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Protecting dogs and cats against the Australian paralysis tick, *Ixodes holocyclus* (Acari: Ixodidae): A review of the Australian acaricide registration process

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ABSTRACT

Tick control is mainly achieved through the use of effective ectoparasiticides that can be either dermally or systemically distributed in/on the host. Before any acaricide can be legally made available to veterinarians and pet owners, it must demonstrate efficacy in a series of well-designed dose confirmation studies. The data generated during these studies are then reviewed by government regulators and used for the registration of the acaricide. In Australia, the most significant tick species is the Australian paralysis tick, *Ixodes holocyclus*. This three-host tick produces a potent neurotoxin (holocyclotoxin) that induces a rapidly ascending flaccid paralysis that can be fatal to companion animals and larger mammals such as cattle and horses. The Australian Pesticides and Veterinary Medicines Authority (APVMA) is the national Australian regulator which sets the data requirements for the registration of acaricides. This paper reviews the requirements set by the APVMA and puts them in direct context with the biology, distribution and reported acaricide susceptibility of *I. holocyclus*. An overview of acaricides currently registered in Australia for the control of *I. holocyclus* in dogs and cats, their reported efficacy data and the conduct of *I. holocyclus* efficacy trials are also provided.

1. Introduction

Ticks are non-permanent blood-feeding ectoparasites that are abundant in the Australian environment and infest a wide range of different native, domestic animal and human hosts. In Australia, the species most commonly associated with dogs and cats are *Rhipicephalus sanguineus*, *Haemaphysalis longicornis* and *Ixodes holocylus* (Greay et al., 2016). Ticks cause damage to their hosts by ingesting blood, transmitting pathogenic agents including bacteria, viruses and protozoans and causing allergy and toxicosis. By far the most virulent and clinically important species is the Australian paralysis tick, *Ixodes holocyclus* (Bagnall and Doube, 1975). This tick is distributed along the east coast of Australia where it infests dogs, cats, sheep, goats, cattle, horses, and a wide range of native animals and humans, predominantly during spring and early summer (Eppleston et al., 2013). The tick produces a potent neurotoxin (holocyclotoxin) that causes a rapidly ascending flaccid paralysis which can be fatal (Masina and Broady, 1999). The toxin is produced by the ticks' salivary gland ~72 h following attachment. Clinical signs in dogs and cats include altered voice, labored respiration, ascending flaccid paralysis eventually leading to respiratory failure and death. The tick has been known to Australian pet owners since early colonial times and every year ~10,000 domestic animals are presented to veterinarians with clinical signs of tick toxicosis (Padula, 2016).

Over the past 100 years treatment and control of this tick has focused on the development of an effective tick antiserum (developed in the 1930's) and advancing veterinary critical care which has led to improved survival of pets (Padula et al., 2020). The biggest advance in the prevention and control of ticks and their associated diseases has been made in the development of highly effective ectoparasiticides such as the isoxazolines (Ozoe et al., 2010; Gassel et al., 2014; Shoop et al., 2014; Curtis et al., 2016; McTier et al., 2016; Rufener et al., 2017). These potent broad-spectrum ectoparasiticides have long-lasting systemic action against ticks (e.g. *I. holocyclus*, *R. sanguineus*), fleas (*Ctenocephalides* spp.), ear mites (*Otodectes cynotis*), demodex mites (*Demodex* spp.) and sarcoptic mange (*Sarcoptes scabiei*) and are available as topical (e.g. spot-ons) or oral (e.g. chews or tablets) formulations (Becskei et al., 2016; Beugnet et al., 2016; Cherni et al., 2016; Six et al., 2016; Packianathan et al., 2017; Taenzler et al., 2018).

Ectoparasiticides continue to be developed by multinational animal health companies in a constant effort for innovation and every drug

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candidate must pass through the stages of discovery, development, registration, and approval, followed by marketing and sales. This is an expensive process that can take 5–15 years for a new drug and patents are generally valid for a period of 20 years from the time they were originally filed, with possibility for extension through the release of new formulations (Hunter et al., 2011). To maximize return on investment, these drugs need to be registered as expeditiously as possible and be sold globally, but because the animal health industry is regulated in most developed countries, new drugs need to be approved by the local government regulator to ensure their quality, safety and efficacy before they can be sold.

In Australia, the government regulator of pesticides and veterinary medicines is the Australian Pesticides and Veterinary Medicines Authority (APVMA). To obtain approval for registration through the APVMA every new acaricide for dogs and cats needs to specifically demonstrate efficacy for the tick species listed on the label. General tick claims have not been accepted by the APVMA since 2014 (APVMA, 2014).

This paper provides an overview of the difficult and expensive process of bringing new acaricides to the Australian pet market with special focus on the Australian paralysis tick, *I. holocyclus*. We review the APVMA registration requirements for label claims for the control of *I. holocyclus* and put that in direct context with its biology and distribution, the requirements for the conduct of *I. holocyclus* efficacy trials, modern acaricides sold in the Australian pet market and their reported efficacies for *I. holocyclus* as well as the biological factors that lead to the development of acaricide resistance in hard ticks.

2. Guidelines for evaluating the efficacy of acaricides used for dogs and cats

Specific guidelines for evaluating the efficacy of parasiticides for the treatment, prevention and control of flea and tick infestations on dogs and cats have been published by the World Association for the Advancement of Veterinary Parasitology (WAAVP) (Marchiondo et al., 2007, 2013). The APVMA sets out its requirements for *I. holocyclus* label claims in its preamble for the WAAVP guideline for fleas and ticks on dogs and cats (https//apvma.gov.au/node/1040) which was last updated in 2014. Two dose confirmation studies demonstrating $>$ 95% efficacy within 72 h of infestation are required for each animal species. Field studies are not required because the animal welfare risk of tick paralysis in untreated control animals is considered too great. Instead, controlled studies should include "groups of animals infested with ticks collected from regions representative of the entire geographical range of *I. holocyclus*" to assess the intraspecific variation in acaricide susceptibility/tolerance between ticks from different geographical localities.

