THE BIOLOGY AND MONITORING OF THE INVASIVE ASIAN LONGHORNED TICK, *HAEMAPHYSALIS LONGICORNIS* NEUMANN, 1901 (IXODIDA: IXODIDAE) IN THE UNITED STATES

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By

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ABSTRACT

The Asian longhorned tick (*Haemaphysalis longicornis*) is a newly introduced, invasive species in the United States. Since the earliest record of its presence in West Virginia in 2010, *H. longicornis* has been collected from twelve states in the US. It is known to transmit disease pathogens, including the severe fever with thrombocytopenia syndrome virus (SFTSV), *Theileria* sp., *Babesia* sp. and *Rickettsia* sp. These pathogens infect livestock and humans, causing economic losses and substantial rates of morbidity. In addition, *H. longicornis* is capable of being infected with pathogens that already exist in the US, namely, *Borrelia* sp. and *Ehrlichia* sp., which compounds the associated health risks. Management and control of this emerging tick requires knowledge of its biology, ecology, and distribution. This research addresses the current need for a standardized and effective collection method, which is important for defining potential tick exposure and disease risks as well as ecological factors relating to the tick distribution.

Average ticks collected using three check distances (5 m, 10 m, and 20 m) and three collection methods (drag sampling, sweeping, and CO_2 traps) were compared to determine the optimal collection technique. Field collections were conducted from June through August 2019, in Westchester County, New York, and ticks were grouped by life stages to assess the collection method efficiency. Results indicated that implementing shorter check distances, using the drag sampling method were ideal for adult collections. Shorter check distances were still the most effective for collecting nymphs. In contrast to the adult collections, the difference between drag sampling and sweeping methods were not statistically significant for nymphs. CO_2 traps attracted *H. longicornis*, but additional research is necessary to devise an effective tick retaining method before CO_2 traps can be implemented in the field. Results are presented to inform and support *H. longicornis* surveillance and control programs across the nation.

Key words: Asian longhorned tick, *Haemaphysalis longicornis*, invasive tick species, drag sampling, sweeping, CO_2 traps, collection methods, check distance

BIOGRAPHICAL SKETCH

Phurchhoki was born in a remote village in the Himalayas of Nepal. Educational opportunities (or lack thereof) led her to attend a boarding school in the capital city, Kathmandu, and then a small liberal arts college, Colby-Sawyer College, in New Hampshire. As an undergraduate she worked on a project that studied the transport of mercury from emerging aquatic invertebrates to the terrestrial ecosystem. That experience and collecting flat flies (Hippoboscidae) off raptors in Cape May, New Jersey, introduced Phurchhoki to the amazing diversity and ecology of insects. However, it was not until her work at a mosquito surveillance program in Vermont when she realized the intersection between curiosity for the natural world and vector-borne diseases. She particularly enjoyed collecting and identifying mosquitoes and sharing her findings with colleagues, family, and friends. Fortuitously, that was when she learned about the Vector-Borne Disease Biology program at Cornell University. Phurchhoki joined the MS program with the Harrington lab in August 2018. In her two years at Cornell, she studied entomology and public health, travelled to Medellín, Colombia and northern New Zealand for winter internships, presented her work at conferences, and met many wonderful and supportive people.

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CHAPTER 1

Literature Review

Vector status

Haemaphysalis longicornis Neumann, 1901, also known as the 'Asian longhorned tick,' 'New Zealand cattle tick,' or 'bush tick,' is a vector for several pathogens (bacteria, viruses, and protozoa) that cause major human and animal diseases. These tick-borne pathogens pose a public health risk, due to the debilitating illnesses they can cause, as well as a social burden from economic losses due to the infection of livestock (Heath 2016). *H. longicornis* is a competent vector for the following pathogens that infect cattle, domestic animals, and humans: *Theileria orientalis* (Heath 2016), *T. sergenti, T. mutans, Coxiella burnetii,* Russian summer-spring encephalitis virus (Hoogstraal et al. 1968), *Babesia* spp. (Chen et al. 2012), and *Rickettsia* spp. (Lee et al. 2013). The potential for *H. longicornis* to transmit other serious pathogens should amplify public health and veterinary concerns as the expansion of this invasive species continues in the US. *H. longicornis* have been found with infections of *Ehrlichia* (Kim et al. 2003), *Anaplasma*, *Bartonella* (Kang et al. 2016), tick-borne encephalitis virus (Yun et al. 2012), *Borrelia burgdorferi* (Sun et al. 2008), and *B. miyamotoi* (Yang et al. 2018).

Research on the role of *H. longicornis* as a vector for a variety of pathogens, in the US, is on-going and indicates that differences in its biology affects vector competency. For example, *H. longicornis* can ingest viable *Borrelia* spirochetes from an infected host in the larval stage, but transstadial transmission of the infection is unsuccessful (Breuner et al. 2019). However, it can acquire *Rickettsia rickettsii* from an infected host, and transmit the infection transstadially, transovarially, and to a naïve host, in a lab setting (Stanley et al. 2020). *Ixodes scapularis*, Say, 1821, an endemic tick in the northeast US, has previously been reported capable of simultaneously transmitting two pathogens (Piesman et al. 1987; Levin and Fish 2000). It remains unclear whether *H. longicornis* can transmit multiple pathogens simultaneously, despite

its capacity to be concurrently infected with multiple pathogens, in one case with *Borrelia*, *Bartonella*, *Anaplasma*, and *Ehrlichia* (Sun et al. 2008). In its native Asian range, *H. longicornis* can transmit a virus that causes severe fever with thrombocytopenia syndrome (SFTS) (Luo et al. 2015). SFTS is an emerging hemorrhagic fever, which is associated with 6.3% to 30% mortality in humans (Liu et al. 2014; Mihara et al. 2018). Collectively, these pathogens and their connection to *H. longicornis* are concerning because several of the associated bacteria and closely related pathogens already exist in the US (Burtis et al. 2017; Wormser et al. 2019), and establishment of *H. longicornis* is likely to expand the current distribution of these pathogens, potentially exacerbating the number of infections. In addition to its role in tick-borne disease transmission, *H. longicornis* is a nuisance parasite to humans and animals. Heavy infestations of this tick can cause economic loss by damaging pelt and hide, reducing dairy yield (Heath 2016), and causing anemia and death of livestock and domestic animals (Yang et al. 2018). Cattle deaths from *H. longicornis* exsanguination have already been reported in the US (Neault 2019). High numbers of tick bites can also cause social malaise and physical ailment, such as dermatitis (Hoogstraal et al. 1968).

