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Susceptibility of *Ixodes scapularis* (Acari: Ixodidae) to Permethrin Under a Long-Term 4-Poster Deer Treatment Area on Shelter Island, NY

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Abstract

Pesticide resistance in medically significant disease vectors can negatively impact the efficacy of control efforts. Resistance research on ticks has focused primarily on species of veterinary significance that experience relatively high degrees of control pressure. Resistance in tick vectors of medical significance has received little attention, in part because area-wide pesticide applications are not used to control these generalist tick species. One of the few effective methods currently used for area-wide control of medically important ticks, including *Ixodes scapularis* Say (Acari: Ixodidae), is deployment of 4-poster devices. Deer self-apply a topical acaricide (permethrin) while feeding on corn from the devices. A 4-poster program using permethrin has been deployed on Shelter Island, NY to control *I. scapularis* populations since 2008. We collected engorged female ticks from deer in this management area and a location in the Mid-Hudson River Valley, NY without area-wide tick control. Larvae were reared from egg masses and their susceptibility to permethrin was tested. Larvae originating from a long-term laboratory colony were used as a susceptible baseline for comparison. Compared against the laboratory colony, resistance ratios at LC-50 for Shelter Island and Hudson Valley *I. scapularis* were 1.87 and 1.51, respectively. The susceptibilities of the field populations to permethrin were significantly lower than that of the colony ticks. We provide the first data using the larval packet test to establish baseline susceptibility for *I. scapularis* to permethrin along with information relevant to understanding resistance emergence in tick populations under sustained control pressure from 4-poster devices.

Keywords

pesticide resistance; larval packet test; acaricide; *Ixodes scapularis* ; 4-poster device

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Pesticide resistance is a common issue in arthropod pathogen vector populations. Considerable research has focused on pesticide resistance in medically significant mosquito species, with well-developed resistance testing guidelines from the World Health Organization (WHO 2016a,b) and the Centers for Disease Control and Prevention (CDC; McAllister and Scott 2019). Pesticide resistance in mosquito populations has been implicated in management failures (Brouqui et al. 2012, Estep et al. 2018) and monitoring is considered an essential component of mosquito control programs (National Association of County and City Health Officials [NACCHO] 2017). This is not the case for ticks, where most management and pesticide resistance guidelines and research have focused on species that impact domestic animals, particularly cattle ticks such as *Rhipicephalus microplus* (Canestrini) (Acari: Ixodidae) and *Rhipicephalus annulatus* (Say) (Food and Agriculture Organization of the United Nations [FAO] 2004). These ticks are one-host species, spending most of their life cycle on cattle, and causing enormous economic damage globally (Jonsson et al. 2001, Kivaria 2006). Cattle tick management often involves applying acaricides directly to cattle and resistance has been reported for multiple pesticide classes (George et al. 2004, Reck et al. 2014). Recently, permethrin resistance also has been detected in an endophilic three-host tick, *Rhipicephalus sanguineus* (Latreille), from multiple populations in Florida and Texas (Eiden et al. 2015).

Most ticks that transmit human disease agents are three-host species, which feed on different vertebrate hosts at each life stage and spend large portions of time off-host in the environment. Consequently, host-targeted methods are challenging for medically important tick species. One exception is deployment of 4-poster devices that can effectively control *Ixodes scapularis* Say and *Amblyomma americanum* (L.) by targeting deer, their primary reproductive host (Stafford and Williams 2017). *Ixodes scapularis* is the most medically significant vector in the eastern United States, transmitting pathogens responsible for Lyme disease, anaplasmosis, babesiosis, and Powassan virus disease. The permethrin-treated application rollers of 4-poster devices treat deer at attached feeding stations offering corn (Pound et al. 2000). This method has effectively reduced questing tick densities, but whether the resulting selection pressure is strong enough for resistance to emerge in tick populations is unclear.