In comparison, the European regulator (European Medicines Agency, EMA) recommends the use of different laboratory tick isolates that are genetically enriched with parasites from field isolates every six years, or tick species from recent field collections, which are multiplied in the laboratory for at least two generations. EMA states that such strains are representative of the current field situation (EMA, 2016).

After application of the acaricide, tick assessments and counts are performed at 24 h, 48 h and 72 h after treatment to assess the immediate curative acaricidal efficacy against pre-attached ticks. This challenge and assessment process is repeated at intervals of ~7–31 days to assess the long-term persistent efficacy, spanning the full duration of the anticipated label claim. There are no clear recommendations regarding the number of animals per study group and/or parasites to be used during laboratory dose determination and confirmation studies. In general, six animals is considered to be the minimum number to provide adequate statistical power to determine significant differences against the untreated control at a 90% level of efficacy but a larger number of animals should be used in products with high animal to animal variability in flea and tick counts (Marchiondo et al., 2013). WAAVP recommends that $\leq$ 50 ticks per animal are used to infest dogs. Sedation of animals during the tick assessments is not permitted, and repellent claims require adequate supporting data (APVMA, 2014).

3. The Australian paralysis tick, *Ixodes holocyclus* (Acari: Ixodidae): biology, habitat, distribution, and genetic differences in Far North Queensland populations

Ixodes holocyclus is a three-host tick. The tick undergoes four life-cycle stages, i.e. egg, larva, nymph and adult, of which the latter three all feed on a separate host (Ross, 1924). In southeastern Queensland, each year there is one major generation of *I. holocyclus* with overlapping smaller cohorts as suggested by the presence of all life-cycle stages throughout most of the year (Doube, 1979). Larvae, nymphs and adult females infest and feed on a wide range of reptile, mammalian and bird hosts, but the bandicoots *Isoodon macrourus* and *Perameles nasuta* seem to be essential to the long-term survival of the population, at least in southeast Queensland, whereas the importance of the bandicoot hosts is unclear in other regions of the distribution of *I. holocyclus* (Barker and Walker, 2014). Based on the observations of Doube (1979), larvae are most common between January to March, nymphs are most abundant in April to September, and adult females between October to December. However, most cases of tick paralysis are reported in Brisbane in the month of September (Doube et al., 1977), before the peak for adult ticks has been reached, and this may reflect a seasonal decline in the acquired immunity of the host rather than being related to the abundance of adult ticks (Marchiondo et al., 2019).

Ixodes holocyclus mates in the grassy nests of bandicoots and not on the hosts and therefore male ticks are rarely encountered on hosts. Also, male *I. holocyclus* feed on the haemolymph of adult females rather than feeding on their mammalian or bird hosts. They have specialized mouthparts that are very different to those of the female paralysis ticks and are specifically adapted to feed on the females, frequently leaving feeding scars on the body of the female tick (Barker and Walker, 2014). Humidity and rainfall are main factors that influence the distribution of *I. holocyclus* as the eggs and larvae are highly susceptible to desiccation (Heath, 1979, 1981) and it is believed that in Queensland *I. holocyclus* can be found in areas that have at least 1000 mm annual rainfall. The other key to its distribution is the presence of bandicoot hosts which probably explains the absence of *I. holocyclus* from the York Peninsula where bandicoots are believed to be scarce or extinct (Barker and Walker, 2014). The distribution of *I. holocyclus* is limited to the coastal areas of Australia's east costs and extends from southeastern Victoria, throughout New South Wales (NSW) to north of Cairns, Queensland (QLD). In Victoria, the distribution of *I. holocyclus* may overlap in areas with those of the closely related species *Ixodes cornuatus* (southern paralysis tick) (Barker and Walker, 2014) (Fig. 1).

The use of molecular methods has allowed detailed insights into the phylogeny and epidemiology of *I. holocyclus*. Mitochondrial cytochrome *c* oxidase subunit 1 (*cox*1) and the second internal transcribed spacer (ITS2) sequences have been useful in differentiating the two species of Australian paralysis ticks, *I. holocyclus* and *I. cornuatus* and in identifying genetic variation in *I. holocyclus* between different geographical ranges (Song et al., 2011). One study that investigated ITS2 sequences of ticks from 17 different locations within the range of this species showed that *I. holocylus* populations in Far North Queensland (FNQ) have an adenine at position 197 of the 793 bp ITS2 sequence whereas populations from other localities had a guanine at this position (Shaw et al., 2002). The authors concluded that a small amount of variation in ITS2 among populations of *I. holocyclus* indicate that the species has moved across the dry habitats that break up the distribution of moist forests and that the largest break has led to the genetic isolation of ticks in FNQ from those further south. The authors also state that if this nucleotide difference is fixed in the genome, the presence of a single nucleotide difference indicates that this population has not been isolated for long (Shaw et al., 2002). This information is of value for phylogenetic analysis but is not predictive of differences in acaricide susceptibility of FNQ ticks. The

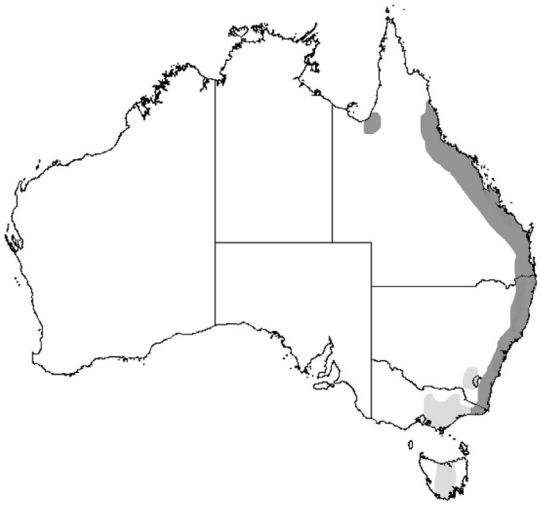

Fig. 1. The geographical distribution of *I. holocyclus* (dark grey) and *I. cornuatus* (light grey) (redrawn from Barker and Walker, 2014).

second internal transcribed spacer of nuclear ribosomal DNA has been widely used in the field of veterinary parasitology as a taxonomic and species-specific marker for identification of e.g. ticks (Barker, 1998), gastrointestinal nematodes infecting ruminants (Roeber et al., 2012, 2017) or horses (Nielsen et al., 2008; Kaspar et al., 2017) and a wide range of other organisms. This is because it generally has a low level of intraspecies variability but a higher level of interspecies variability (i.e. it is the same for individuals of the same species but varies between different species), occurs as tandem repeats with multiple copies within the genome of the organism, and is of small size, which makes it favorable for the amplification and differentiation of closely related species (Baldwin et al., 1995); however, it is non-coding DNA, and differences in its sequence cannot be linked to acaricide resistance.

