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Topical and spatial repellent bioassays against the Australian paralysis tick, *Ixodes holocyclus* (Acari: Ixodidae)

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Abstract The Australian paralysis tick, *Ixodes holocyclus*, is the cause of significant human morbidity. Bites from the tick may result in paralysis, allergic reactions that can include anaphylaxis and death, mammalian meat allergies and the transmission of infectious agents. In recent years, there have been a number of papers published on the microbiome of the species, but to date, there is no published research on *I. holocyclus* management or personal protection options to prevent the bite from the species. The study herein focused on the latter; the use of repellents for the prevention of bites from *I. holocyclus*. Five personal repellents were tested along with coconut oil, and a citronella patch and wristband. These were all tested for repellency in a laboratory assay over the time intervals of 15 min, 1, 2, 3 and 4 h post application. The personal repellents included the active ingredients of picaridin (9.3%), DEET (11.5%), lemon eucalyptus (36.0%), a combined formulation of citronella and tea tree oil (28.4%) and an extract of *Andrographis paniculata* (30% w/v). The coconut oil was 30% v/v. The citronella patch contained 120 mg/patch, whereas the citronella wristband contained 750 mg/band. Two spatial repellents were also tested in the laboratory for repellency and toxicity against *I. holocyclus* and tested for toxicity in the field. These included OFF!® Clip-On™ (metofluthrin 312 g/kg) and Thermacell® (allethrin 219.7 g/kg). For the personal repellents at 4 h, there was no statistical difference in repellency between the formulations of picaridin, DEET and lemon eucalyptus, with over 84% repellency recorded for all. Thus, these would be the personal repellents recommended for preventing tick bites. The citronella patch produced 100% repellency over 4 h; however, as this type of product is known to only provide protection close to the patch, it is not recommended for routine use. For the spatial repellents, both produced significant repellency and toxicity in the laboratory, but failed to produce any tick mortality in the field, and their use cannot be recommended. This is the first published study investigating personal and spatial repellents for the prevention of tick bite from *I. holocyclus*.

Key words *Andrographis paniculata*, citronella, coconut oil, DEET, lemon eucalyptus, OFF!® Clip-On™, picaridin, Thermacell®.

INTRODUCTION

Tick bites and the transmission of tick-borne pathogens continue to be a global public health concern (Jongejan & Uilenberg 2004). The Australian paralysis tick, *Ixodes holocyclus*, is the species most commonly responsible for causing tick-related human morbidity in Australia (Doggett 2004).

This species occurs along the eastern coastal strip of the country and has a seasonal pattern in the activity of its various stages. The larvae are most common during the autumn months, the nymphs during the winter and the adults in the spring (Eppleston *et al*. 2013). The tick is less active in the hot summer months where it over-summers as the egg stage (Eppleston *et al*. 2013; Barker & Walker 2014). As the common name suggests, the tick can induce paralysis, and some 20 human deaths occurred before the development of an effective anti-venene (Doggett 2004). The paralysis is induced by a toxin (known as 'holocyclotoxin') that occurs in the tick saliva that is injected during blood feeding. As the adult female injects larger volumes of saliva, it is this stage that poses greatest risk of paralysis (Doggett 2004). Every year, more than 1000 companion animals are affected with paralysis caused by *I. holocyclus* (Hall-Mendelin *et al*. 2011; Eppleston *et al*. 2013; Mullins *et al*. 2016). Furthermore, the tick saliva can induce allergic reactions, which can be from mild to severe, including anaphylaxis with occasional death (Brown & Hamilton 1998; Rappo *et al*. 2013). Allergic reactions in humans are now considered more significant, and are far more common, than paralysis (Rappo *et al*. 2013; van Nunen 2018). The tick itself can attach to various sites of the body, such as the conjunctiva, making removal very challenging (Teong *et al*. 2015). It also poses a risk to travellers, and there are a number of reports of overseas visitors returning home and finding an attached *I. holocyclus* (Pietzsch *et al*. 2013; Pek *et al*. 2016). Proper tick removal is now considered key to minimising complications from allergic reactions associated with tick bite (Taylor *et al*. 2019).

Around 10 years ago, a syndrome initially known as 'red meat allergy' was recognised in patients bitten by *I. holocyclus* (van Nunen *et al*. 2009). The patients developed symptoms including gut pain, bloating, diarrhoea and occasional anaphylaxis following the consumption of meats of mammal origin, including pork, lamb, bovine, whale and guinea pig. For this reason,

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the condition was renamed as 'mammalian meat allergy' (MMA; van Nunen 2015). It is now recognised that not only can any mammalian meat trigger the condition in affected patients but also animal by-products, such as gelatin and some drugs (van Nunen 2015). Australia is the most affected nation in the world with this condition, with estimates of prevalence in the Sydney basin of 113 patients with MMA per 100 000 population (van Nunen 2018). Other ticks locally and overseas have subsequently been linked with inducing MMA (van Nunen 2018). The condition appears to be triggered as a result of IgE sensitisation of the oligosaccharide galactose-α-1,3-galactose (commonly called 'α-Gal'), which is passed to humans via the tick bite. Disturbingly, α-Gal sensitisation has now been linked to coronary artery disease (Wilson *et al.* 2018).