In an effort to reduce tick populations on Shelter Island, NY, a program deploying 4-poster devices was maintained from 2008 to 2019. A study conducted on the island between 2008 and 2010 tested the efficacy of 60 devices (1.9/km^2) dispersed over the town of Shelter Island and the associated Mashomack Preserve. They observed 85% fewer questing *I. scapularis* nymphs by the final year (Curtis et al. 2011). After study completion, the management program continued with 36 devices. This long running program targeting a relatively isolated island population of *I. scapularis* provides an excellent opportunity to test permethrin resistance emergence. We, therefore, collected engorged female *I. scapularis* from deer on Shelter Island. We similarly collected engorged female *I. scapularis* from deer at the Cary Institute of Ecosystem Studies (CIES) in Millbrook, NY. This site represents a tick population under lower control pressure; no 4-poster devices are in operation and pesticides are not widely applied on the property. Permethrin susceptibility of larvae reared from these specimens was compared with larvae from a long-term laboratory-reared colony maintained by CDC.

Methods

Tick Collection and Rearing

Engorged female *I. scapularis* were collected in November 2019 directly from hunter-killed white-tailed deer [*Odocoileus virginianus* (Zimmermann)] on Shelter Island, NY (N 41° 4′ 4.17′′; W 72° 20′ 19.54′′) and grounds of CIES in Millbrook, NY (N 41° 47’ 6.04′′; W 73° 44′ 1.76′′) in coordination with ongoing deer management programs. Engorged females were removed with tweezers, placed in 50 ml falcon tubes, and transported to the laboratory. Engorged females, egg masses, and larvae were stored in humidified vials inside an incubator set to 24°C, 70% RH, with a 16:8 (L:D) light cycle. The laboratory strain of *I. scapularis* was provided by CDC from a long-term colony and reared as described previously (Troughton and Levin 2007). Original colony material was collected from Rhode Island and has been maintained in colony since 2003. It is periodically refreshed with males from the field. Females were monitored weekly, until egg masses were laid at which point, they were monitored every 24 h. Once larvae hatched, they were aged to 14–18 d and used in bioassays.

Larval Packet Test Bioassay

The larval packet test (FAO 1984) was used to determine permethrin susceptibility of the three *I. scapularis* strains (CDC/CIES/Shelter Island). Technical grade permethrin (99%, Chem Service Inc., West Chester, PA) was dissolved in a 2:1 solution of trichloroethylene (TCE) and olive oil. Six permethrin concentrations between 1.33 and 0.04 mg/ml were prepared for the bioassay. A control, containing only TCE and olive oil mixture with no permethrin, was included for each set of replicates. Control mortality was adjusted using Abbott’s correction (Abbott 1925). Four to eight replicates were conducted at each concentration and tick strain, depending upon availability of larvae.

For each replicate, seven filter papers (Whatman No. 1, Maidstone, England) were cut into uniform packets (7.6 × 8.9 cm). Each paper was inoculated with 1 ml of the target concentration of permethrin solution. The control papers were inoculated with 1 ml of the control solution described above. Papers were suspended in a fume hood for 2 h to evaporate the TCE. Once dry, the filter paper was folded in half and two #2 bulldog clips were used to enclose the sides of the packet. The top was left open and approximately 100 *I. scapularis* larvae were added to each packet using a fine brush. Once the larvae were added, a third bulldog clip was used to seal the packet. Packets were suspended inside an aquarium in an incubator with a 16:8 light cycle at 24°C and 70% RH. After 24 h, packets were opened, and mortality was quantified by counting viable and non-viable ticks. Ticks were categorized as non-viable if they did not respond to being breathed upon.

Data were analyzed using R (version 4.0.2). Three probit analyses (CDC/CIES/Shelter Island) were conducted using the glm() command in the MASS package. Lethal concentration values (LC-50/LC-95/LC-99) and their associated 95% confidence intervals were used to determine whether susceptibility differed significantly between the three tick strains. Resistance ratios (RRs) at the LC-50 value were calculated, using the CDC colony

as a baseline (Yu 2014). Significant differences in susceptibility between *I. scapularis* strains were determined by comparing the LC-50s and their 95% CIs.