The development of molecular markers for the detection and monitoring of acaricide resistance against different drug classes requires the identification of specific single nucleotide polymorphisms (SNP) in genes that are linked to drug resistance. There are several molecular markers that have been developed for the resistance monitoring of *Rhipicephalus microplus* and to lesser extent also for *R. sanguineus* which include mutations in the target genes of sodium channels, acetylcholinesterase, carboxylesterase, β-adrenergic octopamine receptor and octopamine-tyramine (Klafke et al., 2017; Aguilar et al., 2018; Kumar, 2019). These markers allow the detection of resistance-associated genes within tick populations before they reach high levels. However, this is complicated by the fact that acaricide resistance is multifactorial and parasites can develop a number of different mechanisms that lead to the development of resistance which can be behavioral, biochemical or metabolic (Cossío-Bayúgar et al., 2018), e.g. evading the site of drug application, changes in drug metabolism, excretion of drugs before it can reach the target site or structural changes in the target receptors, which can make it difficult to link resistance observed in the field to a single molecular marker or pathway.

4. *Ixodes holocyclus* dose confirmation studies

Efficacy trials for *I. holocyclus* in companion animals require specialized facilities, suitable animals, skilled staff, and access to paralysis ticks. Sourcing paralysis ticks in sufficient numbers to meet the demands of the industry can be a challenging task given that a study may require up to 10,000 ticks (Marchiondo et al., 2019). Artificial breeding of *I. holocyclus* is not currently possible because bandicoots are a protected native species and not available for use in laboratories. Ticks are supplied by tick collectors that find unengorged adult female paralysis ticks in the field. Large numbers of *I. holocyclus* are collected from the Northern Rivers region of NSW because there is a suitable environment and demand created by tick serum producers and veterinary Contract Research Organizations (CROs) that conduct acaricide efficacy trials. Wild-caught unfed, female ticks are collected from October to December from localities within the Northern Rivers region of NSW and to limited extent also from southeast QLD. Ticks are usually collected from vegetation by tick drags, transferred to jars that contain moist moss to provide substrate, and stored until they are used in trials. The storage of ticks is limited, and significant mortality can occur as a result of fungal overgrowth or dehydration (Marchiondo et al., 2019). Far North Queensland ticks also need to be collected every year because the APVMA requires that study animals are infested with ticks collected from both FNQ and from regions representative of the rest of the geographical range of the species. Collection of sufficient numbers of ticks from FNQ has proven difficult despite repeated efforts being made by one of the authors (M.W.) since 2003 to develop infrastructure for the collection of FNQ ticks. Finding enough ticks has become increasingly difficult. Tick collectors report that previously identified collection habitat has become drier or has been destroyed by fire so that new areas need to be identified continuously. Regions in Victoria are usually avoided to prevent the accidental collection of *I. cornuatus* which is morphologically very similar to *I. holocyclus* (A. Muller, pers. comm.).

Using dogs with adequate tolerance to holocylotoxin allows them to be challenged with 30–50 ticks providing more robust data, reduced risk of tick paralysis, and fewer animals are needed to generate estimates of efficacy with tighter confidence limits than can be achieved using naive dogs and reduced challenges (Marchiondo et al., 2013). Trials must be approved by an Animal Ethics Committee (AEC), are generally conducted according to Good Clinical Practice (GCP) (EMA, 2000) and typically involve two groups of animals with 8–12 animals per group to provide enough statistical power in the data. For *I. holocyclus*, two different types of studies can be conducted. For investigational veterinary products that have a repellency (or expellency) effect, ticks are released into the environment or onto the animal and repellency or expellency is measured. Acaricides that are non-repellant/expellant or that have systemic action are tested in studies in which the ticks are manually attached to the animals and the acaricidal effect measured every 12–24 h for 72 h allowing the speed of kill to be estimated. It is critical that ticks are killed well within the 72-h period after attachment before paralysis sets in. For both type of studies ticks are classified during the assessments as live attached (LA), live free (LF), dead attached (DA) and dead free (DF), and the efficacy is determined by the percent reduction of mean numbers of total live ticks (ToL = LA + LF) on the animals in the treated compared to the untreated groups (Marchiondo et al., 2013). Efficacy is calculated as E = (Mc-Mt)/Mc × 100, where E is the percent efficacy and Mc and Mt refer to the number of live parasites counted on the control and treated animals, respectively. Typically, the 72-h post-attachment count is the critical count. For the evaluation of acaricides, this formula is generally used for dermally acting contact acaricides, whereas for systemically acting acaricides the number of live attached ticks is considered more appropriate because efficacy can only occur if the tick bites and ingests blood or tissue fluids (Marchiondo et al., 2013).

Treatments are typically only administered once during these trials (Day 0) and the length of the trial depends on the anticipated label claim - usually extending past the anticipated label claim (Fig. 2).