Ixodes holocyclus has been incriminated as the principle vector of *Rickettsia australis*, the causative agent of Queensland tick typhus (Jongejan & Uilenberg 2004). Very rare deaths from this pathogen have been reported in patients who fail to receive antibiotic therapy; however, recovery is rapid for infected patients who are treated appropriately (Doggett 2004).

More controversial though has been the issue of Lyme disease (LD) in Australia and if the condition is endemic to the country. Over the years since the condition was first recognised globally, numerous patients without a travel history outside of Australia have been diagnosed with the condition (Collignon *et al.* 2016). It is now known that these patients were diagnosed based on non-specific clinical symptomology, usually by general practitioners rather than infectious disease specialists, and supported by questionable pathology tests. Following recent intensive investigations, encompassing sensitive molecular techniques and serological investigations in dogs, there is no scientific evidence to suggest the condition occurs here (Chalada *et al.* 2016; Irwin *et al.* 2017).

What the LD controversy has initiated is a renewed research into potential human and veterinary pathogens that *I. holocyclus* may carry. Modern molecular-based technologies, such as deep sequencing, are being employed, and a variety of microorganisms have been detected within this and other local tick species over recent years (Gofton *et al.* 2015; O'Brien *et al.* 2018; Harvey *et al.* 2019). In a small number of cases, the organisms identified have aligned closely with known human pathogens from overseas (Harvey *et al.* 2019).

In spite of the growth in research focusing on potential pathogens that may be carried by *I. holocyclus*, and the human morbidity this species is known to cause, arguably the most important area of research has been left neglected (and unfunded by all levels of government), namely, the prevention of tick bites.

Current tick management practices suggest the unifying of human and veterinary medicine against tick-borne diseases in the concept of the 'One Health approach' (Dantas-Torres *et al.* 2012). However, *I. holocyclus* may be difficult to manage in the environment because of their complex ecology that involves different animal hosts (Hall-Mendelin *et al.* 2011; van Nunen 2018). Thus, personal protective measures, such as the use of repellents, remain the key method in preventing tick bites in humans (Katz *et al.* 2008; Rahlenbeck *et al.* 2016). However, to date, there has not been one published paper on the effectiveness of repellents against *I. holocyclus*, and thus, it has been necessary to rely on information derived from data obtained on overseas tick species. It is not known if such research directly translates to the Australian situation and our local species.

Repellents can be applied directly on the skin, or to clothing, shoes, bed nets and camping gear, prior to entering tick-infested areas to minimise the risk of bites (Katz *et al.* 2008; Banks *et al.* 2014). Synthetic repellents such as DEET (*N*,*N*-diethyl-3-methylbenzamide) and picaridin (2-(2-hydroxyethyl)-1-piperidinecarboxylic acid 1-methylpropyl ester) are among the most widely used topical repellents for the prevention of bites from mosquitoes, biting flies and ticks (Bissinger *et al.* 2009) and are usually applied to areas of exposed skin. Previous studies have revealed that DEET is highly effective in repelling a range of tick species under laboratory and field conditions, including *Ixodes ricinus* (Jaenson *et al.* 2003), *Ixodes scapularis* and *Amblyomma americanum* (Carroll *et al.* 2004) and *Ixodes persulcatus* and *Ixodes ovatus* (Ogawa *et al.* 2016). Picaridin has also been shown to be effective at repelling a number of tick species including *A. americanum* and *Dermacentor variabilis* (Bissinger *et al.* 2009). Various essential oils have also been demonstrated efficacious in repelling ticks, including lemon eucalyptus oil, lavender oil, geranium oil (Jaenson *et al.* 2006), citronella oil and clove oil (Thorsell *et al.* 2006). These natural-based repellents are becoming increasingly popular because of their low toxicity, comparable efficacy and customer approval (Katz *et al.* 2008).

Topical repellent studies have moved away from being conducted in the field to the laboratory, because of the health risks that ticks pose to field researchers. One simple laboratory assay was developed by Jaenson *et al.* (2003), whereby they allowed ticks to climb up the internal surface of a Falcon® plastic tube covered with repellent-treated cloth in the presence of a human hand. The hand acted as a host attractant, and they found in this assay that DEET at a 10% concentration repelled more than 80% of *I. ricinus* nymphs away from the hand. The study herein adopted this simple method for assessing topical repellents in the laboratory against *I. holocyclus*.

