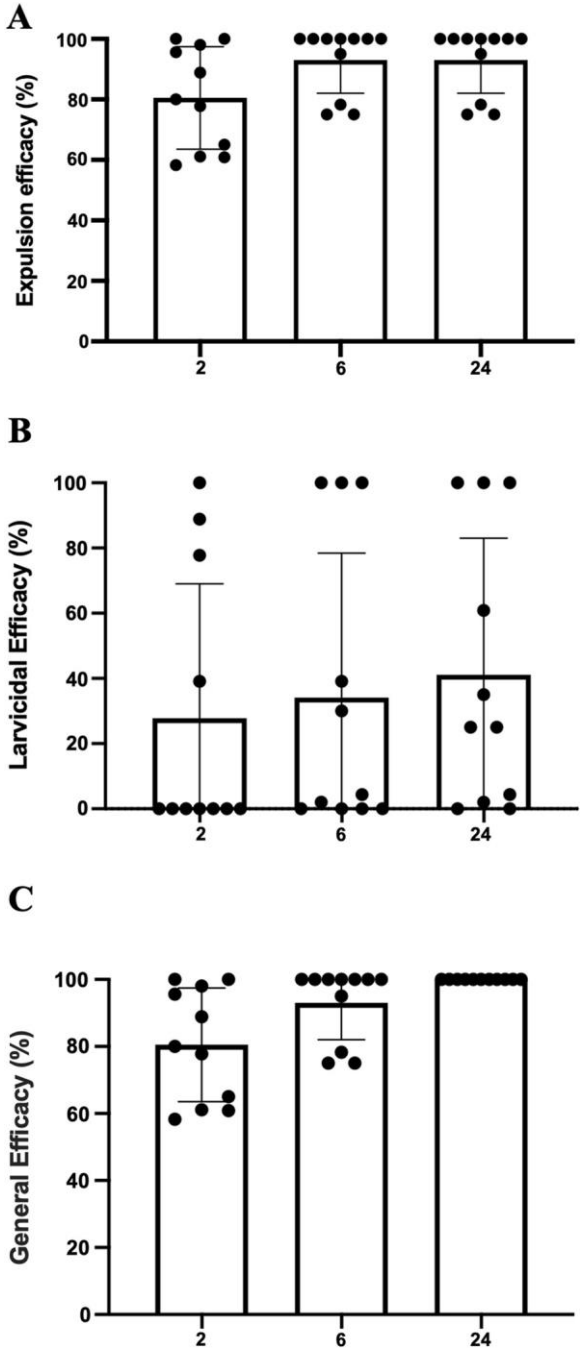

Fig. 2 of a single dose of CredelioExpulsion (**a**), larvicidal efficacy ™ (lotilaner) in dogs at 2, 6 and 24 h (**b**) and general efficacy (**c**)

post-treatment on myiasis by *Cochliomyia hominivorax*. Dots show the individual values for each dog, the bar shows the average value and the horizontal lines show the standard deviation

Sarolaner, also an isoxazoline, was reported to achieve a similar high cumulative larva expulsion of *C. hominivorax* [15], showing that the efficacy against this parasite could be inherent to all registered

compounds of this chemical class. Nitenpyram, a neonicotinoid, also shows a high larval expulsion of *C. hominivorax* after two treatments within 24 h [8], while a single administration of spinosad promoted lower larval expulsion, similar to a single treatment of nitenpyram [13].

Residual protection for at least 30 days against subsequent infestation by *C. hominivorax* should be the focus of further studies. Lotilaner has a terminal half-life of 30.7 days [30] and high insecticide and acaricide efficacy at 30 days of the treatment [18, 22, 31], suggesting that the residual protection against NWS can be expected for the same period of time. Drugs with these characteristics that promote the prevention and control of NWS must be a priority of One Health. Therefore, it is important to encourage the registration of new drugs against *C. hominivorax* and to urge the standardization of protocols for treatment and prevention of myiasis in dogs.

Conclusion

The results of this study show a rapid onset of action and high efficacy against *C. hominivorax*. In conclusion, lotilaner can be recommended for the effective treatment of myiasis in dogs.

Acknowledgements

The authors wish to thank CNPq (Brazilian National Council for Scientific and Technological Development), FAPEMA (Maranhão State Research Foundation) and FINEP (Funding Authority for Studies and Projects) for financial support (PRONEM 01773/14 and IECT (Science and Technology Institute of Maranhão) Biotechnology). The authors also wish to thank the CNPq for awarding a fellowship to L.M. Costa, Júnior and thank FAPEMA and CAPES (Brazilian Federal Agency for support and evaluation of graduate education) for the scholarships to TLV and NCS.

Author contributions

Conceptualisation and design of the study: LMCJ, PVFL. Collection of data: TLV, ARC, LMM, GFS, NCSS, TBL. Writing and preparation of the original draft: TLV, DPC. Data analysis: TLV, HS. Visualisation: DPC. Writing, reviewing and editing: LMCJ, HS. All authors read and approved the final manuscript.

Funding

Elanco Animal Health partially supported this work. This study was financed in part by CAPES, Finance Code 001.

Availability of data and materials

Data supporting the conclusions of this article are included within the article.

Declarations

Ethics approval and consent to participate

The experimental procedures were approved by the animal research ethics committee of the Federal University of Maranhão—UFMA (CIAEP:
02.0341.2019), Brazil, under protocol number 23115.005441/2017-62.

Consent for publication Not applicable.

Competing interests

PL and HS are employees of Elanco Animal Health. All other authors have nothing to declare.

Author details

[1] Postgraduate Program in Health Sciences, Federal University of Maranhão (UFMA), São Luís, MA, Brazil. [2] Department of Pathology, State University of Maranhão (UEMA), São Luís, MA, Brazil. [3] Department of Pathology, Federal University of Maranhão (UFMA), São Luís, MA, Brazil. [4] Elanco Animal Health Inc, Basel, Switzerland. [5] Elanco Animal Health Inc, São Paulo, Brazil.

Received: 17 November 2022 Accepted: 10 January 2023

Published online: 06 March 2023

References

1. Costa-Júnior LM, Chaves DP, Brito DRB, dos Santos VAF, Costa-Júnior HN, Barros ATM. A review on the occurrence of *Cochliomyia hominivorax* (Diptera: Calliphoridae) in Brazil. Rev Bras Parasitol Vet. 2019;28:548–62.

2. Guimarães JH, Papavero N. Myiasis in man and animals in the Neotropical Region: bibliographic database. São Paulo: Plêiade; 1999
3. Hall M, Wall R. Myiasis of humans and eomestic animals. In Baker JR, Muller R, Rollinson D, editors. Advances in parasitology. Cambridge: Academic Press, 1995. p. 257–334.
4. Mastrangelo T, Welch JB. An overview of the components of AW-IPM campaigns against the New World Screwworm. Insects. 2012;12:930–55.
5. Wyss JH. Screwworm eradication in the Americas. Ann N Y Acad Sci. 2000;916:186–93.
6. Vargas-Terán M, Hofmann HC, Tweddle NE. Impact of screwworm eradication programmes using the sterile insect technique. In: Dyck VA, Hendrichs J, Robinson AS, editors. Sterile insect technique principles and practice in area-wide integrated pest management. Berlin: Springer; 2005. p. 629–50.
7. Dyck VA, Hendrichs J, Robinson AS, editors. Sterile insect technique principles and practice in area-wide integrated pest management. Berlin: Springer; 2005.
8. Correia TR, Scott FB, Verocai GG, Souza CP, Fernandes JI, Melo RMPS, et al. Larvicidal efficacy of nitenpyram on the treatment of myiasis caused by *Cochliomyia hominivorax* (Diptera: Calliphoridae) in dogs. Vet Parasitol. 2010;173:169–72.
9. Skoda SR, Phillips PL, Welch JB. Screwworm (Diptera: Calliphoridae) in the United States: response to and elimination of the 2016–2017 outbreak in Florida. J Med Entomol. 2018;55:777–86.
10. Sunny B, Sulthana L, James A, Sivakumar T. Maggot infestation: various treatment modalities. J Am Col Clin Wound Spec. 2016;8:51–3.
11. Han HS, Chen C, Schievano C, Noli C. The comparative efficacy of afoxolaner, spinosad, milbemycin, spinosad plus milbemycin, and nitenpyram for the treatment of canine cutaneous myiasis. Vet Dermatol. 2018;312:e109.
12. Han HS, Sharma R, Jeffery J, Noli C. *Chrysomya bezziana* (Diptera: Calliphoridae) infestation: case report of three dogs in Malaysia treated with spinosad/milbemycin. Vet Dermatol. 2017;28:239-e62.
13. Oliveira PC, Moraes PA, Scott FB, Verocai GG, Correia TR, Fernandes JI. Efficacy of spinosad on the treatment of myiasis caused by *Cochliomyia hominivorax* (Diptera: Calliphoridae) in dogs. Vet Parasitol. 2018;258:53–6.
14. Cutolo AA, Perier N, Menz I, Thyssen P, Silva FO, Beugnet F. Efficacy of ® afoxolaner (NexGard) on the treatment of myiasis caused by the New World screwworm fly *Cochliomyia hominivorax* (Diptera: Calliphoridae) in naturally infested dogs. Vet Parasitol Reg Stud Rep. 2021;24:100569.
15. Oliveira PC, Almeida GPS, Cardoso JD, Tavares RB, Fernandes JI, Correia TR, et al. Efficacy of sarolaner on the treatment of myiasis caused by *Cochliomyia hominivorax* (Diptera: Calliphoridae) in dogs. Vet Parasitol. 2019;276:108966.
16. Rufener L, Danelli V, Bertrand D, Sager H. The novel isoxazoline ectopara-™ siticide lotilaner (Credelio): a non-competitive antagonist specific to invertebrates γ-aminobutyric acid-gated chloride channels (GABACls). Parasit Vectors. 2017;10:530.
17. 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.
18. Lasmar PVF, Murphy M, Nanchen S, Drake J, Coumendouros K, Borges DA, ™ et al. Laboratory evaluation of the efficacy of lotilaner (Credelio) against *Amblyomma cajennense* (sensu lato) infestations of dogs. Parasit Vectors. 2018;11:537.
19. Murphy M, Cavalleri D, Seewald W, Drake J, Nanchen S. Laboratory evalu-™ ation of the speed of kill of lotilaner (Credelio) against *Ixodes ricinus* ticks on dogs. Parasit Vectors. 2017;10:541. ™
20. Snyder DE, Wiseman S, Liebenberg JE. Efficacy of lotilaner (Credelio), a novel oral isoxazoline against naturally occurring mange mite infestations in dogs caused by *Demodex* spp. Parasit Vectors. 2017;10:532.
21. Cavalleri 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;10:521.
22. Young L, Karadzovska D, Wiseman S, Helbig R. Efficacy of lotilaner ™ (Credelio) against the adult cat flea, *Ctenocephalides felis* and flea eggs following oral administration to dogs. Parasit Vectors. 2020;13:25.
23. Han HS, Yasmin L. *Chrysomya bezziana* (Diptera: Calliphoridae) infestation in two Malaysian cats treated with oral lotilaner. Vet Dermatol. 2020;31:335-e87.
24. Stojanovich C, Pratt HD, Bennington EE. Fly larvae: key to some species of public health importance. Atlanta: US Department of Health, Education and Welfare, Public Health Service, Communicable Disease Center, Training Branch; 1962.
25. Cansi ER, Demo C. Ocorrência de miíases em animai de companhia no Distrito Federal. Brasil Acta Sci Vet. 2011;39:982.
26. Souza TM, Fighera RA, Schmidt C, Réquia AH, Brum JS, Martins TB, et al. Prevalência das dermatopatias não-tumorais em cães do município de Santa Maria, Rio Grande do Sul (2005–2008). Pesq Vet Bras. 2009;29:157–62.
27. Cramer-Ribeiro BC, Sanavria A, Oliveira MQD, Souza FSD, Rocco FDS, Cardoso PG. Inquérito sobre os casos de miíase por *Cochliomyia*. Braz J Vet Res Anim Sci. 2002;39:171–17.
28. Cramer-Ribeiro BC, Sanavria A, Monteiro HHMS, Oliveira MQD, Souza FSD. Inquiry of cases of myiasis by *Cochliomyia hominivorax* in dogs (*Canis familiaris*) of the Northern and Western zones of Rio de Janeiro city in 2000. Braz J Vet Res Anim Sci. 2003;40:13–20.
29. Vale TL, Sousa IC, Tavares CP, Silva NC, Luz HR, Gomes MN, et al. Practices employed by veterinary practitioners for controlling canine gastrointestinal helminths and ectoparasites. Rev Bras Parasitol Vet. 2021;30:e007021.
30. Toutain CE, Seewald W, Jung M. The intravenous and oral pharmacokinetics of lotilaner in dogs. Parasit Vectors. 2017;10:1–8.
31. Cavalleri D, Murphy M, Seewald W, Drake J, Nanchen S. Laboratory evalu-™ ation of the efficacy and speed of kill of lotilaner (Credelio) against *Ixodes ricinus* ticks on cats. Parasit Vectors. 2018;11:1–10.

Publisher's Note

***Terpenoids: Recent Advances in Extraction*, 2022, 129-147**

CHAPTER 6

Repellent Potential of Terpenoids Against Ticks

Tássia L. Vale[1], Isabella C. Sousa[1], Caio P. Tavares[1], Matheus N. Gomes[1], Geovane F. Silva[1], Jhone R. S. Costa[1], Aldilene da Silva Lima[2], Claudia Q. Rocha[2] and Livio Martins Costa-Júnior[1,*]

[1] *Departamento de Patologia, Universidade Federal do Maranhão (UFMA), Brazil*

[2] *Departamento de Química, Universidade Federal do Maranhão (UFMA), Brazil*

Abstract: Substances used as repellents to avoid contact with ticks and tickborne disease are essential to control. Several compounds have been developed throughout human history to promote repellent activity, and in the last decades, synthetic repellents have been widely used. However, several humans, animal, and environmental health problems have been related to synthetic compounds. The use of natural molecules with low toxicity becomes an alternative to replace these compounds. The natural terpenoids from secondary plant metabolites are an essential group with repellency activity on different arthropods. This chapter addresses the primary terpenes with repellency activity, briefly identifying the effectiveness of tick repellents, test methodology, primary terpenes tested, and activity. The evaluated compound showed good repellent activity on different tick species and stages. However, through this chapter, we show the variations in the techniques used to evaluate the bioprospection of terpenes with possible repellent activity and a lack of *in vivo* repellency studies with terpenes. Finally, we emphasize the repellent activity of terpenes to encourage the use of natural compounds as a strategy to control ticks.