5. Acaricides used for the control of *Ixodes holocyclus* on/in dogs and cats

There is a range of ectoparasiticides that differ in their route of administration, mode of action, species suitability, length of protection and pest spectrum. At present, there are 25 different formulations registered for dogs and cats that include a label claim for *I. holocyclus* (Table 1). There are 16 (64%) formulations registered for dogs, five (20%) formulations for dogs and cats, and four (16%) formulations registered for cats. Based on their site of distribution, these products

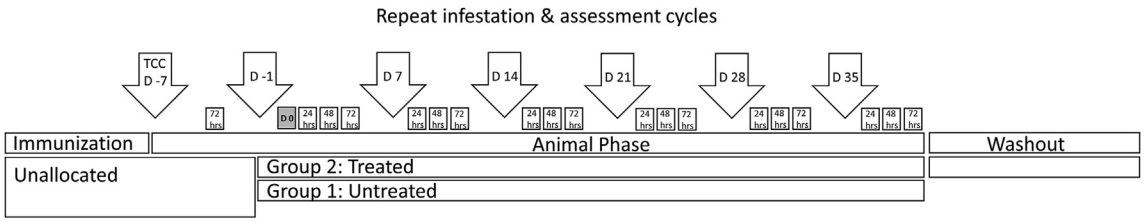

Fig. 2. The design for a typical *I. holocyclus* pen efficacy study including the phases of immunization, animal phase and washout. A larger group of animals is immunized in the weeks leading up to the study by getting exposed to gradually increasing numbers of paralysis ticks. Approximately one week before treatment administration, a tick carrying capacity test (TCC) is conducted to rank animals based on their tick retention ability 72 h following the infestation. Based on the highest tick counts animals are included, ranked, blocked, and randomized into treatment groups. Treatments are given on Day 0 (shown as a grey box) to Group 2 (treated) animals dermally, orally or by injection and tick infest and assessment cycles are performed in regular intervals (shown as arrows; weekly in this example on days −1, 7, 14, 21, 28 and 35) for the duration of the anticipated label claim. Ticks are assessed at 24, 48 and 72 h (shown as white boxes) for their attachment and viability and classified as: LA, live attached; LF, live free; DA, dead attached; and DF, dead free. Efficacy of test compounds is determined by comparing mean parasite numbers on treated and untreated animals at specific time-points. Upon completion of the animal phase, Group 2 dogs must complete a washout period before they can participate in another acaricide efficacy study.

Table 1
Dermally distributed acaricides that have a label claim for the control of *Ixodes holocyclus* in dogs and cats (Source: APVMA – Public Chemical Registration Information System Search; Access date: August 23, 2020).

Type	Trade name	Actives	Target species	Paralysis tick label claim	Manufacturer
Collar	Seresto® for dogs	100 mg/g Imidacloprid, 45 mg/g Flumethrin	Dogs	Repells and controls for up to 4 months	Elanco
	Seresto® for cats	100 mg/g Imidacloprid, 45 mg/g Flumethrin	Cats	Repells and controls for up to 8 months	Elanco
	Kiltix® tick collar	100 g/kg Propoxur, 22.5 g/kg Flumethrin	Dogs	Aids control for up to 6 weeks	Elanco
Spot-on	Advantix®	100 g/l Imidacloprid, 500 g/l Permethrin (cis:trans, 40:60)	Dogs	Provides control for up to 2 weeks	Elanco
	Frontline® Original	100 g/l Fipronil	Dogs	Provides control for up to 2 weeks	Boehringer Ingelheim Animal Health
	Frontline® Plus	100 g/l Fipronil, 90 g/l (S)-Methoprene	Dogs	Provides control for up to 2 weeks	Boehringer Ingelheim Animal Health
	Frontline® Top Spot	100 g/l Fipronil	Dogs	Provides control for up to 2 weeks	Boehringer Ingelheim Animal Health
	Frontline® Spray	2.5 g/l Fipronil	Dogs, cats	Provides control for up to 3 weeks	Boehringer Ingelheim Animal Health
	Purina Total Care Flea and Tick Control	100 g/l Fipronil	Dogs	Provides control for up to 2 weeks	Nestle Purina
Rinse/shampoo	Aristopet Animal Health Flea & Tick Rinse Concentrate	30 g/l N-Octyl Bicycloheptene Dicarboximide, 18 g/l Piperonyl Butoxide, 10 g/l Pyrethrins	Dogs, cats, cage birds	Prevent attachment for up to 3 days	Aristopet
	Cotex Hydrokill Flea & Tick Rinse Concentrate	30 g/l N-Octyl Bicycloheptene Dicarboximide, 18 g/l Piperonyl Butoxide, 10 g/l Pyrethrins	Dogs, cats, cage birds	Prevent attachment for up to 3 days	Deeway Laboratories
	Dermcare Permoxin Insecticidal Spray and Rinse	40 g/l Permethrin (cis:trans, 25:75)	Dogs, horses	Rinse weekly for protection against ticks	Dermcare-Vet
	Fido's Fre-Itch Concentrate	30 g/l N-Octyl Bicycloheptene Dicarboximide, 18 g/l Piperonyl Butoxide, 10 g/l Pyrethrins	Dogs, cats, cage birds	Prevents attachment for up to 3 days	Mavlab
	Purina® Petlife™ Flea Control Shampoo	10 g/l Piperonyl Butoxide, 8 g/l Melaleuca Oil, 2 g/l Permethrin (cis:trans, 25:75)	Dogs	Aids control of paralysis tick	Nestle Purina
	Purina® Total Care™ Flea Control Shampoo	10 g/l Piperonyl Butoxide, 8 g/l Melaleuca Oil, 2 g/l Permethrin (cis:trans, 25:75)	Dogs	Aids control of paralysis tick	Nestle Purina
	Rufus & Coco Flea Free Shampoo	30 g/l N-Octyl Bicycloheptene Dicarboximide, 18 g/l Piperonyl Butoxide, 10 g/l Pyrethrins	Dogs, cats, cage birds	Prevent attachment of paralysis ticks for up to 3 days	Rufus & Coco

can be broadly divided into dermally and systemically distributed ectoparasiticides.

Dermally distributed ectoparasiticides are supplied as collars, spot-on, spray or shampoos. These drugs can only be applied to the external skin of the animal and will exert an effect on the parasite upon direct contact or ingestion of the chemical. In contrast, systemically distributed ectoparasiticides, mainly represented by the macrocyclic lactones and isoxazolines, can be administered either orally, dermally or by injection and these drugs require the parasite to attach and feed on the host to get exposed to the drug (Marchiondo et al., 2013; Selzer and Epe, 2021).