Biogeography and host ecology

A review by Oliver (1977) described three genetic races of *H. longicornis* based on its reproductive strategy: ‘diploid bisexual,’ which requires male fertilization for reproduction (Cane 2010); ‘triploid obligatory parthenogenetic,’ meaning females produce offspring without male fertilization (Suomalainen 1962); and ‘aneuploid,’ which can reproduce both ways. To date, the aneuploid race of *H. longicornis* has only been observed in Cheju Do, South Korea (Chen et al. 2012). Endemic populations of the parthenogenetic race occur in temperate and subtropical regions of the globe, including Australia, Japan, New Zealand, New Caledonia, Fiji,

New Hebrides, Tonga, China, and northeast Russia (Hoogstraal et al. 1968; Herrin and Oliver 1974). Bisexual race is found in Japan, Korea, northeast Russia, and China (Hoogstraal et al. 1968; Herrin and Oliver 1974; Chen et al. 2012). Both parthenogenetic and bisexual races exist in Japan, northeast Russia, and China, however, each race is found in distinct locations, except for Honshu Island in Japan, where both races are reported to co-exist (Hoogstraal et al. 1968; Herrin and Oliver 1974; Chen et al. 2012). As of April 2020, there has not been any confirmed report of male *H. longicornis* in the US, indicating the presence of exclusively parthenogenetic populations in the country.

Since the identification of an established population (all life stages present at the same place) of *H. longicornis* in New Jersey in 2017 (Rainey 2018), the tick populations have been found in twelve US states, including New York (CDC 2020; USDA 2020). Retrospective analysis of archived samples indicates that the tick may have started spreading within the borders of continental US as early as 2010 (Beard et al. 2018). In the US, this tick has been collected from mammalian hosts such as sheep, horses, deer, goats, raccoon, opossum, and humans, and avian hosts (red-tailed hawk) (Burtis et al. 2017; USDA 2019). In other regions of the world, *H. longicornis* has been found on various domestic and wild mammalian hosts such as feral goats and hares (Heath et al. 1987), sheep, horses, dogs, deer, bear, foxes, raccoon (Hoogstraal et al. 1968), and a small percentage of avian hosts (Hoogstraal et al. 1968; Heath et al. 1988). Based on the low number of reported human bite cases, it appears that humans are not the preferred hosts for *H. longicornis* in its invasive ranges, including the US (Foster et al. 2020).

Questing and feeding behavior

All active life stages of *H. longicornis* (larva, nymph, and adult) quest for vertebrate hosts. Adults can aggregate in clusters under leaves and vegetation to prey on hosts (Zheng et al.

2011). Plant height and host body size in the surrounding environment may influence the questing heights of this tick species (Tsunoda and Tatsuzawa 2004). Once attached to a host, *H. longicornis* can imbibe blood for a few days (larva: 3-9 days, nymph: 3-8 days, and adult female: 7-14 days), and the feeding rate is reported to be the fastest during hours preceding detachment (Wharton and Utech 1970; Heath 2016). Reports on the feeding durations for two races of *H. longicornis* are contradictory. Two reports from China and New Zealand found that the parthenogenetic race fed longer than bisexual race (Zheng et al. 2011; Heath 2016). In contrast, another study from China found that the parthenogenetic race fed for shorter time compared to its bisexual counterparts (Chen et al. 2012). The discrepancy in observations could be due to variations in feeding habits in native versus invasive ranges or when co-occurring with other races. Feeding rate also depends on the number of ticks feeding on a host, co-presence of male and female ticks (in bisexual population), and the host's immune status (Heath 2016). *H. longicornis* displays predilection for certain bite sites on its mammal hosts hypothesized to result from differences in vascularization, temperature, grooming behavior, flexibility of hosts, time spent by hosts sitting/lying in pasture, and hosts' feeding behavior (Heath et al. 1987). For instance, *H. longicornis* preferentially attach to the pinnae (ear), head, shoulders, dewlap, belly, and escutcheon of livestock and cattle. In the absence of hosts, adults and nymphs can survive without feeding for eight to ten months (Zheng et al. 2011) and all life stages of the tick can feed on humans (Hoogstraal et al. 1968).

Lifecycle

H. longicornis is a three-host tick, meaning every stage of its life (larva, nymph, and adult) feeds on a different host. It completes one generation per year in its native and invasive ranges (Heath 2016). A fully engorged *H. longicornis* larva or nymph detaches from its host and

falls to the ground, usually among leaf litter, where it eventually molts into its next life stage. When engorged females find a suitable habitat in moist environment, they can lay up to 2000 eggs which hatch into larvae in about 2-3 months, dependent on environmental factors (Hammer et al. 2015; Heath 2016). There is a significant correlation between engorged female weight and the size of an egg mass they lay (Zheng et al. 2011). Bisexual females produce higher daily egg yields compared to the parthenogenetic race (Chen et al. 2012; Hoogstraal et al. 1968). Under optimal conditions in the laboratory (28-29 °C and 100% RH), engorged larvae and nymphs molt into their next life stages within two to three weeks (Khalil 1972). After hatching or molting, larvae and adults take approximately 2-5 days to initiate questing for hosts (Zheng et al. 2011).