Beyond topical repellents is the use of spatial repellents (SRs) that create a pest-free zone by interrupting host-seeking behaviour through a vapour-phase chemical (Achee *et al.* 2012). Most SRs use pyrethroids as the active ingredient, which also have a knockdown effect, thereby stopping the arthropod from reaching the potential blood source. Spatial repellents have been showed to be effective in reducing mosquito (Xue *et al.* 2012; Bibbs *et al.* 2015) and sand fly biting rates (Alten *et al.* 2003). Most commercially available SR products have a dissemination component to help disperse the active ingredient. This includes a fan in the case of OFF!® Clip-On™ (which contains metofluthrin) or a heating unit as in the Thermacell® (the active ingredient being allethrin). Both of these units have been shown to have a repellency and toxic effect against *A. americanum* (Bibbs & Xue 2016; Xue *et al.* 2016). Both OFF!® Clip-On™ and the Thermacell® are available in Australia, although are only currently registered for protection against mosquitoes. To date, neither product has been studied for their protective value against any *Ixodes* species, let alone *I. holocyclus*. Furthermore, the tests

that have been undertaken against ticks with the Thermacell® have been conducted in an artificial environment and not in the field, where the results could be influenced by climatic conditions leading to a markedly different outcome.

The aim of this study was to evaluate the efficacy of a range of commercially available insect repellent products containing different active ingredients against host-seeking adult female *I. holocyclus* in the laboratory. These investigations included both SRs mentioned above, which were also evaluated in the field. The study additionally investigated whether coconut oil (*Cocos nucifera*) and a crude extract of *Andrographis paniculata* would also repel *I. holocyclus*, both of which have been shown to be effective at repelling mosquitoes (Konan *et al.* 2003; Edwin *et al.* 2016). This paper is the first published study that has investigated the effectiveness of a range of topical and spatial repellents against *I. holocyclus*.

MATERIALS AND METHODS

Ticks

Only adult female *I. holocyclus* were used in the experimental trials. Host-seeking (non-bloodfed) ticks were collected via flagging from long grass and bushland habitats in coastal areas of New South Wales, Australia (32°04′S, 152°32′E), during October 2018. Collected ticks were held in 20 mL sample jars containing 1 cm of Plaster of Paris in the base of the tube, which was supplemented with two to three drops of distilled water to maintain humidity. The ticks were transferred back to the laboratory for identification (Roberts 1970; Barker & Walker 2014). Female *I. holocyclus* were also purchased from Australian Veterinary Serum Laboratories (AVSL), Lismore, NSW. The purchased ticks are not from a laboratory colony (no laboratory colony exists of *I. holocyclus*) and were collected in the field by individuals on behalf of the company. As all ticks used in the study were field derived, they were pooled for the experiments.

Repellents

Repellency bioassays were conducted with four registered, commercially available insect topical repellent products: a coconut body oil product, a plant-based crude extract and two spatial repellent devices (Table 1). A citronella patch and a citronella band (Table 1) were also included in the assay. Note that the coconut oil product is not marketed as a repellent and not registered for such use. However, this was included in the trials as recent research has demonstrated that compounds derived from coconut oil provide excellent repellency against ticks and better repellency than DEET against biting flies and bed bugs (Zhu *et al.* 2018).

The commercial topical and spatial repellents, the coconut oils, and the citronella band and patch, were all purchased new from various retail stores and checked to ensure that they had not passed any 'best before' or 'use by' date. The crude extract of *A. paniculata* (100% purity), also known as 'King of Bitters', was purchased from Thai China Flavours and Fragrances (TCFF) Industry Co., Ltd., Ayutthaya, Thailand, just prior to the study. The crude extract was obtained by ethanolic extraction of the plant leaf. Solutions of the extract were prepared by dissolving in absolute ethanol to the concentrations of 30%, 15% and 10% w/v. Coconut oil was two-fold and three-fold diluted to match the *A. paniculata* dilution.

Topical repellent bioassays

A modified bioassay was employed to evaluate the efficacy of each repellent (Fig. 1). The procedures were based on a method developed for testing repellents against the hard tick, *I. ricinus* (Jaenson *et al.* 2003), as mentioned above. The bioassay was as follows: 100 μL of each repellent (and ethanol with the controls) was applied evenly via pipette to individual swatches of chiffon fabric (12.56 cm^2) and allowed to air-dry for 15 min. For each trial, five female ticks were placed into a Falcon® tube (50 mL centrifugal tube, 116 × 28 mm, Becton Dickinson, manufacturer no. 352073) and the treated fabric attached with a rubber band to the open end. For each replicate, the ticks were first tested for repellency with the control fabric for 5 min and then immediately afterward with the repellent; the control fabric was removed and replaced with the treated fabric and again evaluated for repellency over 5 min. A total of 10 replicates for each repellent was performed.

Table 1 Active ingredients and concentrations of repellent products used in tick bioassay