Desirable characteristics of the ideal ectoparasiticide include a broad acaricidal and insecticidal activity, non-toxicity to pets, humans and

environment, easy application and long-lasting residual activity (Stanneck et al., 2012). Ectoparasite control strategies are often poorly implemented and the most common cause for this is non-compliance with recommended treatment regimes. Several studies have highlighted the importance of owner compliance in the care of the veterinary patients and concluded that easier treatment protocols increased owner compliance (Gates and Nolan, 2010; Rohrbach et al., 2011; Van Vlaenderen et al., 2011). Spot-on formulations have been preferred for cats and chews or tablets for dogs. Excellent reviews have been written on the topic of ectoparasiticides (Taylor, 2001; Pfister and Armstrong, 2016) and a detailed review of the different drug classes goes beyond the scope of this paper. In the following, we give account to the commonly used acaricides currently available in the Australian pet market and review the relevant published efficacy data for *I. holocyclus*.

5.1. Dermally distributed acaricides

The main actives in this group are pyrethrin, synthetic pyrethroids and fipronil. Both pyrethrin and synthetic pyrethroids act by disrupting arthropod nerve sodium channels which leads to nerve discharges, incoordination, tremor, paralysis and arthropod death (Narahashi, 1971). This "knockdown" effect is characterized by initial hyper-excitation and disorientation in the arthropod, followed by repellence and/or expellence and death if exposed to a sufficient dose.

Pyrethrin is derived from the *Chrysanthemum* plant and has a very rapid "knockdown" effect on arthropods but only minimal residual activity and is easily removed by water (Crombie and Elliott, 1961). Pyrethrin products are often formulated together with a synergist such as piperonyl butoxide which reduces the insect's natural defense mechanisms and detoxification ability and enhances the efficacy of a given insecticide (Romero et al., 2009). These products are available as pet shampoos (e.g. Aristopet Animal Health Flea & Tick Rinse Concentrate; Cotex Hydrokill Flea & Tick Rinse Concentrate), are generally safe for dogs and cats when used according to label, and prevent the attachment of paralysis ticks for up to three days (Table 1).

Pyrethroids are synthetic pyrethrin derivatives and include, amongst others, permethrin, flumethrin or deltamethrin. They have a slightly slower onset of action compared with pyrethrin but have longer residual activity and repellent action. Permethrin is extremely toxic to cats and clinical signs of pyrethroid intoxication in cats include hypersalivation, tremor, depression, coma, seizures, and if severe and untreated can be fatal (Valentine, 1990). Often intoxication in cats is the result of wrongly applying canine products to cats, or in some cases due to the close contact of cats with treated dogs shortly after the application (Pfister and Armstrong, 2016). Because of these serious risks of intoxication, the APVMA requires that products that contain pyrethroids have clear label warnings that indicate their toxicity to cats (https://apvma.gov.au/node/947). Pyrethroids are also toxic to aquatic environments, fish and amphibians which is of concern given their widespread application in agriculture and the associated contamination of water sources (Thatheyus and Gnana Selvam, 2013).

The pyrethroid flumethrin is generally safe for mammals as it shows a high specificity for arthropod neural tissue (Fourie et al., 2003). It has been used extensively as an acaricide in livestock and is also registered for the use in spot-on and collar preparations for dogs and cats. Pyrethroids are often combined with other compounds for improved insecticidal activity, as for example imidacloprid. Imidacloprid in combination with flumethrin has shown to have strong synergistic action for the treatment of tick and flea infestation (Stanneck et al., 2012). Pyrethroids, or combinations thereof, are available to Australian pet owners as shampoos (e.g. Purina Petlife Flea Control Shampoo; Dermcare Permoxin Insecticidal Spray and Rinse), spot-ons (Advantix, Elanco), or collars (Seresto for dogs or cats, Elanco). These products are often toxic to cats and have a range of different label claims against *I. holocyclus* from aiding in the control (Purina Petlife Flea Control Shampoo) to a repellency and control claim for 8 months (Seresto® for cats, Elanco) which is currently the longest tick label claim in the market. The long-term efficacy of a deltamethrin-impregnated collar (Scalibor® Protector-Band, MSD Animal Health) for the control of *I. holocyclus* was assessed for dogs and demonstrated an efficacy of $>$ 90% for at least 14 weeks (Webster et al., 2011). It took 14 days for the product to reach an efficacy of $>$ 95% and efficacy at the 72-h count was 96% after the day 14 of tick infestation. Efficacy 72 h post-infestation remained above $\geq$ 94% from the day 14 to the day 98 of infestation. Efficacy at 72 h was above 90% after the day 112 of infestation and was still 93% at 72 h after the day 140 of infestation. Scalibor® is no longer available in Australia but is still being sold in other parts of the world (e.g. USA). Two studies were conducted in dogs to assess the long-term efficacy of a imidacloprid/flumethrin polymer matrix collar (Seresto® for dogs, Bayer Animal Health now owned by Elanco) (Smith et al., 2013). In these studies, the acaricidal efficacy of the collar at 72 h post-infestation exceeded 95% on Days 17 (99.3%), 59 (99.7%), 73 (96.6%), 87 (100.0%), 101 (96.4%), 115 (99.1%) and 171 (95.8%), but dropped on Days 45 (94.0%) and 143 (77.8%), and declined from Day 199 (79.9%) to 227 (65.5%). The mean efficacy 72 h post-infestation was 97.9% over the first 115 days and 91.3% over 227 days after application of the collar. In Australia, Seresto® has a registered repellency and control label claim of 4 months in dogs and 8 months in cats (Table 1).

Fipronil belongs to the phenylpyrazoles. This group of ectoparasiticides acts on the ligand-gated chloride channels of the arthropod leading to hyperexcitation and death. Fipronil is a potent adult pulicide with acaricidal efficacy (Gupta and Anadón, 2018). It is available to Australian veterinarians and pet owners as spot-ons (e.g. Frontline Original, Boehringer Ingelheim Animal Health) or sprays (Frontline Spray, Boehringer Ingelheim) with claims for the control of *I. holocyclus* ranging from two to three weeks, respectively.