The usual pre-oviposition and oviposition periods for the bisexual race last for 1-4 weeks and 2-3 weeks, respectively (Zheng et al. 2011). The parthenogenetic race has a longer oviposition period compared to its bisexual counterparts (Chen et al. 2012). However, that can depend on the time of the year and geographic region with variable climatic conditions (Heath 2016).

Reproduction

Parthenogenetic race of *H. longicornis* produces almost exclusively female offspring (Chen et al. 2012). Infertile males can be produced by parthenogenetic females, but the number is relatively low (1 male to 400 females) (Bremner 1958; Hoogstraal et al. 1968).

The mating of bisexual race takes place on hosts. Males remain on the host and keep feeding until they find suitable females (Matsuo and Mori 2000). Mating is important for full engorgement and subsequent egg production among bisexual females (Yano et al. 1989). Males re-feed soon after they have copulated to regenerate their accessory gland secretions for

additional copulations (Matsuo and Mori 2000). Sufficiently and properly re-fed bisexual *H. longicornis* male can copulate with at least six females (Yano et al. 1989).

Bisexual and parthenogenetic females have a similar genital system, including the seminal receptacle (Khalil 1972). However, there are boundaries of gene flow between the races, as cross-insemination between parthenogenetic females and bisexual males does not occur (Chen et al. 2012). Fertilization is possible between bisexual males and parthenogenetic aneuploid females, leading to some inter-race genetic hybridization if the two populations occur in the same area (Oliver 1977). Feeding is required in each life stage for the development of a genital system (Khalil 1972). Therefore, it is possible that without selection for the maintenance of reproductive structures, the parthenogenetic race may lose some of them, specifically seminal receptacle, over time.

Morphology

All life stages have reddish brown or reddish yellow color (Hoogstraal et al. 1968), which contrast well with white corduroy fabrics during collection. *H. longicornis* is morphologically distinctive from other common tick species found at the collection sites, namely, *I. scapularis*, *I. dentatus* Marx, 1899, and *Dermacentor variabilis* Say, 1821. Palps of *H. longicornis* are laterally extended on second segments. Nymphs and adults are larger and more circular than *I. scapularis* and *I. dentatus*. However, the adults are comparable in size to adult *D. variabilis*. Size measurements for parthenogenetic race is provided: unfed adult female *H. longicornis* is approximately 2.65 mm long and 1.8 mm wide, nymph is around 1.76 mm long and 1 mm wide, and larva is about 0.58 to 0.62 mm long and 0.47 to 0.51 mm wide (Hoogstraal et al. 1968). These sizes are comparable to parthenogenetic race from native range, China (Chen et al. 2012).

Other distinguishing features of *H. longicornis* are the size of capitulum and scutum, dental formula, anal groove, and spurs on coxae (Hoogstraal et al. 1968).

Temperature and humidity tolerance

H. longicornis can be readily sampled in areas of full sun and occasionally on manicured lawns differing from *I. scapularis*, which are primarily collected from shady areas (Wormser et al. 2019). *H. longicornis* can tolerate a wide range of temperature and humidity fluctuations, though it prefers warmer temperatures and high relative humidity for reproduction and questing (Heath 1979, 2016). Pre-oviposition, oviposition, and molting periods decreased as temperature progressed from 12°C to 30°C (Yano et al. 1987). The optimum temperature for eggs to hatch is between 28-32°C with survival diminishing above 35°C (Heath 1979). However, the impact of humidity on eggs is complicated. Eggs had faster development and reduced mortality if transferred to a humid atmosphere after a period of dehydration and they survived better under dry conditions if pre-exposed to a humid atmosphere (Heath 1979). Low humidity can slow embryogenesis and development of larva, nymph, and adults (Heath 2016). To survive through environmental variability, *H. longicornis* can acclimatize to its environment by modifying body water, glycerol, and protein content (Yu et al. 2014) and can sustain development at temperatures as low as 12°C (Yano et al. 1987) and as high as ~40°C (Heath 2016).

Unfed nymphs and engorged females can tolerate severe dehydration because of their water retention ability, and the body size and volume of bloodmeal, respectively (Heath 2016). Nymphs are also more cold-tolerant than adults with 100% mortality measured at -20°C and -21°C for adults and nymphs, respectively (Yu et al. 2014). Similarly, in bisexual populations, adult females are more active in cold months (minimum temperature between 0°C to -10°C) compared to their male counterparts (Zheng et al. 2011). *H. longicornis*' tolerance for low

temperatures is concerning because it implies that populations could spread to colder regions of the US.

Phenology

Our current understanding of US populations suggests that the phenology of *H. longicornis* reflects both its native and invasive ranges, though data are limited (Tufts et al. 2019). Tick activity is dependent on humidity, temperature, photoperiod, and host availability (Heath 2016). In NY, the greatest number of nymphs has been collected in late spring to early summer, consistent with knowledge of tick phenology observed in Australia, New Zealand, New Caledonia, Fiji, northern Russia, and Japan (Hoogstraal et al. 1968). Similarly, adult numbers peak in early July and high larval hatching rates occur in August, which agree with reports of increased egg production in July (Zheng et al. 2011; Tufts et al. 2019). Seasonal activity of the tick can occur in two waves, with early season ticks developed from overwintering eggs, larvae, and nymphs (Heath 2016).