Keywords: Animals, Control, Natural Product, Repellent, Tick.

INTRODUCTION

Repellent compounds are volatile chemicals that cause the arthropod to disorient its movements, removing it and thus preventing infestation or attack on the host (Fig. **1**) [1, 2]. Chemical repellents like DEET, IR3535, DEPA, Icaridin (picaridina) and Permethrin (synthetic pyrethroid) have been the most widely used repellents for repelling arthropods, such as insects and ticks [3, 4], with vehicle formulations in the form of a spray, lotion, and gel and can be applied to clothing or skin [5].

* **Corresponding author Livio Martins Costa-Júnior:** Departamento de Patologia, Universidade Federal do Maranhão, Brazil; E-mail: livioslz@yahoo.com

also been described to perform this action [12]. Terpenes represent a diverse chemical group that is part of the secondary metabolism of plants, derivatives of hemiterpene units (C_5) and classified according to the number of carbons found in their chemical skeleton, which can range from monoterpene (C_{10}), sesquiterpenes (C_{15}) to polyterpenes ($>C_{40}$) units [13]. The acyclic and bicyclic isomeric skeletons and functional groups give terpenes the ability to form a wide variety of molecules [14].

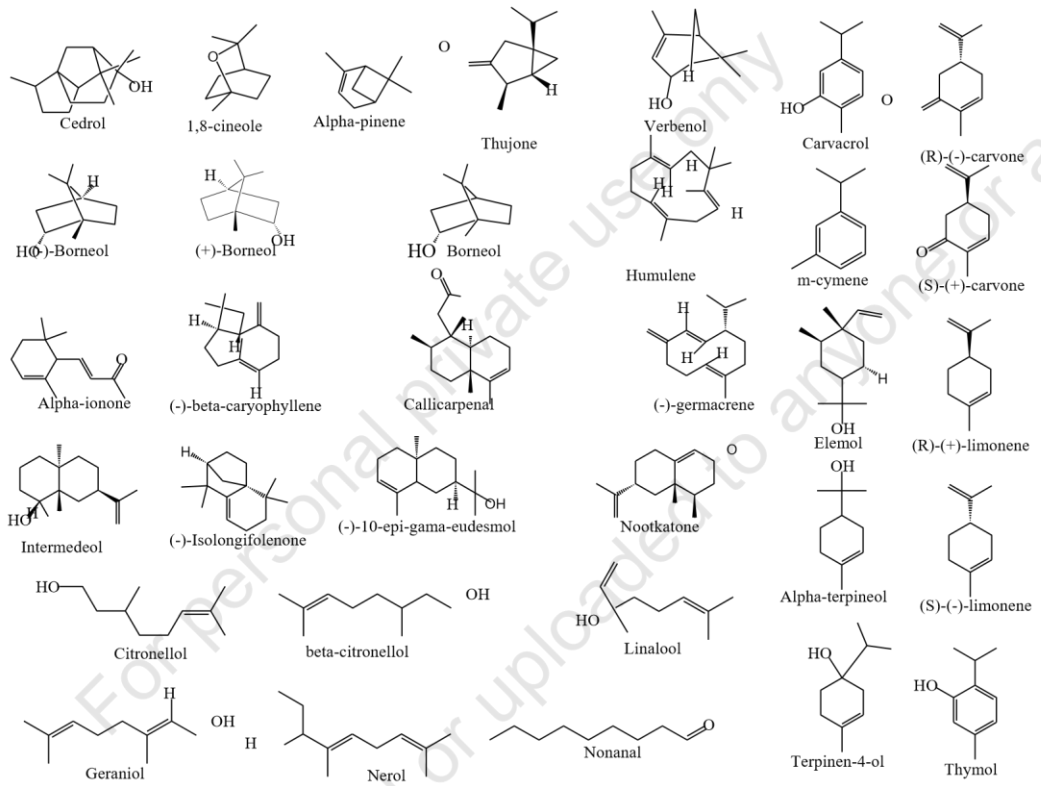

Fig. (2). Terpenoids tested as tick's repellent.

A variety of terpenes have high volatility and lipophilic characteristics, giving them the ability to penetrate the membrane. They are generally colorless and have aromatic odors [15, 16]. Many terpenes are used extensively in the perfumery, cosmetics, and food industries. These compounds show different biological activities; among them, acaricide and repellent against several species of arthropods and have as other functions pollination attractants, herbivore deterrents, antibacterial, anti-inflammatory, allelopathic toxic, antioxidants, thermotolerance, and photoprotection [17 - 20].

The repellent effect is induced by different isoprenoid metabolites, mainly acyclic, monocyclic, bicyclic monoterpenoid, diterpenoid, and sesquiterpenoid. Published data suggests that the repellent effect against arthropods is correlated with oxygenated components, with the occurrence of a hydroxyl group (OH) linked to a primary, secondary or aromatic carbon. However, depending on which carbon the hydroxyl group binds to, the repellent activity can be modulated [21]. These classes can be highly efficient in spatial repellency. However, the monoterpenoid is more volatile when compared to the sesquiterpenoids. Sesquiterpenoids have a higher molecular weight, with a 15-carbon structure, and thus their volatilization is lower, thus promoting a longer duration of their repellent efficiency [12]. This difference in repellency between

the terpenoid classes is directly related to the terpenes' ability to interfere with the vectors' odorous receptors, terpene volatility, molecular weight, polarity, and the intermolecular forces among the molecules of the repellent compounds [22].

Terpenoids Repellent Against Ticks

Arthropods, such as ticks, are responsible for vector-borne diseases to human and veterinary health. These diseases are limiting factors for animal production in tropical and subtropical regions of the world. In humans, they can cause severe toxic conditions and are a neglected public health problem in many parts of the world. Ticks are essential vectors, and tick bites can cause paralyzes, toxicoses, skin irritation, allergy, and secondary infections [23 - 25]. Tick control and disease prevention are mainly dependent on the use of chemical acaricides. Synthetic or natural repellents represent a viable form of protection against tick attacks. Several phytochemical defensives have been heavily exploited in the last decades for repellency against ticks.

Different terpenoids have been described to have repellent effects on tick species, about 33 terpenes (Fig. **2**) have been reported to have this activity (Table **1**). The studies already conducted show that terpenes have been tested on different ticks, such as *Hyalomma* sp., *Ixodes ricinus, Ixodes scapularis*, *Amblyomma americanum*, *Amblyomma sculptum*, *Riphicephalus annulatus, Riphicephalus, appendiculatus*, *Riphicephalus sanguineus*, and *Riphicephalus microplus*, and showed a repellent effect on the larval, nymph, and adult (Table **1**).

Table 1. Terpenoids that show repellency against ticks, concentrations used, percentage of repellency, ticks species and stages repelled.

Compound	Concentration/Repellency (%)	Species	Stage	References
β-Cyclocitral	0.1 μL=90.0	*R. appendiculatus*	*	Lwande *et al*., 1999

(Table 1) cont.....

Compound	Concentration/Repellency (%)	Species	Stage	References
β-Ocimene	0.1 µL=77.8	*R. appendiculatus*	*	Lwande *et al*., 1999
(+)-Borneol	1.3 µg.cm^{-2} = 33.3	*I. ricinus*	N	Schubert *et al*., 2017
(-)-Borneol	1.3 µg.cm^{-2} = 26.6	*I. ricinus*	N	Schubert *et al*., 2017
Borneol	0.5%= 64.3	*I. ricinus*	N	Pålsson *et al*., 2008
Carvacrol	0.078% = 50.0	*I. scapularis*	N	Dietrich *et al*., 2006
	1.0 mg (AI). cm^{-2} = 53.8	*A. americanum*	A	Jordan *et al*., 2012
	0.13 mg.cm^{-2} = 50.0	*R. microplus*	L	Lima *et al*., 2019
	5%=100.0	*I. ricinus*	L	Tabari *et al*., 2017
	1.0 mg (AI).cm^{-2} = 100.0	*I. scapularis*	A	Jordan *et al*., 2012
beta-citronellol	3.6% = 100.0	*A. sculptum*	a Un A	Ferreira *et al*., 2017
	= 90.0	*R. sanguineus*	Un A	Ferreira *et al*., 2017
(-)-beta-caryophyllene	10%= 65.5	*Ixodes ricinus*	N	Ashitani *et al*., 2015
Citronellol	0.206 mg.cm^{-2} =100.0	*A. americanum*	N	Tabanca *et al*., 2013
Callicarpenal	155 nmoles.cm^{-2} = 98.0	*I. scapularis*	N	Carroll *et al*., 2007
β-Caryophyllene	0.1 µL= 27.8	*R. appendiculatus*	*	Lwande *et al*., 1999
1,8-Cineol	0.5%= 70.4	*I. ricinus*	N	Pålsson et al., 2008
m-cymene	0.1µl = 90.0	*R. appendiculatus*	*	Lwande *et al*., 1999
Cedrol	0.1mL=29.7	*R. appendiculatus*	*	Ndungu *et al*., 1999

(Table 1) cont.....

Cedrene	0.1 µL= 86.7	*R. appendiculatus*	*	Lwande *et al*., 1999
(S)-(+)-carvone	4.14 $mg.cm^{-2}$ = 50.0	*R. microplus*	L	Lima *et al*., 2015
(R)-(−)-carvone	4.35 $mg.cm^{-2}$ = 50.0	*R. microplus*	L	Lima *et al*., 2015

(Table 1) cont.....

Compound	Concentration/Repellency (%)	Species	Stage	References
d-Limonene	0.1 µL= 27.2	*R. appendiculatus*	*	Lwande *et al*., 1999
α-Ionone	0.1 µL= 90.0	*R. appendiculatus*	*	Lwande *et al*., 1999
β-Ionone	0.1 µL= 48.7	*R. appendiculatus*	*	Lwande *et al*., 1999
(−)-10-epi-gama-eudesmol Elemol	0.103 mg.cm^{-2} = 90.0	*A. americanum*	N	Tabanca *et al*., 2013
	5.157 nmole.cm^{-2} = 50.0	*I. scapularis*	N	Carroll *et al*., 2010
	14783 nmole.cm^{-2} = 50.0		N	Carroll 2010 *et al*.,
Geraniol	1% = 98.4	*Hyalomma sp*.	*	Khallaayoune *et al*., 2009
	0.206 mg.cm^{-2} =90.0	*A. americanum*	N	Tabanca *et al*., 2013
	5% = 100.0	*A. americanum*	Un, A	Bissinger *et al*., 2014
	5% = 100.0.	*A. americanum*	Un, A	Bissinger *et al*., 2014
	5% = 100.0	*I. scapularis*	Un, A,	Bissinger *et al*., 2014
	5% = 100.0	*R. sanguineus*	Un, A	Bissinger *et al*., 2014
trans-Geranyl-acetone	0.1 µL= 90.0	*R. appendiculatus*	*	Lwande *et al*., 1999
trans-2-Methyl Cyclopentanol	0.1 µL= 90.0	*R. appendiculatus*	*	Lwande *et al*., 1999
(-)-Germacrene	10%=70.2	*I. ricinus*	N	Ashitani *et al*., 2015
Humulene	0%= 96.8	*Ixodes ricinus*	N	Ashitani *et al*., 2015

(Table 1) cont.....

Intermedeol	155mol. cm^{-2} = 96.0	*I.scapularis*	N	Carroll *et al*., 2007
(-)-Isolongifolenone	78 $nmol.cm^{-2}$ =100.0	*I. scapularis*	N	Zhang *et al*., 2009
Alpha-Ionone	0.1µl = 90.0	*R. appendiculatus*	*	Lwande *et al*., 1999
Linalool	5%=50.24	*I.ricinus*	L	Tabari *et al*., 2017

(Table 1) cont.....

(Table 1) cont.....

Compound	Concentration/Repellency (%)	Species	Stage	References
(S)-(−)-limonene	6.56 mg.cm^{-2} = 50.0	*R. microplus*	L	Lima *et al*., 2015
(R)-(+)-limonene	7.81 mg.cm^{-2} = 50.0	*R. microplus*	L	Lima *et al*., 2015
Nerolidol	0.1µl = 100	*R. appendiculatus*	*	Lwande *et al*., 1999
Nootkatone	0.089% = 50.0	*I. scapularis*	N	Dietrich *et al*., 2006
	1.0 mg (AI).cm^{-2} = 100.0	*I. scapularis*	A	Jordan *et al*., 2012
	1.0 mg (AI).cm^{-2} = 100.0	*A. americanum*	A	Jordan *et al*., 2012
Nonanal	0.1µl = 90.0	*R. appendiculatus*	*	Lwande *et al*., 1999
Nerol	0.1µl = 90.0	*R. appendiculatus*	*	Lwande *et al*., 1999
Alpha-Pinene	10%= 83.4	*I. ricinus*	N	Tunón *et al*., 2006
1-α-Terpineol	0.1 µL= 89.9	*R. appendiculatus*	a*	Lwande *et al*., 1999
Alpha-terpineol	65 µg.cm^{-2} = 50.0	*I. ricinus*	N	Schubert *et al*., 2017
Phytol	0.1 mL= 48.4	*R. appendiculatus*	*	Lwande *et al*., 1999
trans-Phytol	0.1 mL= 48.4	*R. appendiculatus*	*	Lwande *et al*., 1999
Terpinen-4-ol	65 µg.cm^{-2} = 66.6	*I. ricinus*	N	Schubert *et al*., 2017
	0.909% = 50.0	*I. scapularis*	N	Dietrich *et al*., 2006
Thymol	0.075 mg.cm^{-2} = 66.7	*A. americanum*	*	Carroll *et al*., 2017
	10%= 85.7	*R. annulatus*	L	Arafa *et al*., 2020
	5%=100.0	*I. ricinus*	L	Tabari *et al*., 2017
Thujone	0.5%= 70.0	*I. ricinus*	N	Pålsson *et al*., 2008
Verbenol	0.5%= 68.6	*I. ricinus*	N	Pålsson *et al*., 2008

L=larvae, N=nymphs, A=adult, Un=unfed, UnA=unfed adult.

Most of the compounds with repellent effect, for example, monoterpenes such as limonene, citronellol, and citronellal, are common secondary metabolites of the essential oils that present arthropod-repellency activity. The repellent effect of terpenoids proves that they can be used as promising alternatives for producing new repellent products based on plant molecules or to assist synthetic products in prolonging their effect [26].