5.2. Systemically distributed acaricides

The isoxazolins are a novel ectoparasiticide class that offers systemic, prolonged and highly specific efficacy against various genera and species of parasitic arthropods (Pfister and Armstrong, 2016). Currently four drugs in this class have received APVMA approval for the use in dogs in Australia, namely, fluralaner (Bravecto, MSD), afoxolaner (NexGard, Boehringer Ingelheim), sarolaner (Simparica, Zoetis) and lotilaner (Credelio, Elanco). Isoxazolins achieve ectoparasiticidal activity by blocking the GABA and glutamate-gated chloride channels and have a highly selective toxicity for arthropod neurons over those of mammals. These drugs are well tolerated by young dogs ($>$ 8 weeks) and cats (8–9 weeks of age depending on the product), are not toxic to cats or Collies and are available as oral formulations (chews or tablets) for dogs or topical (spot-on) formulations for cats and dogs (Table 2). Isoxazolins are also formulated in combination with macrocyclic lactones (e.g. moxidectin, milbemycin oxime) or pyrimidines (pyrantel) for added endoparasite control in dogs (Simparica Trio Chews, Zoetis; NexGard Spectra chewable, Boehringer Ingelheim) and cats (Revolution Plus topical solution, Zoetis; Bravecto Plus Spot-on solution, MSD).

A study that investigated the efficacy of an orally administered fluralaner (Bravecto®) in dogs demonstrated an efficacy of 100% against *I. holocyclus* at all 72 h assessments until Day 115 (Fisara and Webster, 2015). Efficacy remained high at $>$ 95.7% until the last 72 h post-infestation time-point on Day 143. In this study, dogs were infested with 30 paralysis ticks per dog and mean live tick counts on treated dogs at 24 h post-infestation were below 1.0 until 113 days post-treatment indicating the rapid onset of action. This is in line with the observations that fluralaner is rapidly absorbed after oral administration, reaches maximum plasma concentrations within 24 h and is quantifiable in plasma for up to 112 days following a single oral administration (Kilp et al., 2014). Bravecto® chewable tablets for dogs (MSD Animal Health) has been granted a 4-month label claim for the control of *I. holocyclus* which is currently the longest for any orally administered acaricide for dogs.

Table 2
Systemically distributed acaricides that have a label claim for the control of *Ixodes holocyclus* in dogs and cats. Doses shown are for medium size dogs (10–20 kg) and cats (2.6–5.0 kg). (Source: APVMA – Public Chemical Registration Information System Search; Access date: August 23, 2020).

Type	Trade name	Actives	Target species	Paralysis tick label claim	Manufacturer
Tablet/Chew	Simparica® Trio Chews	0.48 mg/chew Moxidectin, 100 mg/chew Pyrantel as Pyrantel Embonate, 24 mg/chew Sarolaner	Dogs	Treats and controls for 5 weeks (35 days)	Zoetis
	Bravecto® chewable tablets	136.4 g/kg Fluralaner	Dogs	Treatment and control for 4 months	Intervet
	Credelio™ chewable tablets	225 mg/TB Lotilaner	Dogs	Treatment and control for 1 months	Elanco
	NexGard Spectra® chewables	7.5 mg Milbemycin Oxime, 37.5 mg Afoxolaner	Dogs	Treatment and control for 1 month	Boehringer Ingelheim Animal Health
	NexGard® chewables	68 mg/chew Afoxolaner	Dogs	Treats and controls for 1 month	Boehringer Ingelheim Animal Health
Spot-on	Revolution® Plus topical solution	60 mg/ml Selamectin, 10 mg/ml Sarolaner	Cats	Treatment and control for 5 weeks	Zoetis
	Bravecto® Plus Spot-on solution	14 mg/ml Moxidectin, 280 mg/ml Fluralaner	Cats	Treatment and control for 10 weeks	Intervet
	Bravecto® Spot-on solution	280 mg/ml Fluralaner	Dogs	Treatment and control for 6 months	Intervet
	Bravecto® Spot-on solution	280 mg/ml Fluralaner	Cats	Treatment and control for 3 months	Intervet

Another study investigated a systemically acting spot-on formulation (Bravecto®) for cats (Fisara et al., 2018). In this study, each cat was infested with 10 paralysis ticks and tick assessments were performed at two weekly intervals until Day 87. The efficacy of the fluralaner spot-on treatment reached 100% at 48 h post-treatment and remained at 100% at 48 and 72 h after all subsequent experimental infestations of *I. holocyclus* for the entire duration of the trial, thus Bravecto® Spot-on solution for cats received a 3-month label claim for *I. holocyclus*.

The acaricidal efficacy of lotilaner (Credelio®) for the control of paralysis ticks in dogs was evaluated in two studies (Baker et al., 2018). These studies were conducted over a 3-month period with repeat challenges occurring in two weekly intervals (Days 14 and 42 in Study 2 only) until Day 84 (Day 91 in Study 1). In Study 1, non-immunized dogs were used and infested with 12 ticks each. In Study 2, immunized dogs were used and infested with 30 ticks each. Lotilaner achieved a 100% efficacy following 48 h of treatment in Study 1 and within 72 h in Study 2. In Study 1, the efficacy remained 100% at all 72-h assessments through Day 87, except for Day 31 when a single tick was found, resulting in a 99.2% efficacy at this time-point. In Study 2, the efficacy remained 100% at each 72-h assessment until Day 59. An efficacy of $>$ 95% was maintained till the final assessments on Day 94 and Day 87 in Study 1 and Study 2, respectively. Interestingly, these results would justify a 3-month label claim but Credelio® is intended to be given by dogs owners in a monthly interval and the additional protection offered by Credelio® is used as a 'grace' period which allows dog owners to miss/or delay a treatment and while their pet remains protected against paralysis ticks (Baker et al., 2018).