Control and management

Commercially available insecticides and repellents are the most prevalent forms of tick control measures in the US. Six active ingredients that are commonly used in tick repellent products are successful against *H. longicornis* ticks, in a lab (Foster et al. 2020). However, it is unknown how that translates to a field setting. There is currently no information available regarding the susceptibility or resistance of this invasive tick to the insecticides applied in the US. Although an extensive number of pesticides are registered in New York to treat tick infestations on cattle and sheep (Helms 2018), most of these pesticides fall under a single insecticide class, pyrethroids. Pesticide resistance is said to be relatively rare in multi-host ticks (Heath and Levot 2015), but the dependence on a limited class of insecticides increases concerns

about the development of resistance by this invasive species. In addition, other factors such as the frequency of chemical application, method and thoroughness of the application, proportion of ticks exposed to treatment, and mobility and generation time of the tick can influence the evolution of resistance (Heath and Levot 2015).

To combat the issue of increasing resistance among arthropods, alternative control methods against *H. longicornis* have been tried, such as a plant-derived acaricide (Nong et al. 2013), avermectin injection in hosts (Doan et al. 2013), tick killing food additives, propylene glycol alginate (Wu et al. 2018), and tick-parasitizing entomopathogenic fungi (Lee et al. 2019; Zhendong et al. 2019). Doramectin and abamectin reduced weight of nymph and adult ticks that fed on treated rabbits (>60% within 3-14 days), and ivermectin inhibited molting of nymphs by 55% in that same period. The toxicity of plant (*Eupatorium adenophorum*), against *H. longicornis*, varied by the concentration of the extract and life stage of the ticks. The highest concentration used, 1.5 g/ml (dried plant powder/95% ethanol), caused 100% mortality among larvae and nymphs within two and six hours, respectively. Entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana*, were effective in causing mortality in *H. longicornis* ticks, but the acaricidal activity varied by strain and concentration of the fungi and time the ticks were exposed to treatments. In both studies it took more than 15 days for fungi to cause more than 60% mortality rate in larvae and nymphs.

Cattle dipping in insecticide or overgrazing of pastures may help control the expansion of *H. longicornis* on farms and reduce numbers on livestock, but aspects of its biology and ecology make it extremely difficult to eradicate (Heath et al. 1987; Heath 2016). The major challenges for eradication include its ability to feed on a wide range of hosts (from wild animals to domestic cattle and pets), tolerance to extreme ranges of temperature and humidity (Heath 2016), its

survival for up to a year without feeding (Heath 1994, 2016), a long period of evasion on pasture (~80% of lifespan) (Heath 1994), and its capacity to reproduce parthenogenetically, shortening generation intervals. Any control measures for *H. longicornis* therefore will require a multi-pronged approach which include environmental management, biological control measures, improvement of farming methods in addition to chemical control measures (Heath 1994), and implementation of regulations and actions that limit the movement and distribution of the tick. The trade and transport of cattle needs to be controlled and monitored. Suitable tick habitats in private properties and public common spaces should be minimized and proper preventative measures for reducing propagation of ticks among domesticated animal and humans should be promoted.

CHAPTER 2

Optimal collection methods for the newly introduced, invasive Asian longhorned tick, *Haemaphysalis longicornis* (Ixodida: Ixodidae), in the northeast US

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INTRODUCTION

The Asian longhorned tick (*Haemaphysalis longicornis*), also known as the 'New Zealand cattle tick' or 'bush tick,' is a newly introduced invasive species in the US and a potential disease vector. As of May 2020, *H. longicornis* has expanded into twelve, mostly eastern states since it was first collected from a deer in West Virginia in 2010 (Beard et al. 2018; USDA 2020). A major established population was first described parasitizing sheep in New Jersey in 2017 (Rainey et al. 2018). This tick is native to Asia (China, Japan, Korea, and Russia) and is an established invasive species in Australia, New Zealand, and surrounding islands (Hoogstraal et al. 1968). Most of the research on *H. longicornis* originates from China, Korea, and New Zealand, out of concern for their impact on the spread of infection among humans and the effects on health and productivity of livestock.

H. longicornis transmits pathogens that cause debilitating health problems among cattle, domestic animals, and humans. The infestation of the tick also leads to other consequences, causing economic loss in livestock and dairy production (Heath 2016) and social burden, including physical discomfort (Hoogstraal et al. 1968). *H. longicornis* is a competent vector for bacterial pathogens, *Theileria orientalis* (Heath 2016), *T. sergenti*, *T. mutans* and *Coxiella burnetii* (Hoogstraal et al. 1968), *Babesia* sp. (Chen et al. 2012), and *Rickettsia* sp. (Lee et al. 2013), and viral pathogens, namely, Russian summer-spring encephalitis virus (Hoogstraal et al. 1968), and severe fever with thrombocytopenia syndrome virus (Luo et al. 2015). Although it has not been incriminated as a competent vector for pathogens circulating among native ticks in the US, natural populations of the tick can be infected with several pathogens of major concern, namely, *Ehrlichia* (Kim et al. 2003), *Anaplasma*, *Bartonella* (Kang et al. 2016), *Borrelia* (Sun et al. 2008; Yang et al. 2018) and tick-borne encephalitis virus (Yun et al. 2012). Moreover, a

highly pathogenic strain of *T. orientalis* (Ikeda), which is detrimental to cattle health and dairy production, has been detected among cattle in Virginia, where the vector, *H. longicornis* is established (Hammer et al. 2015; Lawrence et al. 2016; Watts et al. 2016; Oakes et al. 2019). This increases concerns for the veterinary health and potential impact on cattle industry. Despite these potential threats, it is encouraging to know that *H. longicornis* is refractory to the transmission of *B. burgdorferi sensu stricto*, the causative agent of Lyme disease (Breuner et al. 2019). In their study, Breuner et al. (2019) reported the loss of transstadial transmission of *B. burgdorferi* from larvae to nymphal *H. longicornis* and the tick's reluctance to feed on mice, one of the most successful reservoirs of Lyme disease. This could however change as the tick population spreads and establishes in the US.