The repellent effect may be correlated to the structures of the terpenes and their interactions with a series of biochemical and physiological events that have been little explained to understand the mechanism of action of natural repellent compounds. Many of the terpenes reported the repellent activity in their structure oxygenated components, linked to a hydroxyl group on carbon 1, 2, or aromatic carbons [27]. Another factor that may contribute to repellency is the volatility of the terpenes, which can interfere with the location of their hosts. Arthropods rely predominantly upon the olfactory perception of volatility during host location [9]. Consequently, the terpenes cause a disorder in the tick's behavior in locating its host, preventing it from attacking. Olfactory sensilla ticks can detect volatile molecules, where it can be suggested that olfaction also is involved in the repellency process [21].

Bioassays to Evaluate Repellent Compounds Against Ticks

For many decades, repellent development has been studied using different methods considering the biology and the species' behavior. The tick's behavior, mainly the negative geotropism of the majority of species, is used to evaluate the repellency effect [23]. The repellents could be classified into contact and spatial repellents. For contact repellents, the targets must be in touch with the treated surface before being repelled. In contrast, the spatial repellents must function at a distance from the application site, and the targets do not need to be in physical touch with the treated surface.

Among the disadvantages of researches into new tick repellents is the lack of a standardized test method. These studies differ in 1) species, 2) life stages of the ticks used, 3) the duration in which repellency and toxicity are assessed, 4) the quantity and formulations of the active ingredients of crude extracts, fractions, or essential oils, 5) use of solvent and 6) variability of the ticks' behaviors in assays [3, 28]. These variations in test methodologies and test conditions interfere with comparing studies and selecting molecules with possible repellent potential. Therefore, it is understood that the efficiency of all the bioassays will depend on essential factors such as 1) type of material used, 2) physical-chemical characteristics of the molecule used, 3) time of exposure and 4) the number of ticks used in the chosen method.

Tick Climbing Bioassay

This method is the most used to analyzed tick repellent [29]. The principle is based on the negative geotropism of ticks (Fig. **3**). The tick is added to the base and needs to pass into a treated area with an experimental compound. The substrate used can be filter paper, glass stick, leather, bamboo stick, or other inert material or add the host odor. The number of specimens used depends on the stage and species of the tick.

A variation of the climbing assay is the Fingertip Bioassay, used as an experimental compound to impregnate part of the finger of a human volunteer. The tick is disposable at the base and permits a climb to the finger. The ticks that move up to the treated area are considered non-repelled, while those that retreat or fall off the treated surface are repelled [3, 28].

Fig. (3). Negative geotropism in *Rhipicephalus microplus* ticks and fingertip bioassay with *Amblyomma sculptum.*

Olfactometer Bioassay

Bioassay using olfactometers to test repellent compounds on ticks can be performed. The assay with the attractive stimulus is typically associated with the search behavior of the hosts. The assays that use anemotaxic stimulants such as four-way, arena, or Y-shaped olfactometers, rely on the ectoparasite stimulus to guide themselves according to the prevailing wind plume [30, 31]. The double-choice Y-shaped olfactometer is one of the most used because it allows the insertion of two different odors during the analysis. Although the arena olfactometer is also used for repellency tests, it has a limitation when using only one odor for the test [27, 30].

Petri Dish Bioassay

The Petri dish bioassay is well-known as the “Choice Assay,” where half of the dish is treated and the other controlled (without treatment). After treatments of one side of the evaluated compound and the other with the same amount of solvent, the papers need to dry for ten minutes in a closed chapel to evaporate the solvent. Then, the ticks are placed in the center of the dish in groups of six, three males or three females. The positions found after 5, 10, and 30 minutes of testing are assessed to perform the

analysis. Similar procedures could be achieved using watch glass and impregnating a circular filter paper adding in the glass center.

Although the methodology describes a time for the volatilization of the experimental compounds, these methods are not indicated for compounds that present fumigation activity since they do not have air circulation and kill ticks.

Bioassay of the Falcon Tissue Flask Repellency

Moving Object Bioassay

This method was developed to increase the motivation of ticks to move while requiring search behavior by the host [35]. For this assay, heat, and movement are used to nurture attractions associated with hosts, allowing ectoparasites to exhibit their natural behavior of holding on to a host in the lab. It is necessary to use a vertical drum slowly rotating heated. In the drum, there is a surface that will be used as a base for the effective attachment of the parasite. A glass stick is then placed horizontally, ending in front of the drum, where the ticks will approach.

The distance between the drum and the glass stick is adjusted so that the tick reaches only the desired attachment location. As the drum rotates, the attachment region passes periodically, and the parasite can attach itself to the moving object, simulating the animal that gives. For the test analysis, are accounted for: ticks that approach/ do not approach the drum (spatial repellency), ticks fixed to the drum, and ticks that remain on the treated surface or fall off (contact repellency).

Repellent Compound on Ticks of Medical Importance

The species of ticks usually describe as disease vectors to humans are *A. sculptum*, *A. aureolatum*, *A. ovale*, *Dermacentor andersoni*, and *Dermacentor variabilis* [36, 37]. These species are described as vectors of Rickettsia, the etiologic agent of the Rocky Mountain spotted fever (RMSF). Due to being involved in the transmission of numerous pathogens, these ticks create essential social and economic impacts associated with medical costs, loss of productivity, and death [38]. Considering the many damages created by ticks and the hardships found in effectively instituting pest control, a viable alternative to prevent diseases stemming from parasitism from these species is to reduce interaction with its vectors through the use of repellents [5].

The use of human repellents dates thousands of years ago, but it was only after World War II that it reached its apex, where soldiers employed chemical compounds to prevent bites from insects that were responsible for the transmission of some diseases. To prevent more deaths, numerous researches regarding long-lasting repellent compounds were initiated. One of the first repellent compounds to be developed was Dimethyl phthalate (DMP), followed by Indalone and ethyl hexanediol [10]. The main synthetic repellents found in the market are DEET, IR3535, Icaridin (Picaridin), DEPA, and permethrin (synthetic pyrethroid). But knowledge about the effects related to intoxication caused by synthetic repellents, in addition to the dissatisfaction regarding produce and environmental contamination, leads to a search for more ecologically viable alternatives [39].

Facing the issues occasioned by synthetic repellents, NP, namely plant-based products with volatile constituents, can be an essential source of molecules with repellent action. It is already possible to find citronella-based repellents in the market whose active components are citronellal and Citronellol [5]. Currently, the employment of NP extracted from plants has provoked interest around the world and comprises a promising strategy to tick control, especially essential oils and their chemical constituents. Knowing the importance of the compounds, it is then possible to list some Terpenoids

repellent action over ticks of medical importance regarding public health, as seen in Table **1**.

Repellent Compounds on Dogs' Ticks

Several tick species are capable of infecting dogs, such as *R. sanguineus*, *A. americanum*, *Haemaphysalis longicornis*, *I. scapularis*, *D. variabilis*, and *I. ricinus* [40 - 42]. Besides causing blood loss, irritability, and hypersensibility, these ticks are pathogen vectors [11, 22, 43]. Dogs are exposed to tick infestation in various situations, *e.g*., during walks in parks and forests [44]. To prevent these ticks from transmitting pathogens, it is necessary to institute protection measures against ectoparasites [45]. One action is to avoid contact with ticks by employing parasite-repellent products. Such products are practical tools to reduce the possibility of interaction with ticks and, subsequently, pathogen transmission due to compounds that inhibit the host's attraction [46]. However, the process of parasite-repellent compounds occurs mainly due to interaction with irritant agents, also named deterrents [47] (Fig. **1**).

The main tick-repellent for dogs found in the market are based on synthetic compounds such as Pyrethroids and Phenylpyrazoles in the form of leashes, spray, spot-on, and pour-on [48 - 51]. These compounds are effective and relatively safe. However, there are concerns about their possible adverse effects on human and animal health [52, 53]. Also, there are reports of ticks resistant to the drugs, consequently reducing the acaricidal efficacy [54 - 56] significantly. In light of this issue, the employment of terpenes as repellents has been fully studied (Table **1**). However, studies on the effectiveness of these compounds as repellents have been carried out mainly *in vitro*. Until this work, there is no scientific literature on terpene-based repellents applied directly to dogs [57].

Based on *in vitro* tests, some products using essential oils are already being commercialized as repellents. Since some essential oils, such as clove oil, thyme oil, and cinnamon oil, are listed as "Generally recognized as safe" (GRAS) by Food and Drug Administration (FDA) and exempt from toxicity data requirements from EPA [58, 59]. These products, commercialized as repellents, are composed of essential oil mixtures such as Ultrashield Green (geraniol, citronella, rosemary, lemongrass, cedar e thyme oils), Pyranha Zero-bite (geraniol, thyme, and mint oils), and Flea+Tick Spray repellent (lemongrass, cinnamon, sesame and castor oils) [60]. The list of commercialized products based on natural compounds that produce repellent effects is very extensive [61].

Natural repellents can have a substantial effect against parasites, which could be beneficial in the prevention of tick infestation. Its low toxicity and appeal to be a green product, as they do not act on pollinating insects due to its high volatility, make this type of product increasingly attractive to consumers. When related to pets, such as dogs, due to their proximity to humans [62, 63]. However, it is

necessary to note that the low toxicity does not exempt said products from other adverse effects in dogs and humans altogether [64]. Finally, the efficacy of tick repellents is poorly studied, which further hampers the use of new repellents [65] (Fig. **4**).

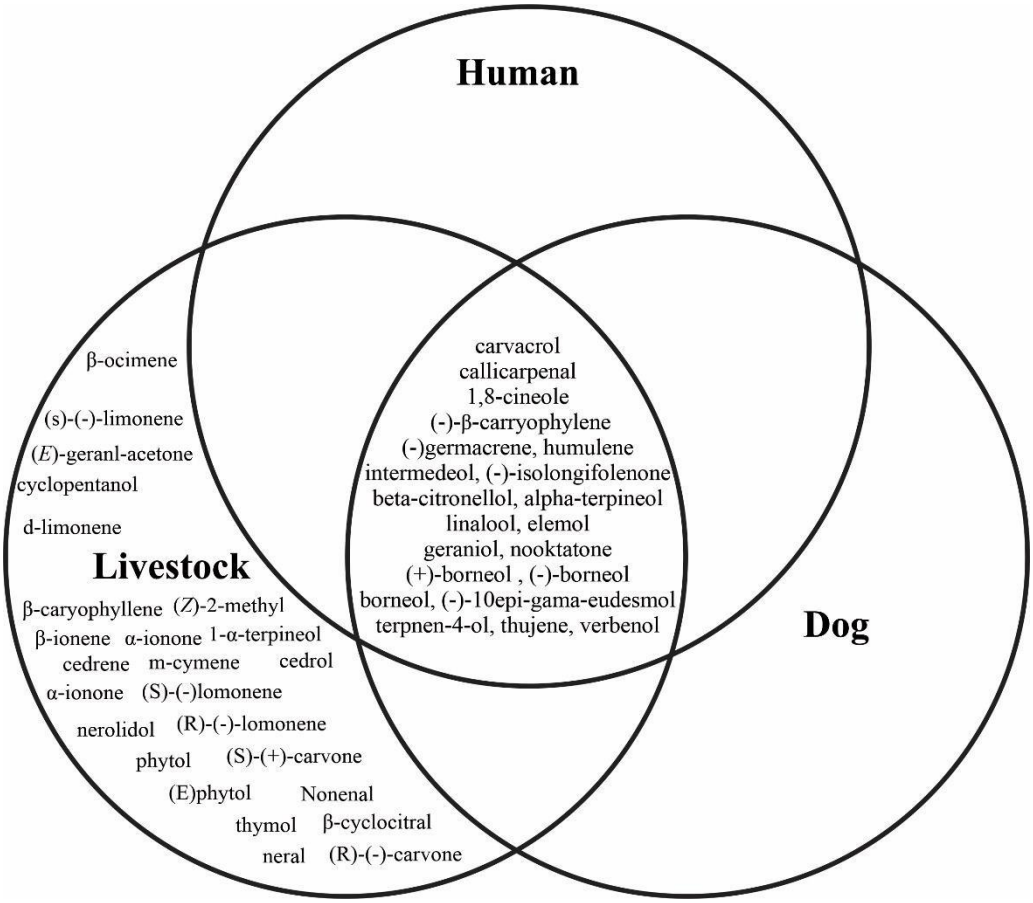

Fig. (4). Venn diagram of terpenes showed with repellent effect on ticks from human, livestock and dog.

Repellent Compounds on Livestock's Ticks

The primary tick species that infest livestock animals are the southern cattle tick, *R. microplus* [54], One-host cattle fever tick, *R. annulatus* [66] and Brown ear tick *R. appendiculatus* [67] infesting cattle as primary hosts and, *A. sculptum* [68] and *A. cajennense* [69] generalist ticks common in horses. For this reason, these five species of ticks are among the most used in studies for the determination of the repellent activity of terpenoids.

A total of 27 terpenoids were tested as a repellent against ticks parasites of livestock animals (Table **1**). All these compounds are monoterpenes, sesquiterpenes, or diterpenes. The monoterpenes have the most compound with repellent activity (17 substances), followed by sesquiterpenes with eight compounds, and finally, the diterpenes with only two compounds. It is known that the constituents of essential oils are highly volatile. For this reason, researchers carried out the encapsulation of the monoterpene carvacrol with the yeast cell wall to increase the time of repellent activity of this molecule, delaying its volatilization [30].

Another species of tick widespread in horses is *Dermacentor nitens* (horse ear tick). However, there is a significant lack of studies to determine the repellent effect of natural products on this tick. The search in the scientific literature revealed only a single study

using eugenol (phenylpropanoid) as a repellent for *D. nitens* larvae [70]. To date, no terpenoids have been evaluated for repellent activity on this tick species

CONCLUDING REMARKS

Terpenoids have repellent activity against different species of ticks. This repellent activity is due to the various biochemical mechanisms involved, conferring degrees of repellency between the terpenoid compounds. Although there are several synthetic repellents for the control of ticks, terpenoids offer a safe alternative with low toxicity. This makes terpenoids attractive for human, animal, and environmental health. In addition, there is a range of molecules available, which can be essential tools as tick repellents. However, the lack of repellent *in vivo* studies with terpenoid compounds impedes a better understanding of how these compounds work in routine use. These problems directly reflect that few terpenes are commercially available, and their use against ticks is not widely used. Thus, the development of products with these molecules deserves to be further explored as a strategy to control ticks.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

The authors acknowledge the financial support received from CNPq (Brazilian National Council for Scientific and Technological Development), FAPEMA (Maranhão State Research Foundation) and FINEP (Funding Authority for Studies and Projects) (PRONEM 01773/14 and IECT (Science and Technology Institute of Maranhão) Biotechnology). This study was financed in part by CAPES, Finance Code 001.