Active ingredient	Product [formulation]
Picaridin (92.8 g/L)	Aerogard® repellent spray, Reckitt Benckiser, West Ryde, NSW, Australia [spray]
DEET (115 g/L)	Aerogard® repellent roll-on, Reckitt Benckiser, West Ryde, NSW, Australia [roll-on]
Lemon eucalyptus (360 g/L)	Bug-grrr Off® natural repellent spray, Selmac Broking Pty. Ltd., Ferny Creek, Victoria, Australia [spray]
Citronellal (28.35 g/L), tea tree oil (18.9 g/L), lemon-scented tea tree oil (9.45 g/L)	Walkabout Insect Repellent, Thursday Plantation, Integria Healthcare (Australia) Pty. Ltd., Eight Mile Plains, QLD, Australia [roll-on]
Coconut oil (100%)	Redwin Sensitive Skin, Coconut Body Oil, PharmaCare Laboratories Pty. Ltd., Warriewood, NSW, Australia [body oil]
A. paniculata crude extract (100%)	Thai China Flavours and Fragrances (TCFF) Industry Co., Ltd., Ayutthaya, Thailand
Citronella patch (120 mg/patch)	Mozzigear™ Mosquito patch insect repellent, Intelligent Health Systems, Clayton South, Victoria, Australia [impregnated patch]
Citronella band (750 mg/band)	Mozzigear™ Mosquito band insect repellent, Intelligent Health Systems, Clayton South, Victoria, Australia [impregnated wristband]
Metofluthrin (312 g/kg)	OFF!® Clip-On™ Mosquito Repellent, S.C. Johnson & Son Pty Lid., Lane Cove, NSW, Australia
Allethrin (219.7 g/kg)	Thermacell® Mosquito repellent, Schawbel Corporation, Bedford, MA, USA

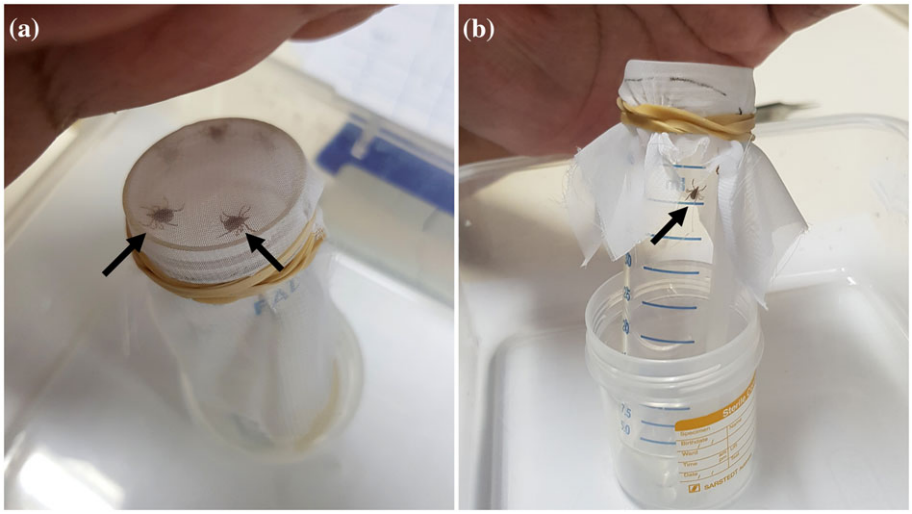

Fig. 1. Modified Falcon® vial bioassay, after Jaenson *et al.* (2003). (a) Ticks clinging to the cloth at 3 min after start of the test were recorded as 'attracted'; (b) ticks that did not were recorded as 'repelled'.

To simulate host stimuli to attract the ticks, the experimenter held their hand close to the surface of the cloth (~5 cm) for each 5 min period. The number of ticks directly in contact with the interior surface of the cloth at the end of the 5 min period was recorded. Ticks clinging to the cloth at 5 min after the start of the test were recorded as 'attracted', whereas those that did not were recorded as 'repelled', as demonstrated in Figure 1. The hands of the observer were washed with non-scented soap and rinsed well with water, between each trial. To evaluate the protection longevity of the repellents, bioassays were performed again at 1, 2, 3 and 4 h after the initial treatment of the fabrics. To investigate potential time-related changes in tick behaviour, two additional tests were performed. Firstly, the ethanol treated control fabric was followed by another ethanol control fabric and recorded for repellency. Secondly, an untreated fabric was followed by a second untreated fabric and repellency recorded. Ten replicates were performed for each of these additional tests.

Two citronella-impregnated products were also evaluated, including a sticky patch and a wristband, using the Falcon® vial bioassay. The products were tested in a similar manner as described above; however, they were attached directly to the outer surface of a fabric swatch.

Spatial repellent bioassays

The laboratory spatial repellency assays were adapted from the study of Xue *et al.* (2016). A plastic tub (26.5 × 21 × 10 cm; L × W × H, Ezy storage, Maidstone, Victoria, Australia) served as an open airflow chamber (OAC) and was connected to a clear vinyl tube (4.3 cm outer diameter × 15 cm, Toro Australia Pty. Ltd., Beverley, SA) on the side of the OAC. The tube was then connected to a 2 L disposable drink bottle through a hole in the centre of the bottle lid. The base of the bottle had been removed to allow insertion of the volunteer's hand. A small fan was placed to allow airflow through from the bottle with the hand into the vinyl tube and then to the OAC (Fig. 2a). For testing, five female ticks were placed into the vinyl tube and secured with fabric mesh, held in place with rubber bands (Fig. 2b). Ticks were allowed to acclimate in the tube for 2 min. A volunteer then placed one hand into the bottle, and ticks were given 5 min to move to their final position. After 5 min, ticks located between the fabric next to the hand and up to 5 cm from this fabric were counted as 'attracted', whereas those that moved away from the fabric next to the hand (>5 cm) were recorded as 'repelled'. This was the process for the controls. For the tests, ticks were given a 2 min rest period, and the assay repeated but with the volunteer holding an activated OFF!® Clip-On™ or Thermacell® device with the hand placed in the bottle. Ticks were then allowed 5 min to orientate towards or away from the volunteer's hand. After 5 min, tick locations were recorded as above. Ten replications were performed, and new ticks were used for each replication.