Aspects of *H. longicornis* biology and ecology allow this tick to multiply at a fast rate and make it an extremely difficult species to eradicate (Heath et al. 1987). It is a three-host tick, which feeds on a wide range of hosts, from domestic to wild mammals, avian hosts, and humans (Hoogstraal et al. 1968; Heath et al. 1987, 1988; USDA 2020). If hosts are unavailable, it can survive for up to a year without feeding (Heath 1994). It can tolerate extremes of temperature and humidity, acclimatize to extreme desiccation by modifying glycerol and protein content (Yu et al. 2014), and continue development at temperatures as low as 12°C (Yano et al. 1987) and as high as ~40°C (Heath 2016). Similarly, this tick spends most of its life (~80%) in pasture, rendering traditional chemical control measures ineffective (Heath 1994). Moreover, *H. longicornis* has three reproductive races: bisexual race (requires males and females to reproduce), parthenogenetic race (females reproduce without male fertilization), and aneuploid race (which reproduces both ways) (Suomalainen 1962; Oliver 1977; Cane 2010). The US populations on record are all females, indicating the presence of parthenogenetic race. This is

worrisome because parthenogenetic race can rapidly increase the number of breeding populations and shorten generation times (Bremner 1958; Hoogstraal et al. 1968; Chen et al. 2012). These characteristics of *H. longicornis* biology are likely to ensure its spread and establishment in other areas of the US, where suitable habitats are available (Raghavan et al. 2019). Therefore, the extent of harm to human health and agriculture caused by this invasive tick depends on its adaptation to the climate, habitat, and hosts in the US, and our ability and willingness to develop appropriate control and monitoring solutions. Currently, the information about the biology, ecology, and distribution of *H. longicornis* populations in the US is minimal.

Studies comparing collection methods for ticks are limited, even more so for *H. longicornis*, and methods vary among published reports describing *H. longicornis* and its surveillance and control (Heath et al. 1987; Chong et al. 2013). To address the need to identify the optimal collection methods that can be standardized for *H. longicornis* in the US, this study provides evidence-based recommendations for enhanced surveillance of *H. longicornis* in the northeast US. We compared the efficacy of three methods (drag sampling, sweeping, and CO_2 traps) and varying check distances (5 m, 10 m, and 20 m). Check distance is a pre-determined distance interval where fabrics are checked for tick specimens. The check distance can impact the number of ticks collected during sampling (Borgmann-Winter and Allen 2019), and there is no data available yet for *H. longicornis*. This work also considers aspects of the biology and ecology of *H. longicornis* which may affect the efficacy of collection methods, with the aim of contributing to the foundation of surveillance and control programs and activities.

MATERIALS AND METHODS

Site description

Haemaphysalis longicornis ticks were sampled from two locations, Armonk (41˚12ˈ74.8N, -73˚73ˈ02W) and Yonkers (40˚96ˈ01N, -73˚89ˈ2W), in Westchester County, in

southern New York State (Figure 1) where the tick population was recently established (Wormser et al. 2019).

In Yonkers, collection sites were located on the southern end of a 42 km public trail. The trail was approximately 5-12 m wide, covered with grass (~10-45 cm) and surrounded by shrubs and vines such as multiflora rose (*Rosa multiflora*) and porcelain-berry (*Ampelopsis* sp.). Predominant trees at the study site included maple (*Acer* sp.), beech (*Fagus* sp.), and oak (*Quercus* sp.). The latter species added leaf litter to the ground serving as potential tick habitat. Vegetation and ground cover were alike on both sides of the trail with minor differences. Most transects were in shade, with some portions exposed to direct sunlight. The trail passed through a residential area and was actively used by people for recreation (biking, running) and walking their dogs.

In Armonk, collection sites were in deciduous forest with predominantly maple (*Acer* sp.), oak (*Quercus* sp.), beech (*Fagus* sp.), and hickory (*Carya* sp.) trees, therefore sampling grounds were covered in leaf litter throughout the collection period.

Both locations provided habitat for wild animals such as white-tailed deer (*Odocoileus virginianus*), eastern chipmunk (*Tamias straiatus*), and gray squirrel (*Sciurus carolinensis*), which serve as potential hosts for *H. longicornis* ticks.