REFERENCES

[1] Dethier, V.G.; Browne, B.L.; Smith, C.N. The designation of chemicals in terms of the responses they elicit from insects. *J. Econ. Entomol.*, **1960**, *53*(1), 134-136.
[http://dx.doi.org/10.1093/jee/53.1.134]

[2] Ferreira, L.L.; Oliveira Filho, J.G.; Mascarin, G.M.; León, A.A.P.; Borges, L.M.F. *In vitro* repellency of DEET and β-citronellol against the ticks Rhipicephalus sanguineus sensu lato and Amblyomma sculptum. *Vet. Parasitol.*, **2017**, *239*, 42-45.
[http://dx.doi.org/10.1016/j.vetpar.2017.04.021] [PMID: 28495195]

[3] Bissinger, B.W.; Roe, R.M. Tick repellents: Past, present, and future. *Pestic. Biochem. Physiol.*, **2010**, *96*(2), 63-79.
[http://dx.doi.org/10.1016/j.pestbp.2009.09.010]

[4] Da Silva Lima, A.; De Carvalho, J.F.; Peixoto, M.G.; Blank, A.F.; Borges, L.M.F.; Costa Junior, L.M. Assessment of the repellent effect of *Lippia alba* essential oil and major monoterpenes on the cattle tick *Rhipicephalus microplus*. *Med. Vet. Entomol.*, **2016**, *30*(1), 73-77.
[http://dx.doi.org/10.1111/mve.12140] [PMID: 26471008]

[5] Tavares, M.; da Silva, M.R.M.; de Oliveira de Siqueira, L.B.; Rodrigues, R.A.S.; Bodjolle-d'Almeida, L.; dos Santos, E.P.; Ricci-Júnior, E. Trends in insect repellent formulations: A review. *Int. J. Pharm.*, **2018**, *539*(1-2), 190-209.
[http://dx.doi.org/10.1016/j.ijpharm.2018.01.046] [PMID: 29410208]

[6] Pitasawat, B.; Choochote, W.; Tuetun, B.; Tippawangkosol, P.; Kanjanapothi, D.; Jitpakdi, A.; Riyong, D. Repellency of aromatic turmeric Curcuma aromatica under laboratory and field conditions. *Journal of vector ecology: journal of the Society for Vector Ecology, 28*(2), 234-240.**2003**,

[7] Elmhalli, F.H.; Pålsson, K.; Örberg, J.; Jaenson, T.G.T. Acaricidal effects of Corymbia citriodora oil containing para-menthane-3,8-diol against nymphs of Ixodes ricinus (Acari: Ixodidae). *Exp. Appl. Acarol.*, **2009**, *48*(3), 251-262.
[http://dx.doi.org/10.1007/s10493-009-9236-4] [PMID: 19169833]

[8] Pohlit, A. M.; Lopes, N. P.; Gama, R. A.; Tadei, W. P.; Andrade Neto, V. F. D. Patent literature on mosquito repellent inventions which contain plant essential oils-a review. *Volume 77*(6), 598-617.**2011**,

[9] Frances, S.P.; Wirtz, R.A. Repellents: past, present, and future. *J. Am. Mosq. Control Assoc.*, **2005**, *21*(sp1) Suppl., 1-3.
[http://dx.doi.org/10.2987/8756-971X(2005)21[1:RPPAF]2.0.CO;2] [PMID: 16921675]

[10] Moore, S. J.; Debboun, M. History of insect repellents. (Insect repellents: principles, methods and uses), 3-29.**2007**,

[11] Rehman, J.U.; Ali, A.; Khan, I.A. Plant based products: Use and development as repellents against mosquitoes: A review. *Fitoterapia*, **2014**, *95*, 65-74.
[http://dx.doi.org/10.1016/j.fitote.2014.03.002] [PMID: 24631763]

[12] da Silva, M.R.M.; Ricci-Júnior, E. An approach to natural insect repellent formulations: from basic research to technological development. *Acta Trop.*, **2020**, *212*105419
[http://dx.doi.org/10.1016/j.actatropica.2020.105419] [PMID: 32119826]

[13] Kiyama, R. Estrogenic terpenes and terpenoids: Pathways, functions and applications. *Eur. J. Pharmacol.*, **2017**, *815*, 405-415.

[http://dx.doi.org/10.1016/j.ejphar.2017.09.049] [PMID: 28970013]

[14] Marmulla, R.; Harder, J. Microbial monoterpene transformationsâ€"a review. *Front. Microbiol.*, **2014**, *5*, 346.
[http://dx.doi.org/10.3389/fmicb.2014.00346] [PMID: 25076942]

[15] Clarke, S., Ed. *Essential Chemistry for Aromatherapy E-Book*; Elsevier Health Sciences, **2009**.

[16] Oz, M.; Lozon, Y.; Sultan, A.; Yang, K.H.S.; Galadari, S. Effects of monoterpenes on ion channels of excitable cells. *Pharmacol. Ther.*, **2015**, *152*, 83-97.
[http://dx.doi.org/10.1016/j.pharmthera.2015.05.006] [PMID: 25956464]

[17] Kozioł, A.; Stryjewska, A.; Librowski, T.; Sałat, K.; Gaweł, M.; Moniczewski, A.; Lochyński, S. An overview of the pharmacological properties and potential applications of natural monoterpenes. *Mini Rev. Med. Chem.*, **2015**, *14*(14), 1156-1168.
[http://dx.doi.org/10.2174/1389557514666141127145820] [PMID: 25429661]

[18] Pateraki, I.; Kanellis, A.K. Stress and developmental responses of terpenoid biosynthetic genes in Cistus creticus subsp. creticus. *Plant Cell Rep.*, **2010**, *29*(6), 629-641.
[http://dx.doi.org/10.1007/s00299-010-0849-1] [PMID: 20364257]

[19] Tholl, D. Biosynthesis and biological functions of terpenoids in plants. *Biotechnology of isoprenoids*, , 63-106.**2015**

[20] Tetali, S.D. Terpenes and isoprenoids: a wealth of compounds for global use. *Planta*, **2019**, *249*(1), 18.
[http://dx.doi.org/10.1007/s00425-018-3056-x] [PMID: 30467631]

[21] Nerio, L.S.; Olivero-Verbel, J.; Stashenko, E. Repellent activity of essential oils: A review. *Bioresour. Technol.*, **2010**, *101*(1), 372-378.
[http://dx.doi.org/10.1016/j.biortech.2009.07.048] [PMID: 19729299]

[22] Norris, E.; Coats, J. Current and future repellent technologies: the potential of spatial repellents and their place in mosquito-borne disease control. *Int. J. Environ. Res. Public Health*, **2017**, *14*(2), 124.
[http://dx.doi.org/10.3390/ijerph14020124] [PMID: 28146066]

[23] Soares, S.F. Repelência de extratos de plantas e do deet (n, ndiethyl-m-toluamide) em amblyomma cajennense (acari: ixodidae). *Dissertação de Mestrado. Veterinária da Universidade Federal de Goiás*, **2008**.

[24] Ghosh, S.; Nagar, G. Problem of ticks and tick-borne diseases in India with special emphasis on progress in tick control research: a review. *J. Vector Borne Dis.*, **2014**, *51*(4), 259-270. [PMID: 25540956]

[25] Solano-Gallego, L.; Sainz, Á.; Roura, X.; Estrada-Peña, A.; Miró, G. A review of canine babesiosis: the European perspective. *Parasit. Vectors*, **2016**, *9*(1), 336.
[http://dx.doi.org/10.1186/s13071-016-1596-0] [PMID: 27289223]

[26] Elmhalli, F. H.; Pålsson, K.; Örberg, J. Jaenson, T. G. Acaricidal effects **2009**.

[27] Pohlit, A. M.; Lopes, N. P.; Gama, R. A.; Tadei, W. P. Andrade Neto, V. F. D.Patent literature on mosquito repellent inventions which contain plant essential oils-a review. **2011**, *77*(6), 598-617.

[28] Adenubi, O.T.; Ahmed, A.S.; Fasina, F.O.; McGaw, L.J.; Eloff, J.N.; Naidoo, V. Pesticidal plants as a possible alternative to synthetic acaricides in tick control: A systematic review and meta-analysis. *Ind. Crops Prod.*, **2018**, *123*, 779-806.
[http://dx.doi.org/10.1016/j.indcrop.2018.06.075]

[29] de Oliveira Filho, J.G.; Ferreira, L.L.; Sarria, A.L.F.; Pickett, J.A.; Birkett, M.A.; Mascarin, G.M.; de León, A.A.P.; Borges, L.M.F. 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 Tick Borne Dis.*, **2017**, *8*(1), 139-145.
[http://dx.doi.org/10.1016/j.ttbdis.2016.10.010] [PMID: 28340941]

[30] Lima, A.S.; Landulfo, G.A.; Costa-Junior, L.M. Repellent effects ofencapsulated carvacrol on the Rhipicephalus (Boophilus) microplus (Acari: Ixodidae). *J. Med. Entomol.*, **2019**, *56*(3), 881-885.
[http://dx.doi.org/10.1093/jme/tjy240] [PMID: 30805609]

[31] Nogueira, J.A.P.; Figueiredo, A.; Duarte, J.L.; de Almeida, F.B.; Santos, M.G.; Nascimento, L.M.; Fernandes, C.P.; Mourão, S.C.; Toscano, J.H.B.; Rocha, L.M.; Chagas, A.C.S. Repellency effect of Pilocarpus spicatus A. St.-Hil essential oil and nanoemulsion against Rhipicephalus microplus larvae. *Exp. Parasitol.*, **2020**, *215*107919
[http://dx.doi.org/10.1016/j.exppara.2020.107919] [PMID: 32442440]

[32] Jaenson, T.G.T.; Pålsson, K.; Borg-Karlson, A.K. Evaluation of extracts and oils of tick-repellent plants from Sweden. *Med. Vet. Entomol.*, **2005**, *19*(4), 345-352. [http://dx.doi.org/10.1111/j.1365-2915.2005.00578.x] [PMID: 16336298]

[33] Dietrich, G.; Dolan, M. C.; Peralta-Cruz, J.; Schmidt, J.; Piesman, J.; Eisen, R. J. Repellent activity of fractioned compounds from Chamaecyparis nootkatensis essential oil against nymphal Ixodes scapularis (Acari: Ixodidae). *J. Med. Entomol.*, **2006**, *43*(5), 957-961.

[34] Ashitani, T.; Garboui, S.S.; Schubert, F.; Vongsombath, C.; Liblikas, I.; Pålsson, K.; Borg-Karlson, A.K. Activity studies of sesquiterpene oxides and sulfides from the plant Hyptis suaveolens (Lamiaceae) and its repellency on Ixodes ricinus (Acari: Ixodidae). *Exp. Appl. Acarol.*, **2015**, *67*(4), 595-606.
[http://dx.doi.org/10.1007/s10493-015-9965-5] [PMID: 26385208]

[35] Nwanade, C.F.; Wang, M.; Wang, T.; Yu, Z.; Liu, J. Botanical acaricides and repellents in tick control: current status and future directions. *Exp. Appl. Acarol.*, **2020**, *81*(1), 1-35.
[http://dx.doi.org/10.1007/s10493-020-00489-z] [PMID: 32291551]

[36] Szabó, M.P.J.; Pinter, A.; Labruna, M.B. Ecology, biology and distribution of spotted-fever tick vectors in Brazil. *Front. Cell. Infect. Microbiol.*, **2013**, *3*, 27.
[http://dx.doi.org/10.3389/fcimb.2013.00027] [PMID: 23875178]

[37] Eisen, R.J.; Kugeler, K.J.; Eisen, L.; Beard, C.B.; Paddock, C.D. Tick-borne zoonoses in the United States: persistent and emerging threats to human health. *ILAR J.*, **2017**, *58*(3), 319-335.
[http://dx.doi.org/10.1093/ilar/ilx005] [PMID: 28369515]

[38] Drexler, N. A.; Traeger, M. S.; McQuiston, J. H.; Williams, V.; Hamilton, C. Medical and indirect costs associated with a Rocky Mountain spotted fever epidemic in Arizona, 2002–2011. *Am. J. Trop. Med. Hyg.*, **2015**, *93*(3), 549-551.

[39] de Siqueira, S.L.; Kruse, M.H. [Agrochemicals and human health: contributions of healthcare professionals]. *Rev. Esc. Enferm. USP*, **2008**, *42*(3), 584-590.
[PMID: 18856129]

[40] Dantas-Torres, F. Biology and ecology of the brown dog tick, Rhipicephalus sanguineus. Parasites & vectors, *3*(1), 1-11.**2010**,

[41] Minigan, J.N.; Hager, H.A.; Peregrine, A.S.; Newman, J.A. Current and potential future distribution of the American dog tick (Dermacentor variabilis, Say) in North America. *Ticks Tick Borne Dis.*, **2018**, *9*(2), 354-362.
[http://dx.doi.org/10.1016/j.ttbdis.2017.11.012] [PMID: 29275873]

[42] Porretta, D.; Mastrantonio, V.; Amendolia, S.; Gaiarsa, S.; Epis, S.; Genchi, C. Effects of global changes on the climatic niche of the tick Ixodes ricinus inferred by species distribution modelling. Parasites & vectors, **2013**, *6*(1), 1-8.

[43] Ferreira Vilela, E. Castro Della Lucia, T. M.Feromónios de insetos (Biología, química e emprego no manejo de pragas. **1987**.

[44] Jennett, A. L.; Smith, F. D. Tick infestation risk for dogs in a peri-urban park. Parasites &amp. *Vectors*, **2013**, *6*(1), 1-10.