Another study evaluated the speed of kill of the two isoxazolins, sarolaner (Simparica®) and afoxolaner (NexGard®) against existing *I. holocyclus* infestations and during weekly reinfestations for a period of 5 weeks after treatment with a single dose (Packianathan et al., 2017). Dogs were infested on Day −1, were examined and live ticks counted at 8, 12, 24 and 48 h after treatment on Day 0, and at 12, 24 and 48 h after subsequent re-infestations on Days 7, 14, 21, 28 and 35. On Day 0, at the 8-h and 12-h time-points, efficacy of sarolaner against existing infestations was 86.2% and 96.9% compared to 21.3% and 85.0% for afoxolaner, respectively. During subsequent weekly re-infestations at 12 h time-points, treatment with sarolaner resulted in an efficacy ranging from 60.2% to 92.2%, compared to 5.8%–61.0% in the afoxolaner-treated dogs. At the 24 h time-points on Days 22 and 36, efficacy of sarolaner was higher at 99.2% and 97.9%, compared to afoxolaner which had efficacy of 92.4% and 91.9% for the same time-points. At the 48 h time-points following each of the five weekly re-infestations, the mean efficacy of sarolaner and afoxolaner was similar on most occasions. Sarolaner (Simparica®) has a label claim against *I. holocyclus* for 5 weeks but in a similar fashion as for lotilaner, the product is intended to be given by dog owners every month and the additional week is allowed so the owner may be late for the treatment by a couple of days without compromising their dog's protection against paralysis ticks.

6. Acaricide resistance in ixodid ticks

Acaricide resistance in ticks infesting dogs and cats has not been as extensively studied compared to ticks infesting cattle, such as *R. microplus* which is resistant to most acaricides and has been the focus of extensive research (Rodriguez-Vivas et al., 2018). There are certain environmental and life-cycle features that increase the likelihood of an organism to develop resistance. The selection pressure exerted on a population drives the rate at which resistance develops. When the entire population is under intense selection pressure, the potential for resistance is increased compared to situations where only a small proportion of the population is exposed to a chemical. The concept of refugia has been well described in the field of veterinary parasitology and is strategically used for the control of various parasites that have developed drug resistance, as for example, gastrointestinal nematodes infecting ruminants and horses. Refugia provide a reservoir of ticks that have not been exposed to acaracides thereby slowing the accumulation of resistance genes within that population. The availability of refugia is influenced by the biology and life-cycle of arthropod parasites. Coles and Dryden (2014) reviewed these factors and provided examples for three species of ixodid ticks that differ in their life-cycles, biology and consequently, also their ability to develop resistance to acaricides.

Rhipicephalus microplus is a one-host tick; larvae, nymphs and adults all attach to and develop on the same host. The total time spent on the host is between 17 and 52 days and the entire life-cycle can be completed within two months (Taylor et al., 2007). This life-cycle provides little refugia as the majority of the population is on the host and the only ticks that are not exposed to a chemical are seed ticks in the vegetation and ticks on untreated cattle (Coles and Dryden, 2014). It is common practice that all cattle in a herd are treated simultaneously which further exacerbates this problem. Because of the high economic importance of this tick species significant efforts for large scale treatment administration and eradication programs have been implemented. The frequent administration of acaricides in combination with a lack of refugia have exerted extreme selection pressure on this tick species which has led to widespread, and rapidly developing drug resistance against many acaricides. The Arthropod Pesticide Resistance Database (Monta-Sanchez and

Wise, 2021) noted 562 reports of resistance in this species against 50 different acaricides, including chlorpyrifos, cypermethrin, deltamethrin, fipronil, flumethrin, and ivermectin.

Rhipicephalus sanguineus is a three-host tick; each life-cycle stage molts in the environment and must find a new host to feed on. Fed larval and nymphal stages are present in the environment, avoiding exposure to the chemical and are available to infest hosts after moulting. However, *R. sanguineus* has a strong preference for dogs which reduces the refugia available to this species in comparison to less host-specific ticks. At present, 26 cases of acaricide resistance in *R. sanguineus* have been reported to amitraz, benzene hexachloride (BHC)/cyclodines, dichlorodiphenyltrichloroethane (DDT), organophospates and permethrin (Monta-Sanchez and Wise, 2021) with additional reports of resistance to deltamethrin, fipronil and ivermectin from Mexico (Rodriguez-Vivas et al., 2017) and Brazil (Becker et al., 2019).

Amblyomma spp., *Dermacentor* spp. and *Ixodes* spp. are three-host ticks that infest a wide range of native and domestic animals as well as humans. This provides them with much more refugia than *R. sanguineus* and therefore, the selection pressure and potential to develop resistance is greatly reduced. In addition, treatments applied against these species are overwhelmingly applied to domestic small companion animals (cats and dogs) and not all animals in a household or location may be treated. If treatments are applied, they may consist of different actives and delivery modes and may also be separated in time. Finally, the timing and frequency of treatments may vary widely from household to household within a small geographical area. Acaricide resistance has rarely been described for these genera and at present there is only one report of *Amblyomma americanum* being resistant to BHC/cyclodienes, two reports of *Dermacentor variabilis* resistant to BHC/cyclodienes and DDT and one report of *Ixodes rubicudus* to sodium arsenite (Monta-Sanchez and Wise, 2021).

Ixodes holocyclus is known to infect a wide range of mammalian and bird hosts. Bandicoots are an important host to the tick, and male specimens do not regularly infest and feed on hosts. Therefore, there are large refugia available to this tick species and no reports of acaricide resistance have been published.

Acaricide resistance of *I. holocyclus* is difficult to evaluate in clinical settings for the veterinary practitioner. There are a number of different possible causes for inefficacy including environmental, host-related or non-compliance by clients. Bathing and swimming can affect the efficacy of topical applications; certain owner and pet behaviors may predispose to higher exposure and infestation pressure, e.g. farm or hunting dogs with access to forested areas and bush land are likely to experience a higher exposure compared to city dogs that only have access to dog parks in the inner city; and finally, seasonal or annual fluctuations in tick populations caused by environmental changes or an influx of wildlife serving as reservoir hosts, can significantly increase infestation pressure and the prevalence of observed tick paralysis (Blagburn and Dryden, 2009; Dryden, 2009). Case reports of individual failures cannot be regarded to be the documentation of acaricide resistance in the absence of more definitive testing (Coles and Dryden, 2014).