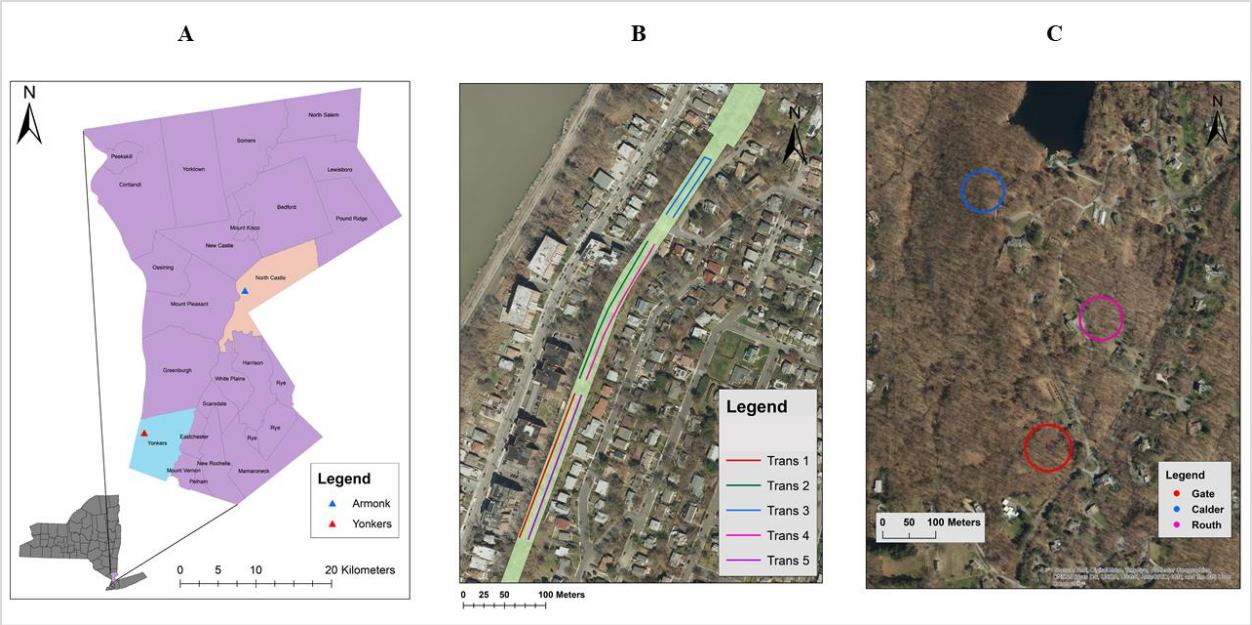


Figure 1: Collection sites in Westchester County- Yonkers (B) and Armonk (C)

Transects

In Yonkers, five 180 m sampling transects were set up, with 2.5 consecutive transects on each side of the trail (Figure 1B). For each 180 m transect, flag markers were set at every 60 m and check distances (5 m, 10 m, and 20 m) were randomized among 60 m sections (Figure 2A). A minimum of 20 m distance was maintained between each 180 m transect.

In Armonk, three 60 m transect were established parallel to one another due to the inability to make straight 180 m transects because of property boundaries and varying landscape (Figure 2B). A buffer distance of 5 m was maintained between each transect to avoid overlap during sampling. Transects were labeled with unique names (a, b, c) to aid in randomization of check distances, which were rotated among transects. Flag markers were placed at every 30 m to guide a collector.

These transects were used for drag sampling and sweeping. CO_2 traps were deployed at different sites on separate days.

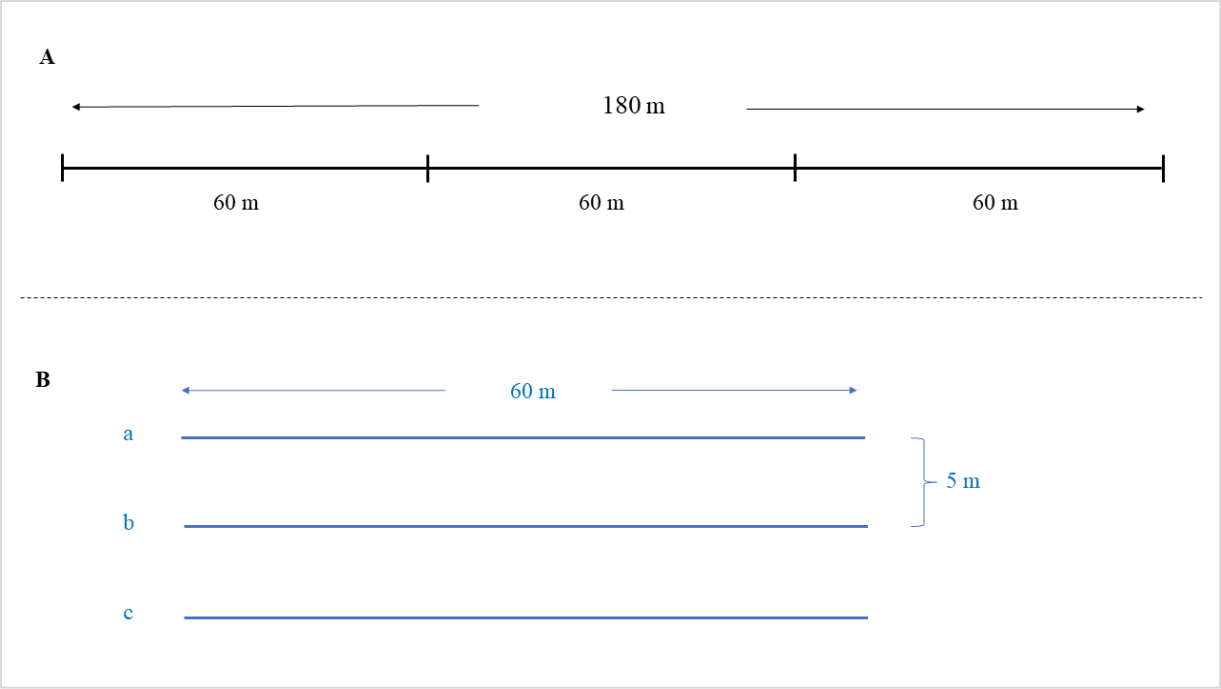


Figure 2: (A) Transect setup in Yonkers, NY. Check distances were rotated among 60 m sections of each transect. (B) Transect setup in Armonk, NY. Check distances were rotated among transects (a, b, and c) every week

Data collection

Field collection was conducted from June 12, 2019 through August 12, 2019 during daylight between 09:00 to 19:00, on dry days. Collection methods were cycled throughout the day to reduce temporal variation in tick activity. Collection was avoided on rainy or windy days. Temperature and humidity were recorded during each sampling event and ranged from 21.2-34.8°C and 30-83% RH.

Drag sampling

Drag sampling was performed using a 1 m^2 double-sided corduroy fabric with a wooden dowel sewn on one edge and a 4 m long nylon rope attached to the dowel (Bouseman 1990; Daniels et al. 1997). Two screw eyes were fitted at each end of the wooden dowel where the rope was attached, to make fabric easily detachable for cleaning.