[45] Dantas-Torres, F.; Ketzis, J.; Mihalca, A.D.; Baneth, G.; Otranto, D.; Tort, G.P.; Watanabe, M.; Linh, B.K.; Inpankaew, T.; Jimenez Castro, P.D.; Borrás, P.; Arumugam, S.; Penzhorn, B.L.; Ybañez, A.P.; Irwin, P.; Traub, R.J. TroCCAP recommendations for the diagnosis, prevention and treatment of parasitic infections in dogs and cats in the tropics. *Vet. Parasitol.*, **2020**, *283*109167
[http://dx.doi.org/10.1016/j.vetpar.2020.109167] [PMID: 32580071]

[46] Piesman, J.; Eisen, L. Prevention of tick-borne diseases. *Annu. Rev. Entomol.,* **2008**, *53*(1), 323-343. [http://dx.doi.org/10.1146/annurev.ento.53.103106.093429] [PMID: 17877457]

[47] Mcmahon, C.; Kröber, T. Ensaios *in vitro* para repelentes e dissuasores para carrapatos: diferentes efeitos dos produtos quando testados com estímulos atrativos ou de detenção. *Med. Vet. Entomol.,* **2003**, *17*(4), 370-378. [http://dx.doi.org/10.1111/j.1365-2915.2003.00453.x] [PMID: 14651650]

[48] Blair, J.; Fourie, J. J.; Varloud, M. Efficacy and speed of kill of a topically applied formulation of dinotefuran-permethrin-pyriproxyfen against weekly tick infestations with Rhipicephalus sanguineus (sensu lato) on dogs. *Parasites & vectors,* **2016**, *6*(1), 1-8.

[49] Cutolo, A. A.; Galvis-Ovallos, F.; de Souza Neves, E.; Silva, F. O.; Chester, S. T. Repellent efficacy of a new combination of fipronil and permethrin against Lutzomyia longipalpis. Parasites & vectors, **2018**, *11*(1), 247.

[50] de Burgh, S.; Hunter, K.; Jackson, C.; Chambers, M.; Klupiec, C.; Smith, V. Repellency Effect of an Imidacloprid / Flumethrin (Seresto®) Controlled Release Polymer Matrix Collar against the Australian Paralysis Tick (Ixodes holocyclus) in Dogs. *Parasitol. Res.,* **2017**, *116*(S1) Suppl. 1, 145-156. [http://dx.doi.org/10.1007/s00436-017-5500-4] [PMID: 28717957]

[51] Dumont, P.; Chester, T. S.; Gale, B.; Soll, M.; Fourie, J. J. Acaricidal efficacy of a new combination of fipronil and permethrin against Ixodes ricinus and Rhipicephalus sanguineus ticks. Parasites & vectors, **2015**, *8*(1), 1-5.

[52] Abdel-Rahman, A.; Shetty, A.K.; Abou-Donia, M.B. Subchronic dermal application of N,N-diethyl m-toluamide (DEET) and permethrin to adult rats, alone or in combination, causes diffuse neuronal cell death and cytoskeletal abnormalities in the cerebral cortex and the hippocampus, and Purkinje neuron loss in the cerebellum. *Exp. Neurol.,* **2001**, *172*(1), 153-171. [http://dx.doi.org/10.1006/exnr.2001.7807] [PMID: 11681848]

[53] Corbel, V.; Stankiewicz, M.; Pennetier, C.; Fournier, D.; Stojan, J.; Girard, E. Evidência para inibição de colinesterases em sistemas nervosos de insetos e mamíferos pelo deet repelente de insetos. *BMC Biol.,* **2009**, *7*(1), 1-11.

[54] Becker, S.; Webster, A.; Doyle, R.L.; Martins, J.R.; Reck, J.; Klafke, G.M. Resistance to deltamethrin, fipronil and ivermectin in the brown dog tick, Rhipicephalus sanguineus sensu stricto, Latreille (Acari: Ixodidae). *Ticks Tick Borne Dis.,* **2019**, *10*(5), 1046-1050. [http://dx.doi.org/10.1016/j.ttbdis.2019.05.015] [PMID: 31175029]

[55] Eiden, A.L.; Kaufman, P.E.; Oi, F.M.; Allan, S.A.; Miller, R.J. Detection of permethrin resistance and fipronil tolerance in Rhipicephalus sanguineus (Acari: Ixodidae) in the United States. *J. Med. Entomol.,* **2015**, *52*(3), 429-436. [http://dx.doi.org/10.1093/jme/tjv005] [PMID: 26334817]

[56] Eiden, A.L.; Kaufman, P.E.; Oi, F.M.; Dark, M.J.; Bloomquist, J.R.; Miller, R.J. Determination of metabolic resistance mechanisms in pyrethroid-resistant and fipronil-tolerant brown dog ticks. *Med. Vet. Entomol.,* **2017**, *31*(3), 243-251. [http://dx.doi.org/10.1111/mve.12240] [PMID: 28639697]

[57] Soutar, O.; Cohen, F.; Wall, R. Essential oils as tick repellents on clothing. *Exp. Appl. Acarol.,* **2019**, *79*(2), 209-219. [http://dx.doi.org/10.1007/s10493-019-00422-z] [PMID: 31578646]

[58] EPA US. http://www.epa.gov/oppbppd1/biopesticides/regtools/25b_list.htm**2004**.

[59] Jiang, Z.L.; Akhtar, Y.; Zhang, X.; Bradbury, R.; Isman, M.B. Insecticidal and feeding deterrent activities of essential oils in the cabbage looper, Trichoplusia ni (Lepidoptera: Noctuidae). *J. Appl. Entomol.,* **2012**, *136*(3), 191-202. [http://dx.doi.org/10.1111/j.1439-0418.2010.01587.x]

[60] Eastep, J. *Efficacy of Natural Repellents in Ticks.,* **2018**.

[61] George, D.R.; Finn, R.D.; Graham, K.M.; Sparagano, O.A.E. Present and future potential of plantderived products to control arthropods of veterinary and medical significance. *Parasit. Vectors,* **2014**, *7*(1), 28. [http://dx.doi.org/10.1186/1756-3305-7-28] [PMID: 24428899]

[62] Isman, M.B. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annu. Rev. Entomol.,* **2006**, *51*(1), 45-66. [http://dx.doi.org/10.1146/annurev.ento.51.110104.151146] [PMID: 16332203]

[63] Kiss, T.; Cadar, D.; Spînu, M. Tick prevention at a crossroad: New and renewed solutions. *Vet. Parasitol.,* **2012**, *187*(3-4), 357-366. [http://dx.doi.org/10.1016/j.vetpar.2012.02.010] [PMID: 22424918]

[64] Trumble, JT Caveat emptor: considerações de segurança para produtos naturais usados no controle de artrópodes. *Entomologista americano*, *48*(1), 7-13.**2002**,

[65] Bissinger, B.W.; Schmidt, J.P.; Owens, J.J.; Mitchell, S.M.; Kennedy, M.K. Activity of the plantbased repellent, TT-4302 against the ticks Amblyomma americanum, Dermacentor variabilis, Ixodes scapularis and Rhipicephalus sanguineus (Acari: Ixodidae). *Exp. Appl. Acarol.*, **2014**, *62*(1), 105-113. [http://dx.doi.org/10.1007/s10493-013-9719-1] [PMID: 23907554]

[66] Esteve-Gasent, M.D.; Rodríguez-Vivas, R.I.; Medina, R.F.; Ellis, D.; Schwartz, A.; Cortés Garcia, B.; Hunt, C.; Tietjen, M.; Bonilla, D.; Thomas, D.; Logan, L.L.; Hasel, H.; Alvarez Martínez, J.A.; Hernández-Escareño, J.J.; Mosqueda Gualito, J.; Alonso Díaz, M.A.; Rosario-Cruz, R.; Soberanes Céspedes, N.; Merino Charrez, O.; Howard, T.; Chávez Niño, V.M.; Pérez de León, A.A. Research on Integrated Management for Cattle Fever Ticks and Bovine Babesiosis in the United States and Mexico: Current Status and Opportunities for Binational Coordination. *Pathogens*, **2020**, *9*(11), 871. [http://dx.doi.org/10.3390/pathogens9110871] [PMID: 33114005]

[67] De Meneghi, D.; Stachurski, F.; Adakal, H. Experiências no controle do carrapato por acaricida no setor tradicional de gado na Zâmbia e Burkina Faso: possíveis implicações ambientais e de saúde pública. *Fronteiras em saúde pública*, **2016**, *4*, 239.

[68] Muraro, L.S.; Nogueira, M.F.; Borges, A.M.C.M.; Souza, A.O.; Vieira, T.S.W.J.; de Aguiar, D.M. Detection of Ehrlichia sp. in Amblyomma sculptum parasitizing horses from Brazilian Pantanal wetland. *Ticks Tick Borne Dis.*, **2021**, *12*(3)101658 [http://dx.doi.org/10.1016/j.ttbdis.2021.101658] [PMID: 33556777]

[69] Pires, M.S.; Santos, T.M.; Santos, H.A.; Vilela, J.A.R.; Peixoto, M.P.; Roier, E.C.R.; Silva, C.B.; Barreira, J.D.; Lemos, E.R.S.; Massard, C.L. Amblyomma cajennense infestation on horses in two microregions of the state of Rio de Janeiro, Brazil. *Rev. Bras. Parasitol. Vet.*, **2013**, *22*(2), 235-242. [http://dx.doi.org/10.1590/S1984-29612013005000017] [PMID: 23778827]

[70] Zeringóta, V.; Senra, T.O.S.; Calmon, F.; Maturano, R.; Faza, A.P.; Catunda-Junior, F.E.A.; Monteiro, C.M.O.; de Carvalho, M.G.; Daemon, E. Repellent activity of eugenol on larvae of Rhipicephalus microplus and Dermacentor nitens (Acari: Ixodidae). *Parasitol. Res.*, **2013**, *112*(7), 2675-2679. [http://dx.doi.org/10.1007/s00436-013-3434-z] [PMID: 23636308]

Experimental Parasitology 242 (2022) 108364

Contents lists available at ScienceDirect

Experimental Parasitology

journal www.elsevier.com/locate/yexp

In vitro assessment of the acaricidal activity of a carvacrol shampoo on tick larvae

AnildesI.P. Sousa [a], GlayaneJ.S. Castro [a], Caio P. Tavares [b], Tassia L. do Vale´ [b], Livio M. Costa-Junior [b,*], AlexandraM.S. Soares [a,**]

[a] Laboratory of Plant Biochemistry, Federal University of Maranhao, S˜ao Luís, MA, Brazil ˜ [b] Laboratory of Parasite Control, Federal University of Maranhao, S˜ao Luís, MA, Brazil ˜

ARTICLEINFO

Keywords:

Shampoo

Monoterpene

Acaricide

ABSTRACT

Ticks are a widely distributed arthropod of veterinary importance. Resistance of ticks to synthetic acaricides has become widespread, warranting the development of new drugs for tick management. Carvacrol is a volatile monoterpene, with promising results against various species of ticks; however, to be used for therapeutic purposes, carvacrol must be included in a formulation that makes its application feasible. This study aims to develop a formulation of a carvacrol-containing shampoo that is effective against two species of ticks: *Rhipicephalus sanguineus* and *R. microplus*. Shampoo sensory characteristics and pH were evaluated at 37, 25 and 5 °C, for a maximum of 15 days. The shampoo remained stable at 25 and 5 °C. The efficacy of the carvacrol-containing formulation against two species of ticks was assessed by the larval immersion test. Mortality of both tick species was significantly higher for the carvacrol shampoo than for a carvacrol hydroalcoholic solution. In conclusion, the carvacrol-containing shampoo showed larvicidal efficacy on ticks.

* Corresponding author. Laboratory of Parasite Control, Department of Pathology, Federal University of Maranhao, São Luís, MA, 65080-805, Brazil. ˜

** Corresponding author. Laboratory of Plant Biochemistry, Department of Chemical Engineering, Federal University of Maranhao, S˜ao Luís, MA, 65080-805, ˜ Brazil.

E-mail addresses: livio.martins@ufma.br (L.M. Costa-Junior), alexandra.ufma@gmail.com (AlexandraM.S. Soares). https://doi.org/10.1016/j.exppara.2022.108364

Received 6 December 2021; Received in revised form 22 July 2022; Accepted 18 August 2022
Available online 24 August 2022

1. Introduction

The brown dog tick, *Rhipicephalus sanguineus* sensu lato, is one of the most widely distributed tick species globally and preferentially parasitizes dogs, but may also be found feeding on other hosts, including humans (Dantas-Torres, 2008). The management of *R. sanguineus* is mostly based on the use of synthetic chemical acaricides. However, the intensive use of these compounds has led to the selection of acaricide-resistant tick strains (Borges et al., 2007; Rodriguez-Vivas et al., 2017; Becker et al., 2019). Consequently, there is a need for the development of alternative approaches for tick management, and some plant-based compounds have been evaluated (Ellse and Wall, 2013).

Carvacrol is a volatile plant monoterpene which is currently classified as GRAS (Generally Recognized As Safe) and approved for use in human food (EAFUS, 2006; Hyldgaard et al., 2012; European Parliament and Council, 1996). Importantly, carvacrol has shown promising results when used against various species of ticks, including *R. sanguineus* (Araújo et al., 2016; Novato et al., 2015; Tabari et al., 2017). Natural or synthetic chemical substances can be included in pesticide formulations in order to make their application feasible (York, 2016). Thus, effectiveness, stability, and ease of application are among the criteria that these formulations must satisfy (Díaz et al., 2019; Ferreira et al., 2017). For the development of an acaricide formulation containing carvacrol, its low solubility in water must be considered, and, for example, the use of colloidal distribution systems, may be required (Ryu et al., 2018). Because acaricides are typically applied topically (Dantas-Torres, 2008), shampoo formulations emerge as viable alternatives, having already shown good efficiency in the delivery of other acaricides (Franc and Cadiergues, 1999; Heukelbach et al., 2006; Schuele et al., 2008).

The current study aimed to formulate a shampoo containing carvacrol against the brown dog tick *R. sanguineus* and the cattle tick *Rhipicephalus microplus*, used here as model organism. The cattle tick *R. microplus* is an excellent model for testing acaricide formulations, due to its ease of maintenance in experimental animals and the large numbers of ticks that can be obtained for testing, when compared to other tick species. Even though it is uncommon to use shampoos in cattle, we tested the acaricide formulation on these two species as a proof of concept, and because it is of the utmost importance to test

whether the acaricide formulation is tick species-specific.

2. Materials and methods

2.1. Shampoo formulation

The carvacrol shampoo was formulated at room temperature with the following raw materials: 14.0 g sodium lauryl ether sulfate, 1.0 g 30% cocamidopropyl betaine, and 0.5 g lauryl glucoside as surfactants; 0.75 g carboxymethyl cellulose as gelling agent; 0.2 g methylparaben as preservative; 10% citric acid (q.s.) as acidulant; 3.0 g carvacrol (W224502, Sigma-Aldrich, St. Louis, MO, USA), and ultrapure water (q.

s. 100 g) as base solution. The surfactants and carvacrol were mixed until completely homogenized. Next, citric acid was added to adjust the pH to 5.0. The carboxymethyl cellulose and methylparaben (previously solubilized in water at 100 °C) were then added. Finally, the mass of formulation was adjusted to 100 g with ultrapure water. At each addition, the

formulation was completely homogenized with a glass rod. A shampoo formulation without carvacrol was also prepared.

