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Toxicity and persistence of permethrin-impregnated clothing against the Australian paralysis tick, *Ixodes holocyclus* (Acari: Ixodidae)

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Abstract Permethrin is a pyrethroid insecticide that has been widely used for the impregnation of fabrics to prevent the bites from arthropods such as mosquitoes and ticks. There are two types of permethrin impregnation: Do-It-Yourself (DIY) packs and pre-impregnated fabrics. Both forms have been on sale for many years in Australia, however to date, no data are available regarding their efficacy against *Ixodes holocyclus*. The aims of this study were to test and compare the contact toxicity of a DIY product versus two pre-impregnated fabrics (shirt and trousers) against *I. holocyclus*, and to test the efficacy of the fabrics after repeated laundering. All treated and untreated fabrics were tested before laundering (0 washes) and after 10, 30 and 50 washes. Five ticks were placed onto each test fabric for 2 min, with four replicates for a total of 20 ticks per test. Both knockdown and mortality of *I. holocyclus* ticks were recorded at 60 min and 24 h, respectively. Logistic regression analysis was used to assess the relationship between knockdown and mortality in ticks exposed to each fabric product, before and after laundering. All of the unwashed treated fabrics were effective in causing 100% knockdown. With 10 washes, the percentage of knockdown with the pre-impregnated shirt and trousers was 95% and 90%, respectively. The DIY product was less effective with only 15% knockdown. The levels of knockdown with the pre-impregnated shirt and trousers further decreased with 30 washes to 70% and 20%, respectively, and then further declined to 45% and 20% with 50 washes. For the DIY product, knockdown was only 10% and 5% with 30 and 50 washes, respectively. There was minimal mortality recorded at 24 h even with the unwashed fabrics. This study indicated that the pre-impregnated product was more effective in repelling *I. holocyclus* ticks than the DIY impregnation pack and the efficacy of this product degrades notably after 10 washes.

Key words efficacy testing, permethrin protection durability, tick bite prevention, tick management, tick repellent, washing stability.

INTRODUCTION

Ticks are an important vector in the transmission of a variety of pathogens to humans and animals (Colwell *et al*. 2011). Worldwide, there are 896 species of ticks including 702 Ixodidae, 193 Argasidae and 1 Nuttalliellidae (Guglielmone *et al*. 2010). Of these, only 72 species are endemic to Australia (Barker *et al*. 2014; Ash *et al*. 2017; Kwak *et al*. 2018), and six out of 17 ticks that bite humans act as competent vectors of pathogen transmission to humans (Barker & Walker 2014). Among these six species, 97% of tick bites in humans are caused by the Australian paralysis tick, *Ixodes holocyclus* (Taylor *et al*. 2019; Geary *et al*. 2020). This tick is considered the most medically significant species in Australia because of its ability to induce paralysis, trigger a variety of allergic reactions, and for the transmission of infectious diseases (Doggett 2004). The species is involved in inducing mammalian meat allergy and the spread of tick-borne diseases such as Australian tick typhus, *Rickettsia australis* (van Nunen *et al*. 2007; Graves & Stenos 2017; van Nunen 2018). To combat this public health threat, tick bite prevention measures that are both effective and easy to use are required. However, up until recently, little research had been undertaken on strategies in this area.

One method of preventing tick bites promulgated by some companies is the use of ultrasonic devices, which are commonly sold in Australia and around the world. However, several previous studies have suggested these devices are relatively ineffective, and they cannot be recommended in the prevention of tick bites. A more effective method is the application of personal repellents that are applied directly to the skin prior to entering tick-infested areas (Katz *et al*. 2008; Rahlenbeck *et al*. 2016). To date, only one paper has been published on the effectiveness of topical and spatial repellents at repelling *I. holocyclus*, and recommendations were made on which were the most effective products (Sukkanon *et al*. 2019).

For people who regularly engage in outdoor activities or have the potential for occupational exposure to ticks, impregnation of clothes with a long-lasting insecticide can provide additional protection from the bite of ticks (Banks *et al*. 2014; Rahlenbeck *et al*. 2016). Insecticide-treated clothing has been used for many years by the military for protection against the bites from a variety of arthropods including ticks, chigger mites, sand flies, and mosquitoes (Snyder & Morton 1946; Breeden *et al*. 1982; Schreck *et al*. 1982; Gupta *et al*. 1989; Evans *et al*. 1990; Harbach *et al*. 1990; Fryauff *et al*. 1996; Rowland *et al*. 1999;

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