During sampling, the fabric was pulled behind a collector, at a slow walking pace, making sure the drag cloth was parallel and in contact with the ground and vegetation (Figure 3A). For each 60 m transect, the collector stopped at randomly assigned check distance (5 m, 10 m, or 20 m) to meticulously scan the fabric from top to the bottom for ticks (Goddard and Piesman 2008). Ticks were collected using fine tipped forceps and stored in glass vials, labeled with collection date, location, collection method, and check distance. The numbers and life stage of ticks were recorded. When all the ticks on one side of the cloth were collected, the cloth was gently flipped to the other side and the aforementioned processes were repeated. Collection vials were stored in a sealed plastic bag to provide secondary containment during transportation to the laboratory, where they were stored at -20°C until identification.

Sweeping

Sweeping is similar to flagging, another method employed in tick collection, with differences in fabric size, collection process, and construction- bent PVC pipe used for sweeping instead of straight wooden dowel (Ginsberg and Ewing 1989). Sweep construction for this project was modified from that described by Carroll and Schmidtmann (1992). A 0.5 m^2 double-sided corduroy fabric was attached to a PVC pipe handle. The cloth was swept at a walking pace, to the side of a collector, making sure to keep the flag in contact with the ground (Figure 3B). Randomization of check distances, sample collection, storage methods, and data recording were performed as described previously for drag sampling.

Figure 3: (A) Drag sampling: a 1 m^2 corduroy cloth was dragged behind a collector ; (B) Sweeping: a 0.5 m^2 corduroy cloth attached to a PVC pipe handle was dragged on the side of a collector; (C) CO_2 baited trap setup with dry-ice reservoirs placed at least 10 m apart

CO_2 trapping

Several designs exist for using CO_2 baited traps in tick collection. Previously described designs can be expensive or require specialized construction, such as using large cylinders (Garcia 1962), fitting pipe dispensers to containers (Miles 1968) and constructing fiberglass lined or plywood boxes (Wilson et al. 1972; Falco and Fish 1991). To reduce the cost and labor associated with trap construction, we used styrofoam boxes (outer dimension: 28 cm long x 23 cm wide x 16 cm tall with an inner dimension: 20.5 x 15.5 x 12 cm) for CO_2 reservoirs (Figure 3C). Two small holes were drilled on each side of the box, allowing continued diffusion of CO_2 from pelleted dry ice to attract ticks (Wilson et al. 1972; Falco and Fish, 1992). Traps were deployed without lids to prevent clogging of holes from condensation and to improve dispersal of CO_2.

Two to three traps were set out at once; boxes were placed on a 1 m^2 white corduroy cloth at a 10 m distance from one another. Observations were conducted on four different dates in July and August. From initial observations, *H. longicornis* were attracted to the traps, but

crawled away within 30 min. Therefore, reservoirs were checked at five to ten-minute intervals. Tick scanning was performed as described for drag sampling and sweeping, with an exception that the underside of the fabric was scanned by gently lifting each corner, with the CO_2 boxes in place. The number of ticks and their life stages were recorded, ticks were marked on their scutum using fine point DecoColor paint markers (Marvy Uchida, California, USA) unique to each tick, and returned to their spot on the fabric. After one hour, ticks were collected, processed, and stored as previously described.

Tick identification and measurement

Ticks were sorted by life stages, sex, and identified to species level using published identification guides (Durden and Keirans 1996; Egizi et al. 2019). To compare *H. longicornis* body sizes to those recorded from its native and other invasive ranges, a subset of ticks was measured using cellSens imaging software (Olympus Corporation, Pennsylvania, USA). Body length was measured from the tip of a hypostome to the posterior margin of festoons and width was measured between third and fourth pairs of legs, or, in the case of larvae, the widest part of the entire body. Following identification and measurements, specimens were stored in 70% ethanol at room temperature.

Data analysis

Statistical testing for differences among collection methods and check distances was performed using a negative binomial generalized linear mixed effects model in R using the following packages: lme4 (Bates et al. 2015), ggplot2 (Wickham 2016), tidyr (Wickham and Henry 2019), dplyr (Wickam et al. 2019), and ggthemes (Arnold 2019). Check distances and collection methods were used as fixed variables and transect was a random effect. Statistical significance was set at $\alpha = 0.05$. Statistical analysis was only possible for Yonkers due to the low

number of *H. longicornis* collected in Armonk (n = 13). Larvae were excluded from all the analyses because of their limited mobility and difference in questing behavior. Two extreme outliers were removed from the nymph data. Extreme outliers were determined as numbers that were greater than two times the 'outer fence' value, which is calculated as $\{Q_3 + (3 * IQR)\}$ (Norman and Streiner 2014). Data from CO_2 traps were not included in the analysis due to low number of ticks retained.

RESULTS

Demographics and phenology

A total of 3,798 *H. longicornis* were collected (adult = 159, nymph = 387, larva = 3239). In addition to *H. longicornis*, we collected three species of native ticks, namely, *I. scapularis*, *I. dentatus*, and *D. variabilis*. The highest number of *H. longicornis* was collected in Yonkers, whereas we collected predominantly *I. scapularis* in Armonk (Table 1).

Table 1: Species, life stages, and number of ticks collected during June-August 2019

Site	Tick species	Adult	Nymph	Total
Armonk	*Ixodes scapularis*	2	153	155
	Haemaphysalis longicornis	1	12	13
	Dermacentor variabilis	1	0	1
Yonkers	*Haemaphysalis longicornis*	158	375	533
	Ixodes scapularis	0	11	11
	Ixodes dentatus	0	1	1
	Dermacentor variabilis	1	0	1
Species Total		163	552	715

Larva, nymph, and adult populations peaked at different points during the collection period (June 12- August 12, 2019). Nymphal life stage was the first to emerge, which peaked in June then molted into adults that were in greatest numbers in July, and larvae appeared in August (Figure 4). All three life stages co-occurred at several points during the summer, although larval populations were virtually non-existent between late-June and mid-July. Nymph populations steadily declined over the summer.