2.2. Sensory evaluation and pH stability

Sensory evaluation and pH stability tests were performed in triplicate, at 37 ± 2 °C, 25 ± 2 °C or 5 ± 2 °C, without direct light exposure, using 100 mL of shampoo sealed hermetically in amber glass flasks. The results were recorded after 1, 7, and 15 days of preparation. The sensory evaluation tests consisted of judging any changes in color, odor, and appearance and results were ranked in three categories: normal, without alteration; slightly modified; and intensely modified (ANVISA, 2004). The pH was measured with universal pH indicator strips (gradation 1.0, range 0–14). The accuracy of the indicator strips was confirmed by testing them against buffer solutions of known pH.

2.3. Tick collection and maintenance

Naturally detached engorged *R. sanguineus* and *R. microplus* females were obtained from artificially-infested New Zealand rabbits and calves, respectively, and maintained at 27 ± 1 °C, ≥ 80% humidity (Biological Oxygen Demand) until oviposition was completed. Eggs were collected and incubated for hatching. Larvae aged 14–21 days were used in larval immersion tests. The experimental procedures were approved by the Federal University of Maranhao (UFMA) ethics committee under pro˜ tocol number 23115.005443/2017–51.

2.4. Larval immersion test

The larval immersion test was performed according to Klafke et al. (2006). Carvacrol shampoo and the shampoo without carvacrol were prepared and immediately diluted in water to be used in the assay. A carvacrol hydroalcoholic solution (3.0 g carvacrol diluted in 50% ethanol solution, q.s. 100 g) was also used. Water, 50% ethanol, and the non-carvacrol shampoo were considered as negative controls. The formulation samples were diluted at a ratio of 1:16, 1:19, 1:23, and 1:47 in water. Approximately 500 larvae were immersed for 10 min in each treatment solution and then transferred to a filter paper to dry. Subsamples of approximately 100 larvae were transferred to a clean dry filter paper (8.5 × 7.5 cm) that was folded and closed with plastic clips. The packets were incubated at 27 ± 1 °C and relative humidity ≥80% for 24 h. After incubation, dead and live larvae were counted: immobile ticks were considered dead. Three independent repetitions of the experiment were conducted for each experimental group.

The statistical analysis of mortality data from the larval immersion test was performed using GraphPad Prism 8.0 software (version 8, GraphPad Inc., San Diego, CA, USA). The mean values for each treatment were compared by analysis of variance (ANOVA), followed by Tukey's test ($p < 0.05$) to compare differences between specific groups.

3. Results and discussion

In this study, the shampoo containing carvacrol exhibited a pearl-like color, the characteristic carvacrol odor, pH 5.0, and the same color at 5, 25 and 37 °C. After 1, 7, and 15 days of preparation, at 5 and 25 °C, it remained homogeneous, and was rated normal, without alteration. However, a phase separation occurred at 37 °C after 7 days and the shampoo was rated as slightly modified after 7 days and intensely modified after 15 days. The incorporation of carvacrol in formulations is important for the stability of the product, by preventing

degradation and microbiological contamination, facilitating its application, and enhancing the effect of the active compound (Díaz et al., 2019; Ferreira et al., 2017).

The carvacrol-containing shampoo was highly effective against both tick species, leading to 100% mortality in *R. microplus* and *R. sanguineus* after treatment with 0.125% of carvacrol shampoo (1:23 dilution of the shampoo in water) and with 0.15% of carvacrol shampoo (1:19 dilution of the shampoo in water), respectively (Table 1). The carvacrol hydroalcoholic solution had no efficacy against *R. sanguineus* at the tested concentrations and showed only low efficacy (21.6 ± 2.5% mortality at 0.175%, 1:16 dilution ratio) against *R. microplus* at the highest concentration used (Table 1).

Carvacrol has been studied for several years as a bactericide, fungicide, acaricide, and insecticide because of the growing interest for active compounds that are safe for human and animal health and the environment and that exert weak selection pressure leading to resistance (Abbaszadeh et al., 2014; Araújo et al., 2016; Park et al., 2017; Ryu et al., 2018). Even though the acaricidal activity of carvacrol against ticks including *R. sanguineus* has already been demonstrated (Araújo et al., 2016; Costa-Júnior et al., 2016; Cruz et al., 2013; Novato et al., 2018; Senra et al., 2013a, 2013b), carvacrol-containing formulations have been poorly explored against ticks, especially *R. sanguineus* (Lima et al., 2017, 2019; Novato et al., 2019).

The bioactivity of carvacrol, which is frequently diluted in ethanol for the larval immersion test against unengorged *Rhipicephalus* larvae (Coelho et al., 2020; Daemon et al., 2012; Scoralik et al., 2012), can be

Table 1
Mortality (mean ± SD) of *Rhipicephalus sanguineus* and *Rhipicephalus microplus* larvae, treated with different amounts of the carvacrol shampoo and with the experimental controls.

Treatment	Dilution ratio	Carvacrol concentration (%)	Mortality (%)	
			R. sanguineus	R. microplus
Water	–	–	0.0 ± 0.0[a]	0.0 ± 0.0[a]
50% Ethanol	–	–	0.0 ± 0.0[a]	0.0 ± 0.0[a]
Non-carvacrol shampoo	1:16	–	0.0 ± 0.0[a]	0.0 ± 0.0[a]
	1:19	–	0.0 ± 0.0[a]	0.0 ± 0.0[a]
	1:23	–	0.0 ± 0.0[a]	0.0 ± 0.0[a]
	1:47	–	0.0 ± 0.0[a]	0.0 ± 0.0[a]
Carvacrol hydroalcoholic solution	1:16	0.175	0.0 ± 0.0[a]	21.6 ± 2.5[b]
	1:19	0.15	0.0 ± 0.0[a]	3.4 ± 1.5[a]
	1:23	0.125	0.0 ± 0.0[a]	16.6 ± 5.4[b]
	1:47	0.0625	0.0 ± 0.0[a]	0.0 ± 0.0[a]
Carvacrol shampoo	1:16	0.175	100.0 ± 0.0[c]	100.0 ± 0.0[d]
	1:19	0.15	100.0 ± 0.0[c]	100.0 ± 0.0[d]
	1:23	0.125	97.3 ± 1.4[b]	100.0 ± 0.0[d]
	1:47	0.0625	97.3 ± 1.8[b]	39.4 ± 5.7[c]

Mean values, followed by different letters in the same column, are significantly different ($p < 0.05$).

partly explained based on its interaction with the tick's surface. The cuticle, the outermost part of the integument covering ticks, is composed mainly of lipids, polyphenols, proteins, and chitin (Hackman and Filshie, 1982; Lees, 1947). Because of its liposolubility, carvacrol has been suggested to interact strongly with the cuticle.

The mortality rates found in the tests comparing the carvacrol- containing shampoo and the carvacrol hydroalcoholic solution (Table 1) suggest a possible synergistic effects of the components of the shampoo formulation. The intrinsic properties of some compounds may have acaricidal effect or contribute to it. For instance, carboxymethyl cellulose (at 0.1%) alone is capable of inhibiting larval hatching in engorged *R. microplus* females (de Mendonça et al., 2019). Similarly, high mortality rates of *R. sanguineus* were observed when glycerin, which also forms a film on the surfaces where it is applied, was used in formulations associated with the terpene thymol (Delmonte et al., 2017). The authors suggested that this effect may be attributable to the increased water loss through the cuticle or to the film produced, which causes occlusion of the gas exchange channels. Most water loss in ticks occurs through the cuticle and spiracles (Lees, 1947), and the lipid layer in the cuticle plays an important role in regulating this occurrence. The higher efficacy of carvacrol shampoo against ticks compared to hydroalcoholic solution may be explained by the use of surfactants in the former increasing the terpene efficacy.

This is a proof-of-concept study, in which only the *in vitro* efficacy of one shampoo formulation was tested. Nonetheless, given the high efficiency of the carvacrol shampoo, even at low concentrations, the data presented here provide considerable support for new studies including, the assessment of *in vivo* effectiveness, allergenicity, and potential to cause dermatitis, contributing to the validation of integrated tick management strategies. In conclusion, the carvacrol shampoo developed was effective against *R. sanguineus* and *R. microplus* larvae under an *in vitro* study.

Credit author statement

Anildes Iran Pereira Sousa, carried out the experiment, collected samples, and data, and performed laboratory analyses.

Glayane de Jesus Soares Castro, collected samples and data, performed laboratory analyses, and prepared the first draft of the manuscript.

Caio Pavao Tavares and T˜ assia Lopes do Vale, carried out the ´ experiment, collected samples, and data, and performed laboratory analyses.

Livio Costa-Junior was responsible for conceptualization, funding acquisition, visualization, and writing - review & editing.

Alexandra Martins dos Santos Soares, was responsible for project administration, conceptualization, funding acquisition, supervision, visualization, and writing - review & editing.

Declaration of competing interest

The authors declare no conflict of interest related to this work.

Acknowledgments

The authors thank FINEP (Funding Authority for Studies and Projects) and FAPEMA (Maranhao Research Foundation) for supporting the ˜ IECT (Science and Technology Institute of Maranhao) Biotechnology. ˜ This study was financed in part by the Coordination for the Improvement of Higher Education Personnel (CAPES/Brazil) – Finance Code 001.

References

Abbaszadeh, S., Sharifzadeh, A., Shokri, H., Khosravi, A.R., Abbaszadeh, A., 2014. Antifungal efficacy of thymol, carvacrol, eugenol and menthol as alternative agents to control the growth of food-relevant fungi. J. Med. Mycol. 24 (2), e51–e56.

ANVISA, 2004. Guia de estabilidade de produtos cosm´eticos (Brasília, Agencia Nacionalˆ de Vigilancia Saniˆ taria (ANVISA)´).

Araújo, L.X., Novato, T.P.L., Zeringota, V., Maturano, R., Melo, D., Da Silva, B.,´ Daemon, E., De Carvalho, M.G., Monteiro, C.M.O., 2016. Synergism of thymol, carvacrol and eugenol in larvae of the cattle tick, *Rhipicephalus microplus*, and brown dog tick, *Rhipicephalus sanguineus*. Med. Vet. Entomol. 30, 377–382.

Becker, S., Webster, A., Doyle, R.L., Martins, J.R., Reck, J., Klafke, G.M., 2019. Resistance to deltamethrin, fipronil and ivermectin in the brown dog tick, *Rhipicephalus sanguineus* sensu stricto, Latreille (Acari: ixodidae). Ticks Tick Borne Dis 10 (5), 1046–1050.

Borges, L.M.F., Soares, S.F., Fonseca, I.N., Chaves, V.V., Louly, C.C.B., 2007. Resistˆencia acaricida em larvas de *Rhipicephalus sanguineus* (Acari: ixodidae) de Goiania-GO.ˆ Brasil. Rev. Patol. Trop. 36 (1), 87–95.

Coelho, L., Paula, L.G.F., Alves, S.G.A., Sampaio, A.L.N., Bezerra, G.P., Vilela, F.M.P., Matos, R.S., Zeringota, V., Borges, L.M.F., Monteiro, C., 2020. Combination of´ thymol and eugenol for the control of *Rhipicephalus sanguineus* sensu lato: evaluation of synergism on immature stages and formulation development. Vet. Parasitol. 277, 108989.

Costa-Júnior, L.M., Miller, R.J., Alves, P.B., Blank, A.F., Li, A.Y., P´erez de Leon, A.A.,´ 2016. Acaricidal efficacies of Lippia gracilis essential oil and its phytochemicals against organophosphate-resistant and susceptible strains of *Rhipicephalus (Boophilus) microplus*. Vet. Parasitol. 228, 60–64.

Cruz, E.M.O., Costa-Junior, L.M., Pinto, J.A.O., Santos, D.A., de Araujo, S.A., Arrigoni- Blank, M.F., Bacci, L., Alves, P.B., Cavalcanti, S.C.H., Blank, A.F., 2013. Acaricidal activity of Lippia gracilis essential oil and its major constituents on the tick *Rhipicephalus (Boophilus) microplus*. Vet. Parasitol. 195, 198–202.

Daemon, E., Monteiro, C.M.O., Maturano, R., Senra, T.O.S., Calmon, F., Faza, A., Prata, M.C.A., Georgopoulos, S.L., de Oliveira, L.F.C., 2012. Spectroscopic evaluation of thymol dissolved by different methods and influence on acaricidal activity against larvae of *Rhipicephalus microplus* (Acari: ixodidae). Parasitol. Res. 111, 1901–1906.

Dantas-Torres, F., 2008. The brown dog tick, *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: ixodidae): from taxonomy to control. Vet. Parasitol. 152, 173–185.

de Mendonça, A.E., Moreira, R.G., do Amaral, M.P.H., Monteiro, C.M.O., de Mello, V.,´ Vilela, F.M.P., Mendonça Homem, F.C., Furlong, J., Dolinski, C., Prata, M.C.A., das Chagas, E.F., 2019. Entomopathogenic nematodes in pharmaceutical formulations for *Rhipicephalus microplus* (Acari: ixodidae) control: in vitro evaluation of compatibility, thermotolerance, and efficiency. Ticks Tick Borne Dis 10, 781–786. Delmonte, C., Cruz, P.B., Zeringota, V., de Mello, V., Ferreira, F., Amaral, M.P.H.,´ Daemon, E., 2017. Evaluation of the acaricidal activity of thymol incorporated in two formulations for topical use against immature stages of *Rhipicephalus sanguineus* sensu lato (Latreille, 1806)(Acari: ixodidae). Parasitol. Res. 116, 2957–2964.

Díaz, E.L., Camberos, E.P., Herrera, G.A.C., Espinosa, M.E., Andrews, H.E., Buelnas, N.A. P., Ortega, A.G., Velazquez, M.M., 2019. Development of essential oil-based phyto-´ formulations to control the cattle tick *Rhipicephalus microplus* using a mixture design approach. Exp. Parasitol. 201, 26–33.

EAFUS, 2006. A Food Additive Database. Centre for Food Safety, and Applied Nutrition (US Food and Drug Administration, Washington, DC, USA.