7. Discussion

Regulators such as the APVMA protect consumers' interests and ensure the quality, safety and efficacy of products. Registration of new products should be based on up-to-date scientific knowledge and with careful consideration of vector biology, epidemiology and other diseases specifics, and be achievable within reasonable time and effort. *Ixodes holocyclus* is a three-host tick with a sylvatic life-cycle that involves a wide range of native and domestic animal hosts. The species mates off the host, males are rarely encountered on the hosts and only feed off female ticks. These life-cycle features provide the species with more refugia than is available for most other tick species. The selection pressure for resistance in this species is low which reduces the risk of resistance genes accumulating to significant levels within the population. The ability of resistant ticks to reproduce is further limited as ticks that survive treatment for more than 72 h are likely to induce paralysis in the host and subsequently be found during ensuing veterinary care which includes a tick search and removal. It is generally accepted that different populations or species of parasites can vary in their susceptibility/tolerance towards different ectoparasiticides and therefore it is prudent to assess an acaricide on more than one population of ticks (Marchiondo et al., 2013; Pfister and Armstrong, 2016). Tolerance is a natural tendency rather than the result of selection pressure and certain individuals within a population may be more tolerant of a specific parasiticide dose than others. This range of natural pesticide susceptibility can be expressed as a bell curve in every population of pests and can vary between different species or even life-cycle stages of the same organism (Coles and Dryden, 2014).

Phylogenetic studies using ITS2 of nuclear ribosomal DNA showed that *I. holocyclus* populations in FNQ are genetically distinct from those in the rest of distribution area by a single base pair difference in the ITS2 region (Shaw et al., 2002). Even though this information is of interest from a phylogenetic point of view, the use of ITS2 does not provide any information about the resistance or tolerance status of a parasite as it is non-coding DNA and any changes in its sequenced cannot be linked to acaricide susceptibility.

There are several factors that may influence the acaricide susceptibility/tolerance of ticks towards a particular acaricide which include parasite related factors, e.g. intraspecies variability (i.e. different tolerance between individuals of the same species), host related factors (e.g. differences in the bioavailability, distribution and persistent efficacy of an acaricide in different hosts) or environmental factors related to the collection, transport and storage of ticks for the conduct of pen efficacy studies (e.g. humidity, temperature or photoperiod), which makes the interpretation of results difficult. It can be hypothesized that acaricide resistance is more likely to occur in regions were ticks experience higher exposure to acaricides. These would be areas with higher treated host density such as the suburbs of developed metropolitan areas that are within the distribution area of *I. holocyclus*. Suburbs with access to bushland and scrub would be prime locations where treated domestic pets are exposed to *I. holocyclus*. Exposure would be less frequent in remote areas of FNQ where there is a lower canine population density and subsequently fewer acaricide-treated dogs and cats. Taken together, these factors reduce the risk of FNQ ticks having a different susceptibility to other more readily available ticks and do not support the requirement for inclusion of FNQ ticks in dose confirmation studies. On the other hand, the authors support the view that the assessment of new veterinary pharmaceuticals should be done prudently, and that consideration be given to requiring a field study across the range of *I. holocyclus* for those products that have demonstrated acceptable efficacy in pen studies as is the case for products seeking registration to control paralysis ticks in cattle. Such data would provide more assurance of efficacy than current guidelines. The exact number of animals and parasites to be used in a study will be influenced by the acaricide and parasite species; however, recommendations should be based around confidence intervals to provided increased clarity and to establish a more standardized approach. Confidence intervals refer to the probability that a population parameter will fall between a set of values for a certain proportion of times and measures the degree of certainty or uncertainty in a sampling method. This is directly influenced by the number of parasites per animal and number of animals included in a study (i.e. if the number of parasites per animal is increased less animals need to be used to achieve the same confidence interval and *vice versa*).

There is a range of highly effective dermally and systemically distributed acaricides available for dogs and cats in Australia with label claims varying from "aid in the control of *I. holocyclus*" to an eight-month repellency and control claim. All the currently registered acaricides that have a *I. holocyclus* control claim have the demonstrated capacity to kill paralysis ticks within 72 h and have shown to provide $>$ 95% protection over the duration of the label claim in well-designed dose confirmation studies. The high standard required for registration of acaricides in

Australia means that only the highly efficacious products become available to the Australian pet market. However, no acaricide is guaranteed to be 100% effective all the time (Baker et al., 2018) and there is a wide range of possible reasons for failure in acaricide efficacy in the field (Blagburn and Dryden, 2009; Dryden, 2009). Therefore, pet owners should always remain vigilant, especially during peak periods (spring and early summer) and search their pets for ticks. Additionally, compliance with recommended treatment regimens is essential to providing effective ectoparasite control and any drug can only be effective if it is administered appropriately by the pet owners (i.e. to the right animal species, at the right dose and at the correct re-treatment interval).

8. Conclusion

Based on the detailed review of current literature, the authors suggest that the requirement for inclusion of FNQ ticks in pen efficacy studies is unnecessary and should be removed. This would significantly facilitate the sourcing of paralysis ticks and reduce the economic burden on sponsors, but still provides an adequate efficacy assessment of new acaricides. Setting requirements for registration should involve the dialogue with major stakeholders including sponsors, parasitologists and CROs to ensure that requirements are scientifically sound as well as achievable within reasonable effort. Registration requirements must be set sensibly and with foremost focus on ensuring the quality, efficacy and safety of new veterinary pharmaceuticals, but at the same time it must not be excessively restrictive which would stifle the advancement of the veterinary pharmaceutical industry, and potentially reduce the number of products developed, or prevent the registration of products that would actually be beneficial.

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FR conceptualized and wrote the paper with further edits and review from MW.

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