In all the trials with the spatial repellents, the devices were activated according to the manufacturer's instructions. Thus, OFF!® Clip-On™ was activated for 60 min prior to use, and the Thermacell® was activated for 60 min prior to use. For each trial, new chemical cartridges were used.

Pilot mortality assays were undertaken to demonstrate that the volatile chemical from both spatial repellent devices could pass through the chiffon cloth. Using the spatial repellency assay system as described above, five female ticks were placed into cylindrical clear vinyl tubes (2.5 cm interior diameter × 4 cm, Toro Australia Pty. Ltd., Beverley, SA) that served as bioassay cages (Fig. 2b). The ends were covered in chiffon cloth, held in placed with elastic bands. Then the bioassay cage was placed inside the larger vinyl tube connected to the OAC. The spatial repellent device was then allowed to operate inside the disposable drink bottle for a period of 5 min. Knockdown and mortality were observed at 5 min and 24 h post exposure, respectively. Four replicates were performed. The results show that both spatial repellent devices produced 95–100% knockdown and 75–100% mortality in the caged ticks with 5 min exposure, indicating that the volatile chemicals, of allethrin and metofluthrin, were able to readily pass through the chiffon cloth.

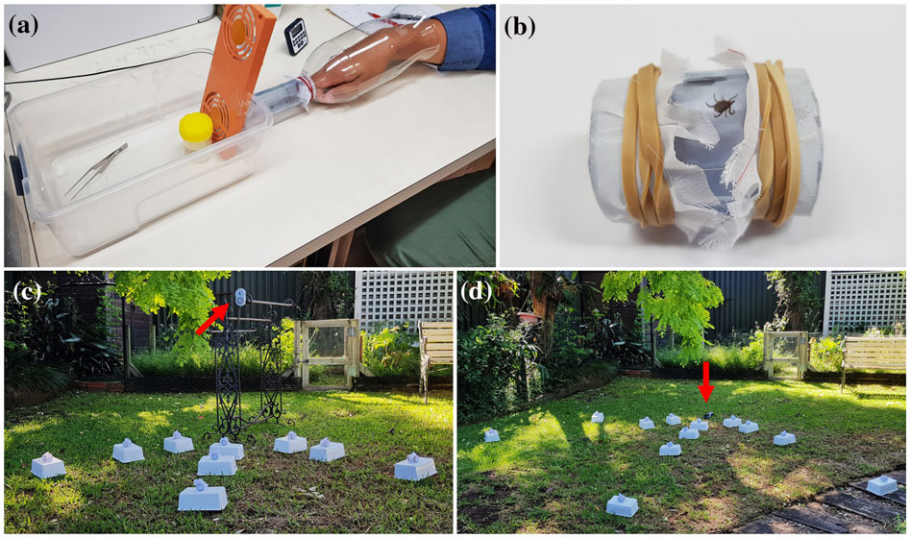

Fig. 2. Spatial repellent bioassays: (a) laboratory bioassays, (b) bioassay cage containing five female ticks and outdoor bioassays for (c) OFF!® Clip-On™ and (d) Thermacell®. The arrows indicate the spatial repellent devices.

A modified outdoor mortality assay was adapted from the study of Bibbs and Xue (2016) to test the effectiveness of the SRs at killing ticks in the field. Five female ticks were placed into each bioassay cage, as described above (Fig. 2b). Cages were then placed on top of a plastic tray (10 × 16 cm) producing a 65 mm height from the ground (Fig. 2c,d). Placing the cages in this manner allowed for airflow uninterrupted by ground vegetation. Treatments used included an activated OFF!® Clip-On™ device suspended 1 m above the ground (Fig. 2c) and an activated Thermacell® positioned at ground level (Fig. 2d). These heights were based on the manufacturer's recommendations. For the OFF!® Clip-On™ trials, three cages containing ticks were placed each at 0.25, 0.5 and 1.0 m away from the device (one cage for each point), and one cage serving as the control was placed more than 5 m away. These distances are based on those used from the study of Bibbs and Xue (2016). For the Thermacell® trials, four cages containing ticks were placed at 0.25, 0.5, 1.0 and 2.0 m away from the device, based on the protection zone of 4.5 m suggested by the manufacturer, with one control cage placed 5 m away. Five replications were performed for both devices. Bioassay cages were exposed to the treatments for 30 min. The cages were removed from the treatment area and held in laboratory conditions for 24 h to observe mortality. The assays were carried out in an open backyard during 8:00–12:00 h, with 27–28°C, 60% RH and <1 km/h winds.