Results

Nine fully engorged *I. scapularis* females were collected from deer on Shelter Island, seven of which laid viable egg masses. At CIES, six fully engorged females were collected, four of which laid viable egg masses. All five engorged females supplied by CDC produced egg masses and larvae. Bioassays included totals of 3,569 larvae from seven egg masses from Shelter Island, 2,347 larvae from four egg masses from CIES, and 5,324 larvae from five egg masses from the CDC colony (Fig. 1). Results for probit analyses for Shelter Island (t = 18.3; df = 34; $P < 0.001$), CIES (t = 17.4; df = 46; $P < 0.001$), and the CDC colony (t = 14.8; df = 22; $P < 0.001$) are shown in Table 1, along with the LC values and LC-50 RRs.

Discussion

Ixodes scapularis collected from Shelter Island and CIES were less susceptible to permethrin than the CDC colony, with RRs of 1.87 and 1.51, respectively. Furthermore, specimens from Shelter Island were significantly less susceptible than those from CIES, but the difference in RRs was relatively small. It appears that field populations of *I. scapularis* may be less susceptible than the CDC laboratory colony, regardless of selective pressure from 4-poster devices. No other studies reporting *I. scapularis* RRs have been published. For other species, the RR threshold values for a population to be considered resistant have been set between 2 and 10 (Castro-Janer et al. 2011, Coles and Dryden 2014, Eiden et al. 2015). When connected to field efficacy, RR thresholds can vary by species, active ingredient, and bioassay method (Barré et al. 2008, Lovis et al. 2011). Field populations with RR values < 2 are rarely considered resistant, but further evaluation is needed to connect these thresholds to *I. scapularis* control efficacy. The absence of established susceptible colonies for *I. scapularis* is another limitation. The detection of pesticide resistance in mosquitoes generally employs the use of well-established susceptible lines that have been maintained under laboratory conditions, often for decades (Vontas et al. 2012). Unfortunately, no such established susceptible colonies exist for *I. scapularis* or other medically significant tick species.

Although, we found no permethrin resistance in the Shelter Island *I. scapularis*, it is possible that resistance might emerge under more intensive management regimes using 4-poster devices. Recent 4-posters densities on Shelter Island are approximately 1.2 devices/km^2, down from the 1.9 devices/km^2 in the original study, when a significant reduction in questing *I. scapularis* was observed (Curtis et al. 2011). Remaining devices were placed so that at least one device was within the majority of the home ranges of deer on the island. Tick sampling has continued on Shelter Island and tick densities do not appear to have rebounded, but data collection was less rigorous after the initial study (B. Payne, personal communication). There is evidence that both deer and 4-poster density influence the efficacy of 4-poster devices (Stafford and Williams 2017). A large-scale study observed significant reductions in questing tick densities with 4–5 devices/km^2 (Pound et al. 2009). Other studies using lower device densities (1.65 devices/km^2) have shown limited efficacy

(Grear et al. 2014). Permethrin susceptibility of tick populations should be monitored from other 4-poster control areas so that guidelines for managing pesticide resistance in the field can be developed.

The emergence of resistance in *I. scapularis* populations is likely to differ from that in mosquitoes, which may ultimately make resistance in ticks easier to track in the field. Hard ticks have significantly longer life cycles and therefore longer generation times than mosquitoes. This should reduce the amount of temporal variability in resistance status. The 11-yr deployment of the 4-poster program on Shelter Island only represents approximately six generations, so the emergence of resistance would require a high degree of control pressure. It is common for resistance to emerge rapidly in mosquito populations in response to targeted control efforts (Barbosa et al. 2011, Matowo et al. 2019), but for ticks this will occur on a longer timescale. This may reduce the frequency of sampling needed to monitor resistance in *I. scapularis* populations. Furthermore, ticks can move long distances attached to their hosts (Madhav et al. 2004, Diuk-Wasser et al. 2020), potentially increasing population mixing and reducing the probability that resistance will emerge due to localized control efforts.