Ellse, L., Wall, R., 2013. The use of essential oils in veterinary ectoparasite control: a review. Med. Vet. Entomol. 28 (3), 233–243.

European Parliament and Council, 1996. Regulation (EC) No 2232/96 the European parliament, and of the Council on 28 october 1996, commission decision of 23 february 1999 adopting a register of flavouring substances used in or on foodstuffs. Off. J. Eur. Commun 1–37. L84:1999/217/EC.

Ferreira, F.M., Delmonte, C.C., Novato, T.L.P., Monteiro, C.M.O., Daemon, E., Vilela, F. M.P., Amaral, M.P.H., 2017. Acaricidal activity of essential oil of Syzygium aromaticum, hydrolate and eugenol formulated or free on larvae and engorged females of *Rhipicephalus microplus*. Med. Vet. Entomol. 32 (1), 41–47.

Franc, M., Cadiergues, M.C., 1999. Activity of a deltamethrin shampoo against *Ctenocephalides felis* and *Rhipicephalus sanguineus* in dogs. Vet. Parasitol. 81, 341–346.

Hackman, R.H., Filshie, B.K., 1982. The tick Cuticle. In: Obenchain, F.D., Galun, R. (Eds.), Physiology of Ticks. Pergamon Press, Oxford, New York, Toronto, Sydney, Paris, Frankfurt, pp. 1–42.

Heukelbach, J., Oliveira, F.A.S., Speare, R., 2006. A new shampoo based on neem (*Azadirachta indica*) is highly effective against head lice in vitro. Parasitol. Res. 99, 353–356.

Hyldgaard, M., Mygind, T., Meyer, R.L., 2012. Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. Front. Microbiol. 3 (12).

Klafke, G.M., Sabatini, G.A., de Albuquerque, T.A., Martins, J.R., Kemp, D.H., Miller, R. J., Schumaker, T.T., 2006. Larval immersion tests with ivermectin in populations of the cattle tick *Rhipicephalus (Boophilus) microplus* (Acari: ixodidae) from State of Sao Paulo, Brazil. Vet. Parasitol. 142, 386–390.

Lees, A.D., 1947. Transpiration and the structure of the epicuticle in ticks. J. Exp. Biol. 23 (3–4), 379–410.

Lima, A.S., Landulfo, G.A., Costa-Júnior, L.M., 2019. Repellent effects of encapsulated carvacrol on the *Rhipicephalus (boophilus) microplus* (Acari: ixodidae). J. Med. Entomol. 56, 881–885.

Lima, A.S., Maciel, A.P., Mendonça, C.J.S., Costa-Junior, L.M., 2017. Use of encapsulated carvacrol with yeast cell walls to control resistant strains of *Rhipicephalus microplus* (Acari: ixodidae). Ind. Crop. Prod. 108, 190–194.

Novato, T., Gomes, G.A., Zeringota, V., Franco, C.T., de Oliveira, D.R., Melo, D., de´ Carvalho, M.G., Daemon, E., de Oliveira Monteiro, C.M., 2018. In vitro assessment of the acaricidal activity of carvacrol, thymol, eugenol and their acetylated derivatives on *Rhipicephalus microplus* (Acari: ixodidae). Vet. Parasitol. 260, 1–4.

Novato, T.L.P., Marchesini, P., Muniz, N., Prata, M.C.A., Furlong, J., Vilela, F.M.P., Daemon, E., Maturano, R., Monteiro, C., 2019. Evaluation of synergism and development of a formulation with thymol, carvacrol and eugenol for *Rhipicephalus microplus* control. Exp. Parasitol. 207, 107774.

Novato, T.P.L., Araújo, L.X., de Monteiro, C.M.O., Maturano, R., Senra, T.O.S., Matos, R. S., Gomes, G.A., de Carvalho, M.G., Daemon, E., 2015. Evaluation of the combined effect of thymol, carvacrol and (E)-cinnamaldehyde on *Amblyomma sculptum* (Acari: ixodidae) and *Dermacentor nitens* (Acari: ixodidae) larvae. Vet. Parasitol. 212, 331–335.

Park, J.H., Jeon, Y.J., Lee, C.H., Chung, N., Lee, H.S., 2017. Insecticidal toxicities of carvacrol and thymol derived from *Thymus vulgaris* Lin. against *Pochazia shantungensis* Chou & Lu., newly recorded pest. Sci. Rep. 7, 40902.

Rodriguez-Vivas, R.I., Ojeda-Chi, M.M., Trinidad-Martinez, I., Perez de Leon, A.A., 2017.´ First documentation of ivermectin resistance in *Rhipicephalus sanguineus* sensu lato (Acari: ixodidae). Vet. Parasitol. 233, 9–13.

Ryu, V., McClements, D.J., Corradini, M.G., Yang, J.S., McLandsborough, L., 2018. Natural antimicrobial delivery systems: formulation, antimicrobial activity, and mechanism of action of quillaja saponin-stabilized carvacrol nanoemulsions. Food Hydrocolloids 82, 442–450.

Schuele, G., Barnett, S., Bapst, B., Cavaliero, T., Luempert, L., Strehlau, G., Young, D., Moran, C., Junquera, P., 2008. The effect of water and shampooing on the efficacy of a pyriprole 12.5% topical solution against brown dog tick (*Rhipicephalus sanguineus*) and cat flea (*Ctenocephalides felis*) infestations on dogs. Vet. Parasitol. 151, 300–311.

Scoralik, M.G., Daemon, E., Monteiro, C.M.O., Maturano, R., 2012. Enhancing the acaricide effect of thymol on larvae of the cattle tick *Rhipicephalus microplus* (Acari: ixodidae) by solubilization in ethanol. Parasitol. Res. 110, 645–648.

Senra, T.O.S., Calmon, F., Zeringota, V., Monteiro, C.M.O., Maturano, R., Matos, R.S.,´ Melo, D., Gomes, G.A., de Carvalho, M.G., Daemon, E., 2013a. Investigation of activity of monoterpenes and phenylpropanoids against immature stages of *Amblyomma cajennense* and *Rhipicephalus sanguineus* (Acari: ixodidae). Parasitol. Res. 112, 3471–3476.

Senra, T.O.S., Zeringota, V., Monteiro, C.M.O., Calmon, F., Maturano, R., Gomes, G.A.,´ Faza, A., de Carvalho, M.G., Daemon, E., 2013b. Assessment of the acaricidal activity of carvacrol,(E)-cinnamaldehyde, trans-anethole, and linalool on larvae of *Rhipicephalus microplus* and *Dermacentor nitens* (Acari: ixodidae). Parasitol. Res. 112, 1461–1466.

Tabari, M.A., Youssefi, M.R., Maggi, F., Benelli, G., 2017. Toxic and repellent activity of selected monoterpenoids (thymol, carvacrol and linalool) against the castor bean tick, *Ixodes ricinus* (Acari: ixodidae). Vet. Parasitol. 245, 86–91.

York, P., 2016. Delineamento de formas farmacˆeuticas. In: Aulton, M.E., Taylor, K.M.G. (Eds.), Aulton - Delineamento de formas farmaceuticas. Elsevier, Rio de Janeiro,ˆ pp. 17–28.

Experimental and Applied Acarology (2019) 78:173–179 https://doi.org/10.1007/s10493-019-00383-3

Feeding and respiratory gas exchange of *Rhipicephalus sanguineus* sensu lato (Acari: Ixodidae)

Gabriel A. Landulfo[1] · Andrew Y. Li[2] · Aldilene S. Lima[1] · Naylene C. S. Silva[1] · Tassia L. Vale[1] · Livio M. Costa-Junior[1]

Received: 9 April 2019 / Accepted: 27 May 2019 / Published online: 30 May 2019

Abstract

Ticks are subject to various environmental constrains, such as dehydration, desiccation and long-waiting for hosts to attach. These factors are crucial for tick survival in the environment. Ticks have developed physiological mechanisms and/or strategies that allow adaptability and survival in the environment in which they live, such as spiracle control and cyclical or discontinuous gas exchanges. However, details of gas exchange profile have been reported only in a few tick species in the past. The present study aims to identify and describe respiratory gas exchange patterns in a tropical population of the brown dog tick *Rhipicephalus sanguineus* sensu lato and effects of blood feeding. Adult female ticks were fed on rabbit hosts. Partially fed (4 to 6 days) and completely fed (> 9 days) ticks were collected daily during feeding, weighed and subjected to C O_2 emission measurement at 25 °C using flow-through respirometry. Unfed adult females showed a well-defined periodical burst of CO_2 emissions, followed by short periods of low-emission intercepts. The fed groups had drastic changes in respiratory profiles with semi-engorged females showing a high-intensity respiratory pattern alternating between continuous and discontinuous and the engorged females showing a continuous respiratory pattern with high frequency and intensity. The findings from this study contribute to a better understanding of the respiratory physiological process of a tropical population of the dog tick, which may help future investigations on other biological aspects of this ectoparasite and development of control measures.

Keywords Tick feeding · CO_2 exchange · Respiration · Metabolic rate

This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation by the USDA for its use. The USDA is an equal opportunity provider and employer.

✉ Livio M. Costa-Junior livio.martins@ufma.br

[1] Laboratory of Parasite Control, Department of Pathology, Center for Biological and Health Sciences, Federal University of Maranhão, São Luís, MA, Brazil

[2] United States Department of Agriculture, Agricultural Research Service, Invasive Insect Biocontrol and Behavior Laboratory, Beltsville, MD, USA

Introduction

Ticks are ectoparasites of medical and veterinary importance because they can act as vectors of pathogenic organisms and cause lesions and injuries to the host at the site of the bite during hematophagy (Barros-Battesti et al. 2006). Ticks are subject to various environmental, such as humidity and temperature, and physiological factors, such as dehydration, desiccation and fasting (Needham and Tell 1991; Fielden et al. 1999; Sonenshine and Roe 2013). These factors are crucial for tick survival in the environment. Ticks have developed mechanisms and/or strategies that allow adaptability and survival in the environment in which they live, such as reduction of metabolic rate, diapause, impermeability of the integument, control of the spiracles and a discontinuous cycle of gas exchange (Needham and Teel 1991; Lighton et al. 1993; Fielden et al. 1999; Sonenshine and Roe 2013).

The discontinuous cyclic gas exchange pattern of arthropods, observed in insects and ticks, is a very effective behavioral and physiological strategy against water loss, oxidative stress and survival in hypoxic and hypercapnic environments (Lighton et al. 1993; Fielden et al. 1993, 1999; Lighton 1996, 1998; Fielden and Lighton 1996; Hetz and Bradley 2005; Chown et al. 2006; Zheng et al. 2013, 2015). The typical cycle for CO_2 release in bursts described for insects comprises three sequential stages: (1) the closed phase during which the spiracles are closed with negligible external gas exchange; (2) the flutter phase, triggered by endotracheal hypoxia, during which rapid fluttering of the spiracles allows diffusive and convective ingress of O_2 but little egress of CO_2 or water vapor; and (3) the burst phase in which hypercapnia (caused by the accumulation of CO_2 from respiring tissue) triggers the spiracles to open resulting in rapid release of C O_2 and water vapor by diffusion without/with convection (Lighton 1996; Fielden et al. 1999; Fielden and Duncan 2013). In ticks, the discontinuous pattern of gas exchange does not have the three evident phases, being thus characterized only by bursts of C O_2 emission, which coincide with the opening of the spiracles, followed by periods of low emissions caused by the closure of the spiracles (Fielden and Duncan 2013).

Amblyomma marmoreum (Fielden et al. 1993; Lighton et al. 1993), *Amblyomma hebraeum* (Fielden et al. 1993), *Amblyomma americanum* (Zheng et al. 2013), *Dermacentor andersoni* (Fielden and Lighton 1996), *Dermacentor variabilis* (Fielden et al. 1999) and *Ornithodoros turicata* (Zheng et al. 2015) are among tick species that have been investigated in term of gas exchange. Little has been studied on this subject in tick species belonging to the Neotropical region.

The brown dog tick, *Rhipicephalus sanguineus* sensu lato, is an African tick species that has spread throughout the world along with humans and their dogs (Walker et al. 2000). It has a worldwide distribution, being the only tick species that is currently established in North, Central, and South America, Europe, Africa, Asia, and Oceania (Walker et al. 2000; Estrada-Peña et al. 2004). In South America, a possible different vectorial competence for *Ehrlichia canis* is showed, elucidating the taxonomic position of the *R. sanguineus* group species complex in the world, which includes at least two species in South America, designated as 'tropical lineage' and 'temperate lineage' (Nava et al. 2015; Moraes-Filho et al. 2015; Labruna et al. 2017; Valim et al. 2017). The present study aims to identify and describe the respiratory gas exchange pattern in a tropical population of *R. sanguineus* s.l. and the effect of blood feeding.

Materials and methods

Source of ticks

Female ticks of *R. sanguineus* s.l. were collected from a dog naturally infested in São Luís city, Maranhão, Brazil. The tick colony was maintained in the laboratory using New Zealand (*Oryctolagus cuniculus*) rabbits aged 60 to 120 days and weighing between 1.5 and 2.5 kg of both sexes as hosts for all biological stages (larva, nymphs and adults) of *R. sanguineus* s.l.

The experimental group of *R. sanguineus* s.l. was divided into adult female unfed (fasted), partially fed (4 to 6 days) and completely fed (> 9 days). The unfed adult females used in the analysis were between 5–30 days of age. The partially fed females were collected/removed from the rabbit host with 4 to 5 days of attachment, while the completely fed were collected after the natural detachment from the host (9 days after infestation).

Respirometry

The measurement of gas exchange of *R. sanguineus* s.l. was performed using a carbon dioxide and water (CO_2/H_2O) analyzer (LI-7000, Li-COR, Inc., Lincoln, NE). Ticks were placed individually in a cylindrical 5 mL glass respirometer chamber with activity detector (AD2, Sable Systems, NV, USA) covered with aluminum foil. Air flow was generated using an air pump that was regulated by using a mass air flow controller to maintain a constant air flow rate (40 mL min-1). Room air was scrubbed of C O_2 and H_2O vapor by running room air through drierite and ascarite columns. All experiments were performed at room temperature at 28 ± 2 °C.

The Li-COR Li-7000 data acquisition software (v.2.0.0) was used to record C O_2. Each session of recording lasted for 18 h and data were recorded with sampling rate of once every 5 s. Data analysis was performed using the ExpeData data analysis software (Sable Systems). The CO_2 recording data were corrected to obtain the baseline and converted to the µL h^{-1} units. The parameters of data analysis included mean C O_2 emission volume (Vco_2), mean of CO_2 emission volume by peak (Burst V co_2), burst length and interburst length times. Were excluded of the analysis the data obtained during the time of activity of the ticks.