Data analysis

Percentage repellency was calculated as follows:

$$\frac{\text{No.of ticks recorded as attracted in the control vial} - \text{No.of ticks recorded as attracted in the test vial}}{\text{No.of ticks recorded as attracted in the control vial}} \times 100$$

The Wilcoxon signed rank test was used to compare the number of attracted ticks in the products with that of the controls for both topical and spatial repellent bioassays. Mann–Whitney *U* test was used to compare between each test concentrations and between each time points for topical repellents and mortality response in the laboratory spatial repellent bioassay. All statistical analyses were performed using SPSS Statistics version 22 (IBM Corp., Armonk, NY), and a *P* value of 0.005 or less was considered statistically significant. As no mortality was observed in the field trials of the SRs, so statistical analyses were undertaken.

RESULTS

Topical repellents

The percentage of attracted ticks and repellencies exhibited against female *I. holocyclus* with the different products at 15 min are shown in Table 2. The percentage of ticks attracted to the treated fabrics were significantly lower in all repellents tested compared with the control fabrics ($P < 0.05$). For the formulated products, repellent products containing lemon eucalyptus and Walkabout Insect Repellent (containing a combination of citronella, tea tree oil and lemon-scented tea tree oil) provided 100% repellency within 15 min after treatment, whereas picaridin and DEET exhibited 91–97% repellency. However, no significant difference between the formulated products was found ($P > 0.05$) at 15 min after treatment (Table 2). The -citronella-impregnated patch (100% repellency) provided significantly higher repellency ($P < 0.001$) compared with the wristband (66.7% repellency) (Table 2). Additionally, the citronella-impregnated wristband provided significantly lower repellency compared with other formulated products ($P < 0.05$). For the plant-based substances, coconut oil at 30% (v/v) repelled 94.9% of the ticks, while the ethanol extract of *A. paniculata* at 30% (w/v) exhibited lower repellency, but not

Table 2 Percentage repellency of the products based on percentages of *I. holocyclus* female adults attracted in a laboratory bioassay at 15 min after fabric treatment

Test substance	AI conc.	% attracted ticks: Control	Test	*P*	% repellency
Formulated products					
Picaridin	92.8 g/L	66	6	0.004	90.9[a]
DEET	115 g/L	86	2	0.004	97.7[a]
Lemon eucalyptus	360 g/L	82	0	0.005	100.0[a]
Citronella + Tea tree	28.35 g/L	76	0	0.004	100.0[a]
Citronella patch	120 mg/patch	78	0	0.005	100.0[a]
Citronella band	750 mg/band	84	28	0.005	66.7[b]
Plant-based					
Coconut oil	30% v/v	78	4	0.011	94.9[a]
	15% v/v	66	12	0.005	81.8[a]
	10% v/v	70	38	0.005	45.7[b]
Plant extract					
A. paniculata	30% w/v	70	18	0.007	74.3[a]
	15% w/v	84	18	0.007	78.6[a]
	10% w/v	80	44	0.007	45.0[b]

Repellency is based on percentages of *I. holocyclus* female adults ($n = 50$) attracted in a laboratory bioassay to the test and control substances. AI, active ingredient; *P*, the probability frequencies between the test and the control. Under '% repellency', the superscript letters indicate a significant difference in percentage repellency.

significantly different ($P = 0.056$), at 74.3% (Table 2). When the concentration of the active ingredient was decreased three-fold, the repellency also decreased in both coconut oil and *A. paniculata* indicating a dose-dependent relationship (Table 2). No statistically significant changes in tick behaviour were observed when a control fabric (treated with ethanol only) was tested followed by another control fabric ($P = 0.480$) and an untreated fabric followed by an untreated fabric ($P = 0.705$).

The mean percentage repellencies for each product across all the time points from 15 min to 4 h after treatment are presented in Figure 3. Picaridin and DEET provide high repellency with 85–94% and 98–100%, respectively, for up to 4 h. There was no significant difference between both products for up to 3 h ($P > 0.05$), while DEET was significantly more effective at repelling ticks than picaridin at 4 h after treatment ($P = 0.012$) (Fig. 3a). Lemon eucalyptus (360 g/L) remained effective (98–100% repellency) in repelling female ticks for up to 4 h after treatment and was not significantly different to DEET at this time point ($P > 0.05$). For citronella in combination with tea tree oil (Walkabout Insect Repellent), the repellent at 28.35 g/L exhibited high repellency (89–100%) over 4 h (Fig. 3a); however, the level of repellency was significantly lower after 2 h ($P = 0.029$). Coconut oil also exhibited a relatively high repellency (70–95%) at 15–30% concentration (Fig. 3b), although repellency was significantly lower after 1 h with the 30% concentration ($P = 0.005$). For *A. paniculata* crude extract at 15–30% w/v, high repellency (74–79%) was observed only at first 15 min, which then decreased to 36–69% repellency the following hour (Fig. 3c), being significantly lower. The citronella-impregnated patch exhibited