Our sample sizes were limited due to the cessation of the Shelter Island 4-poster program, but results presented here provide the first field-based pesticide susceptibility assessment for *I. scapularis* collected from an area with potential long-term pressure. Future efforts to establish susceptible reference colonies, monitor field susceptibility, and develop operational resistance thresholds are critical to ensure long-term efficacy of existing and new pesticide-based tick control strategies.

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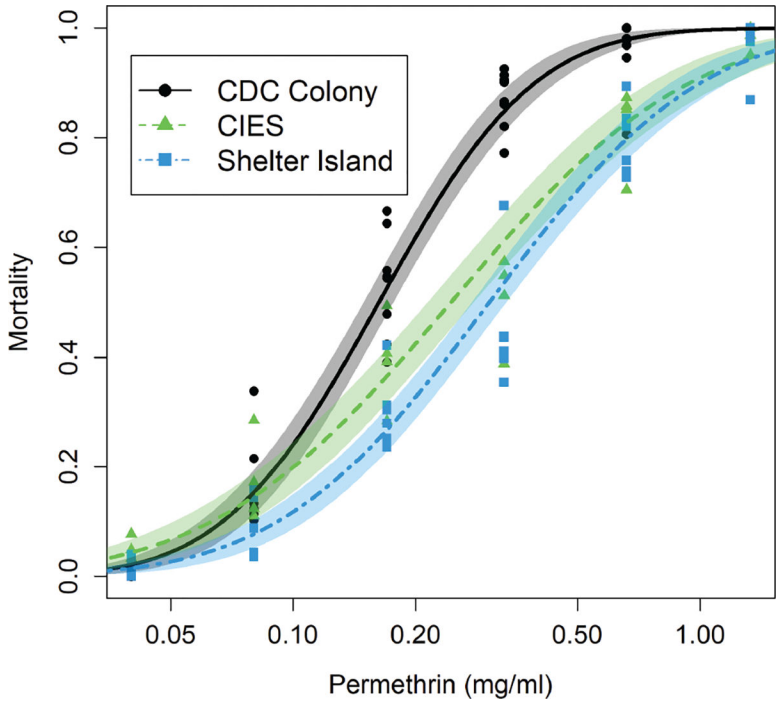

Fig. 1.

Scatterplots displaying permethrin susceptibility curves for *Ixodes scapularis* larvae from the two field populations (CIES/Shelter Island) and the CDC laboratory colony. Lines represent the predictions resulting from probit analyses and the shaded areas are 95% confidence intervals. A jitter was used to add a small amount of noise to these data to reveal overlapping points on this figure.

Table 1.

Results of the probit analyses for *Ixodes scapularis* larvae from the two field populations for which susceptibility to permethrin was tested (CIES/Shelter Island), and the CDC laboratory colony serving as the susceptible baseline

	No. egg masses included	No. replicates tested	df	Slope (SE)	LC-50 (± 95% CI)	LC-95 (± 95% CI)	LC-99 (± 95% CI)	RR (LC-50)	χ^2
CDC colony	5	8	46	3.34 (0.19)	0.162 (± 0.01)	0.505 (±0.08)	0.808 (± 0.18)	1	303
CIES	4	4	22	2.47 (0.13)	0.244 (± 0.03)	1.39 (± 0.42)	2.84 (± 1.19)	1.51	220
Shelter Island	7	6	34	2.18 (0.14)	0.303 (± 0.03)	1.41 (± 0.30)	2.66 (± 0.78)	1.87	337

LC values are presented in milligrams per milliliter. 'No. replicates tested' represents the number of replicate packets (each with ~100 larvae) included for the six tested permethrin concentrations for the field populations and susceptible control colony. RR is the resistance ratio at LC-50 (determined by dividing the LC-50 of the field colonies by that of the CDC colony). χ^2 values represent the goodness-of-fit for the probit analyses.