Statistical analysis

The CO_2 release/respiration parameters were compared between feeding stages of the female ticks. The normality was verified using the Shapiro–Wilk test. The effect of blood feeding on various respiration parameters was analyzed with ANOVA and Student's t-tests (pairwise comparison of means) ($\alpha = 0.05$). All tests were performed using the software BioEstat (v.5.0).

Results

Unfed females of *R. sanguineus* showed a discontinuous cyclic gas exchange pattern, characterized by short burst interspersed by low emission levels (interburst), which are at the baseline level of CO_2 emission (Fig. 1a). The mean burst and inter burst durations were 1.12 ± 0.6 and 1.79 ± 1.01 min, respectively (Table 1). In addition to short interbursts, long intercepts were observed, which are considered to be a long period of low CO_2 emission (Fig. 1a). These long interburst lasted for on average 105 ± 1575 min (77–126) in time (Table 1). A single long interburst was recorded typically during the recording in unfed females, with exception of two females which demonstrated two long intercepts during the recording. Recording of the long interburst occurred between 21:00 and 00:00 during the overnight recording period of the analyzes. The mean V co_2 of unfed females was 0.47 ± 0.2 µL h r^{-1} (Table 1), while the mean specific V co_2 was 0.22 ± 0.1 µL hr^{-1}. The mean gas exchange frequency was 7.2 ± 4 cycles per hour.

The partially fed females showed a continuous pattern mainly in the initial periods of the recording, which then changed to a discontinuous profile comprised in high-intensity respiratory cycles and frequencies (Fig. 1b). The mean durations of the bursts and interbursts (short and long) of the fed group were significantly ($p < 0.05$) lower than those of the unfed ticks (Table 1). In addition to reducing the duration of the interbursts, the gas exchange of females partially fed was characterized by the high occurrence of bursts per hour (Table 1).

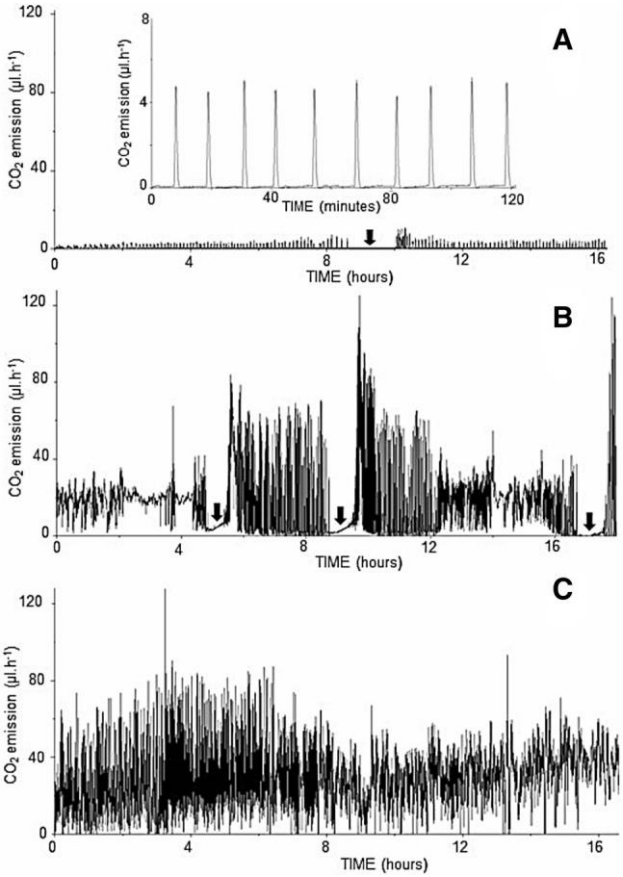

Fig. 1 Gas exchange (CO_2 emission) of *Rhipicephalus sanguineus* s.l. females, unfed (**a**), partially fed (**b**) and completely fed (**c**). Black arrows indicate long interbursts

Table 1 Mean and standard deviation of C O_2 release in unfed, partially fed and engorged females of *Rhipicephalus sanguineus* s.l.

Metabolic characteristics	Unfed	Partially fed (4–6 days)	Engorged female
Number of ticks	10	10	10
Mean weight (mg)	2.41 ± 0.60	28.50 ± 7.7	175.47 ± 37.20
Vco_2 (µL h^{-1})	0.47 ± 0.20[a]	16.30 ± 5.85[b]	59.55 ± 13.45[c]
Specific Vco_2 (µL h^{-1} mg^{-1})	0.20 ± 0.10[a]	0.55 ± 0.11[b]	0.34 ± 0.05[a]
Cycle frequency (h^{-1})	7.20 ± 4.00[a]	43.24 ± 10.10[b]	46.50 ± 7.40[b]
Burst Vco_2 (µL)	0.04 ± 0.02[a]	0.22 ± 0.07[b]	0.32 ± 0.09[b]
Burst V_{CO2} specific (µL)	0.016 ± 0.01	0.007 ± 0.001	0.001 ± 0.00
Burst duration (min)	1.12 ± 0.60[b]	0.60 ± 0.06[a]	0.60 ± 0.20[a]
Interbusrt length (min)	1.79 ± 1.01[c]	0.14 ± 0.07[b]	0.03 ± 0.03[a]
Long interburst length (min)	105.30 ± 15.70[b]	40.00 ± 12.70[a]	–

Different letters indicate significant differences by *t*-tests or ANOVA

The gas exchange pattern of the engorged females resembled the continuous profile of the partially fed females, in which bursts and interburst cannot be clearly defined, with high-frequency of C O_2 emissions (Fig. 1c). The long interbursts were not observed in the engorged

females. The total Vco_2 and the burst Vco_2 of the engorged females were higher than those observed in unfed ticks ($p < 0.05$), and the engorged females had the highest total Vco_2 (Table 1). The increase in Vco_2 is likely associated with an increase in the metabolic rate in response to blood sucking and reproductive activity of the fed specimens.

Discussion

In the present study we described the cyclic pattern of gas exchange of *R. sanguineus* s.l. females at different times of blood feeding. Unfed females showed a well-defined discontinuous pattern of bursts of CO_2 emissions, followed by short periods of low-emission. The fed groups had changes in respiratory profiles with partially fed females showing a high intensity respiratory pattern alternating between continuous and discontinuous, while the engorged females showing a continuous respiratory pattern of high frequency and intensity.

The increase in Vco_2, the high frequency and intensity and the reduction in the duration of the bursts and interbursts are the main changes in the respiratory patterns of fed females in comparison to non-fed specimens. These modifications in females ticks after feeding were previously reported in *A. marmoreum* and *D. variabilis* (Lighton et al. 1993; Fielden et al. 1999). The increase in body mass during blood feeding and consequently of the metabolic rate and production of metabolites are factors that contribute to the change from the discontinuous to the continuous pattern. The increase of the metabolic rate leads to a greater generation of CO_2 and its concentration in the hemolymph and in the tracheal trunks of the engorged specimens results in increased frequency of the spiracular opening for CO_2 release and absorption of O_2 (Fielden et al. 1999; Fielden and Duncan 2013).

Several interspecific variations among tick species in the frequency of spiracular opening are found, which causes variations in patterns of gas exchange that can range from a single burst to several bursts per hour (Fielden and Duncan 2013). In the present study we found that unfed females of *R. sanguineus* was characterized by short burst and interburst durations. These measurements were smaller than what found in *A. habraeum* (5 min. of burst), *A. marmoreum* (5 to 7 min of burst), *A. americanum* (6 min of burst), *D. variabilis* (3 min of burst) and *O. turicata* (13 min of burst) (Fielden et al. 1993; Lighton et al. 1993; Zheng et al. 2013, 2015). One possible explanation for such differences is that the inherent adaptability of *R. sanguineus* to the tropical environmental conditions where they inhabit, such as temperature above 27 °C, which eventually require higher respiratory rates.

In addition to short interbursts between CO_2 bursts, it was observed in unfed and partially fed females of *R. sanguineus* s.l. a long period of low CO_2 emission greater than the duration of the interbursts, which we call the long interburst. Physiologically, this long interburst is probably a period of closure of the spiracle, which causes a long period of hypoxia and low metabolic rate. The duration of the long interburst of *R. sanguineus* s.l. (95 min), were similar to those observed in other species of ticks, such as *A. hebraeum* (95 min), *A. marmoreum* (114 min), *A. americanum* (115 min) and *D. andersoni* (100 min) (Fielden et al. 1993; Lighton et al. 1993; Fielden and Lighton 1996; Zheng et al. 2013). We believe that a long interburst could be a mechanism to compensate for the short and fast CO_2 emission, since the respiratory intensity of *R. sanguineus* s.l. is higher than showed in other species, necessitating a closed period of the spiracles and hypoxia to compensate for the fast respiratory process and loss of water.

The discontinuous gas exchange cycle of insects is an effective mechanism against oxidative stress in environments where the oxygen level may be toxic. The insects close the spiracles to control the level of internal oxygen to avoid oxygen toxicity (Hetz and Brandley 2005). This mechanism can be another compensation strategy for *R. sanguineus* s.l. to control the level of oxygen with long intercepts.

The present study described for the first time the respiratory pattern of gas exchange of the brown dog *R. sanguineus* s.l. at different blood feeding stages, evidencing a change of respiratory profile in response to the blood sucking. Our findings contribute to a better understanding of the respiratory physiological process of a tropical species of tick, which may help future investigations on different aspects of this ectoparasite, such as biology and development of control measures.

Acknowledgements This research was supported through grants from FAPEMA (Maranhão State Research Foundation, Brazil) and CAPES (Coordination for the Improvement of Higher Education Personnel, Brazil). G.A. Landulfo and N.C.S. Silva received a post doc fellowship from CAPES, and A.S. Lima and T.L. Vale received an PhD scholarship from FAPEMA. The authors would also like to thank CNPq for a fellowship to L.M. Costa-Júnior.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

References

Barros-Battesti DM, Arzua M, Bechara GH. (2006) Carrapatos de Importância Médico-Veterinária da Região Neotropical: Um Guia Ilustrado Para Identificação de Espécies. São Paulo: Vox/ICTTD-3/ Butantan, 5-7

Chown SL, Gibbs AG, Hy SK, Ky CJ, Ly JR, Marais E (2006) Discontinuous gas exchange in insects: a clarification of hypotheses and approaches. Physiol Biochem Zool 79:333–343

Estrada-Peña A, Quíez J, Sánchez Acedo C (2004) Species composition, distribution, and ecological preferences of the ticks of grazing sheep in north-central Spain. Med Vet Entomol 18(2):123–133

Fielden LJ, Ducan FD (2013) Respiratory system: structure and function. In: Sonenshine DE, Roe RM (eds) Biology of ticks, vol 1, 2nd edn. Oxford University Press, New York, pp 240–257

Fielden LJ, Lighton JRB (1996) Effects of water stress and relative humidity on ventilation in the tick *Dermacentor andersoni* (Acari: Ixodidae). Physiol Zool 69:599–617

Fielden LJ, Duncan FD, Lighton JRB, Rechav Y (1993) Ventilation in the adults of *Amblyomma hebraeum* and *A. marmoreum* (Acarina, Ixodidae), vectors of heartwater in southern Africa. Rev Elev Med Vet Pays Trop 46:335–338

Fielden LJ, Jones RM, Goldberg M, Rechav Y (1999) Feeding and respiratory gas exchange in the American dog tick, *Dermacentor variabilis*. J Insect Physiol 45:297–304

Hetz SK, Bradley TJ (2005) Insects breathe discontinuously to avoid oxygen toxicity. Nature 433(7025):516–519

Labruna MB, Gerardi M, Krawczak FS, Moraes-Filho J (2017) Comparative biology of the tropical and temperate species of *Rhipicephalus sanguineus* sensu lato (Acari: Ixodidae) under different laboratory conditions. Ticks Tick-Borne Dis 8:146–156

Lighton JRB (1996) Discontinuous gas exchange in insects. Ann Rev Entomol 41:309–324

Lighton JRB (1998) Notes from underground: towards ultimate hypotheses of cyclic, discontinuous gasexchange in tracheate arthropods. Am Zool 38:483–491

Lighton JR, Fielden LJ, Rechav YIGAL (1993) Discontinuous ventilation in a non-insect, the tick *Amblyomma marmoreum* (Acari, Ixodidae): characterization and metabolic modulation. J Exp Biol 180:229–245

Moraes-Filho J, Krawczak FS, Costa FB, Soares JF, Labruna MB (2015) Comparative evaluation of the vector competence of four South American populations of the *Rhipicephalus sanguineus* group for the bacterium *Ehrlichia canis*, the agent of canine monocytic ehrlichiosis. PLoS ONE 10(9):e0139386

Nava S, Estrada-Peña A, Petney T, Beati L, Labruna MB, Szabó MP, Venzal JM, Mastropaolo M, Mangold AJ, Guglielmone AA (2015) The taxonomic status of *Rhipicephalus sanguineus* (Latreille, 1806). Vet Parasitol 208:2–8

Needham GR, Teel PD (1991) Off-host physiological ecology of ixodid ticks. Ann Rev Entomol 36:659–681

Sonenshine DE, Roe RM (eds) (2013) Biology of ticks, vol 1, 2nd edn. Oxford University Press, New York, p 560

Valim JR, Rangel CP, Baêta BA, Ribeiro CC, Cordeiro MD, Teixeira RC, Cepeda PB, Fonseca AH (2017) Using plastic tips in artificial feeding of *Rhipicephalus sanguineus* sensu lato (Acari: Ixodidae) females. Rev Bras Parasitol Vet 26(1):110–114

Walker JB, Keirans JE, Horak IG (2000) The genus *Rhipicephalus* (Acari, Ixodidae): a guide to the brown ticks of the world. Cambridge University Press, Cambridge, p 643

Zheng H, Li AY, Fielden LJ, Liu J, Seshu J, León AAP (2013) Effects of permethrin and amitraz on gas exchange and water loss in unfed adult females of *Amblyomma americanum* (Acari: Ixodidae). Pest Biochem Physiol 107(2):153–159

Zheng H, Li AY, Teel PD, León AAP, Seshu J, Liu J (2015) Biological and physiological characterization of in vitro blood feeding in nymph and adult stages of *Ornithodoros turicata* (Acari: Argasidae). J Insect Physiol 75:73–79