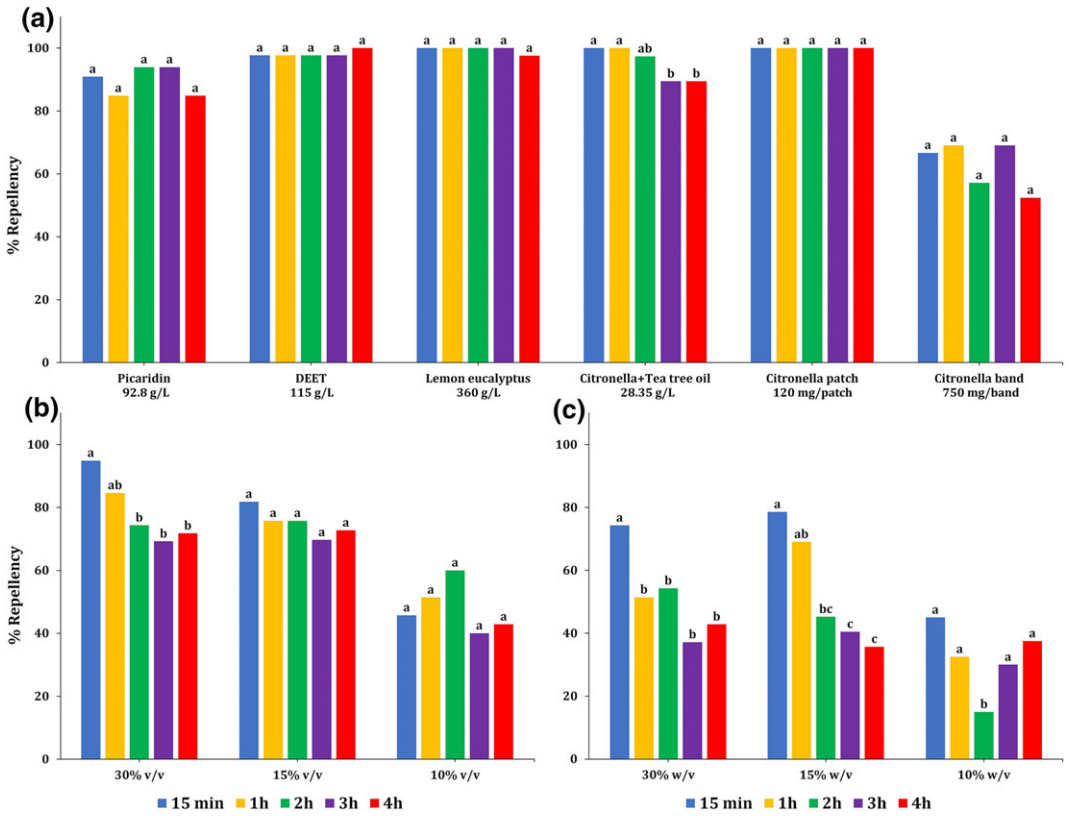

Fig. 3. Percentage repellency of (a) formulated products, (b) coconut oil and (c) *A. paniculata* at three different concentrations against *I. holocyclus* female adults at five time points after treatment. Different letters indicate a statistical difference.

complete repellency (100%) for 4 h, while the wristband provided only 57–69% repellency. No significant differences were found between the time frames tested for both citronella-impregnated products (Fig. 3a).

Spatial repellents

In the laboratory testing of the SRs, there was a significant difference in repellency between the treatments and the controls for both OFF!® Clip-On™ (P = 0.004) and Thermacell® (P = 0.002). Only 12% of ticks were attracted to the volunteer holding the OFF!® Clip-On™ device, while 40% of ticks were attracted to the control indicating 70% repellency was provided by OFF!® Clip-On™ device. Moreover, 92% of ticks were found knocked down after 5 min exposure to OFF!® Clip-On™, with 22% mortality observed at 24 h post exposure. For the Thermacell® device, 28% of ticks were attracted to the volunteer holding the device, while 56% were attracted to the control. Comparing with the control, Thermacell® device provided 50% repellency against the tick. However, 5 min exposure to the Thermacell® resulted in 100% knockdown and complete mortality at 24 h.

The outdoor assay to evaluate both OFF!® Clip-On™ and Thermacell® devices was carried out in an open backyard during 8:00–12:00 h with 27–28°C, 60% RH and <1 km/h winds. However, no knockdown or any 24 h mortality was found with either spatial repellent over any distance.

DISCUSSION

As noted in the introduction, *I. holocyclus* is the source of considerable human morbidity, causing paralysis, mild to severe allergic reactions including mammalian meat allergy, anaphylaxis and even the occasional death (Doggett 2004; Rappo *et al*. 2013; van Nunen 2018). Furthermore, the tick is known to transmit infectious agents such as rickettsia (Doggett 2004). Recent research investigating the microbiome of *I. holocyclus* has shown that this tick contains a range of bacteria and viruses, some of which are closely related to pathogens that are known to cause human disease (Harvey *et al*. 2019). In spite of the extensive recent investigations into the pathogens that this species may carry and the work on the allergies that the tick can induce, to date, there has not been one piece of published research that has investigated management options for this tick, which includes personal protection measures.

Currently, a range of repellents are registered for preventing tick bite (APVMA 2019) by the Australian Pesticides and Veterinary Medicines Authority (APVMA), the federal government body responsible for the registration of all pesticides and veterinary products. A requirement of registration is that efficacy data must be provided to support the claim that the product actually repels the tick (APVMA 2018). However, such data are commercially confidential and not available for public scrutiny (APVMA 2014). Thus, no public data exist on the comparative efficacy of either personal (or spatial) repellents against *I. holocyclus*. The information herein represents the first published paper that attempts to address this deficiency.

For the formulated products evaluated at 15 min, the active ingredients of DEET, lemon eucalyptus and the citronella/tea tree oil combination, all provided greater than 97% repellency. The coconut oil provided almost 95% repellency, and picaridin provided just over 90% repellency, although none of these five were significantly different. The *A. paniculata* derived product produced just under 75% repellency, which was significantly lower than the other topical repellents and the coconut oil. The ethanol treated fabrics produced 0% repellency. Thus, for the comparison undertaken at 15 min, the products containing DEET, lemon eucalyptus, the citronella/tea tree oil combination and coconut oil provided comparable protection.

However, most people are likely to spend more than 15 min in a high-risk tick area and in the case of bush regenerators and bush walkers, usually many hours. Thus, there was a need to test the repellents for efficacy over a longer duration, and this was undertaken for up to 4 h. For the formulated products over 4 h, there was no change in repellency for DEET, lemon eucalyptus or picaridin. In contrast, at 4 h, there was a significant reduction in repellency with the remaining essential oils. For DEET and lemon eucalyptus, repellency was close to 100% at 4 h and just under 90% for picaridin. These results follow closely to those of other studies that show that DEET tends to offer better repellency against ticks than essential oils (Meng *et al*. 2015), although in our investigation, lemon eucalyptus provided high protection.

For the citronella patch and wristband, both products remain effective in repelling ticks with 100% and 52–69% repellency, respectively, up to 4 h. Considering that past repellency studies have found citronella performs very poorly against haematophagus arthropods in comparison with DEET (Fradin & Day 2002), the 100% repellency result with the patch was unexpected. However, from previously published studies, citronella patches and wristbands provide only a very limited area of protection against arthropod bites (Fradin & Day 2002; Revay *et al*. 2013). In order to have any level of protection, it would be necessary to wear hundreds of these and completely cover the body. Such an impractically would raise questions as to why the APVMA allowed registration of these devices, as the wearing of a single patch or band could provide a false sense of security, and increase the risk of tick bites and associated health impacts. The authors urge the APVMA to reconsider the registration status of citronella wristbands and patches.

As noted above, there has been a move away from field testing of topical repellents because of the health risks imposed by biting arthropods. Furthermore, protocols have been developed that do not involve the administration of repellents onto the skin of volunteers (Jaenson *et al*. 2003). Obviously, this reduces the risk of chemical exposure and also obviates the need for human ethics approvals. Naturally, however, laboratory assays and assays that do not involve the application of repellents to the skin are going to introduce limitations. For example, there may be repellent/skin interactions that could affect repellency, plus sweating and the rubbing of treated skin from clothing that may degrade the repellent over time. Thus, there may be some

discordance between laboratory results and what is observed in the field. Such limitations are unlikely to be resolved with the decline in field evaluations of repellents.

The SRs employed in this study both use pyrethroids as the active ingredient. While such products are sold as 'repellents', strictly speaking, pyrethroids have contact irritancy and act as toxicants and are not true repellents (Prose *et al*. 2018). The laboratory trials employed with the SRs herein demonstrated that the devices were fully operational as toxic, knockdown, and repellency effects were all recorded. In contrast with the laboratory results however, neither SR managed to produce any mortality in the field situation. To date, the only field trial so far conducted with the OFF!® Clip-On™ against ticks, compared the effect of the device with the unit running with the fan on compared with the fan being off (Xue *et al*. 2016). With the fan on, 89% of host-seeking *A. americanum* ticks were repelled when on a volunteer when seated and 28% protection was achieved when the volunteer was walking through a tick-infested area. It is worth noting that *A. americanum* is a tick species that is an active hunter of hosts, compared with *Ixodes*, which are typically more passive in their questing behaviour. Whether these behavioural differences contributed to the contrasting result in our study where we found no direct toxic effect in the field is unknown. At present, the only published data on the effectiveness of the Thermacell® against ticks were a trial undertaken in a vented garage against *A. americanum* (Bibbs & Xue 2016). These authors found significant mortality was produced by the Thermacell®. In contrast with our trial (and the procedures were based on the Bibbs & Xue paper), no mortality was recorded. Our experiments were conducted in the field on a moderately warm day and perhaps convention heat prevented the insecticide from reaching the ticks. Further research is required to confirm this was the case; however, our results indicate that site and species-specific testing should be undertaken before SRs are routinely recommended for personal protection against ticks.

This paper provides the first published data on the effectiveness of topical and spatial repellents as part of a personal protection strategy for the prevention of tick bites from *I. holocyclus*. The finding herein suggests that topical repellents containing picaridin, DEET or lemon eucalyptus are the most effective at repelling *I. holocyclus* and are effective for at least 4 h. Spatial repellents are not recommended for the prevention of bites from *I. holocyclus*.

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