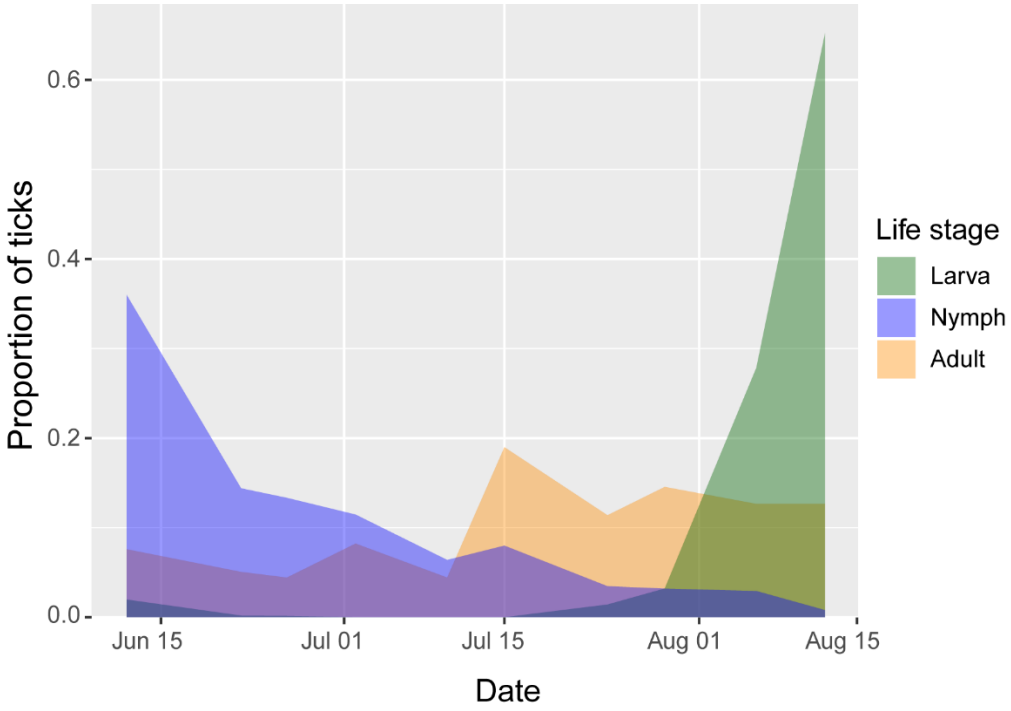


Figure 4: Phenology of *H. longicornis* collected in Yonkers, NY, 2019

Comparison of collection methods

Drag sampling method was significantly better at collecting adult *H. longicornis* than sweeping (z value = -2.744, p = 0.006) (Figure 5). Drag sampling yielded an average of 2.2 times more adult *H. longicornis* than sweeping. The difference between the methods was not statistically significant for nymphs, though a greater number of nymphs (1.6 times more) were recovered by drag sampling, consistent with the data for adults. CO_2 trap method proved ineffective without efficient tick containment system around the CO_2 reservoirs. Additionally, the traps did not recover any larvae, indicating a bias towards life stages with greater mobility favoring adults, followed by nymphs.

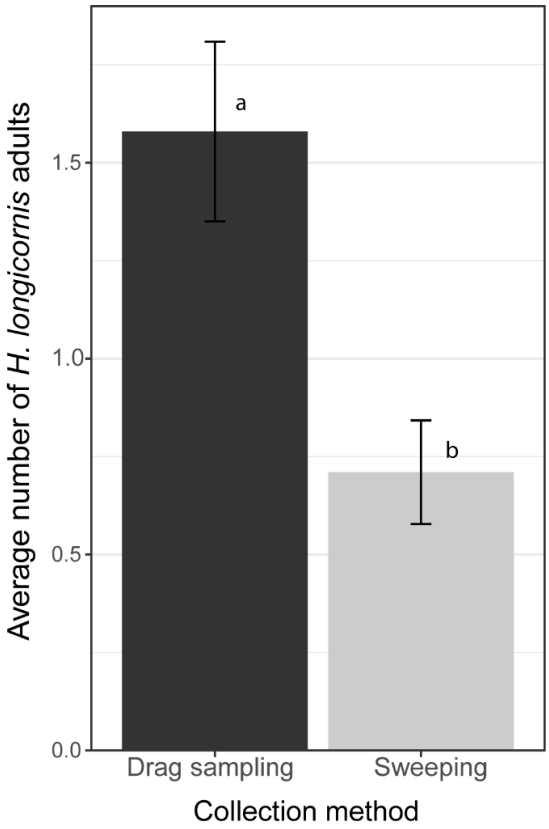


Figure 5: Average number of adult *H. longicornis* collected by drag sampling and sweeping

Comparison of check distances

Overall, the 5 m check distance yielded higher numbers of adult *H. longicornis* compared to longer intervals. The five-meter check distance recovered 1.8-fold more adult ticks than 10 m and 2.5 times more than 20 m. When compared per collection method, the differences were statistically significant between 5 m and 10 m ($z = 2.811$, $p = 0.01$) for drag sampling, and between 5 m and 20 m ($z = 3.069$, $p = 0.006$) and 10 m and 20 m ($z = 2.811$, $p = 0.01$) for sweeping (Figure 6). The five-meter check distance also recovered a greater number of nymphs on average than longer intervals: 1.2 times more than 10 m and 1.6 times more than 20 m. However, when compared per collection method, the differences did not have strong statistical significance (Figure 7).

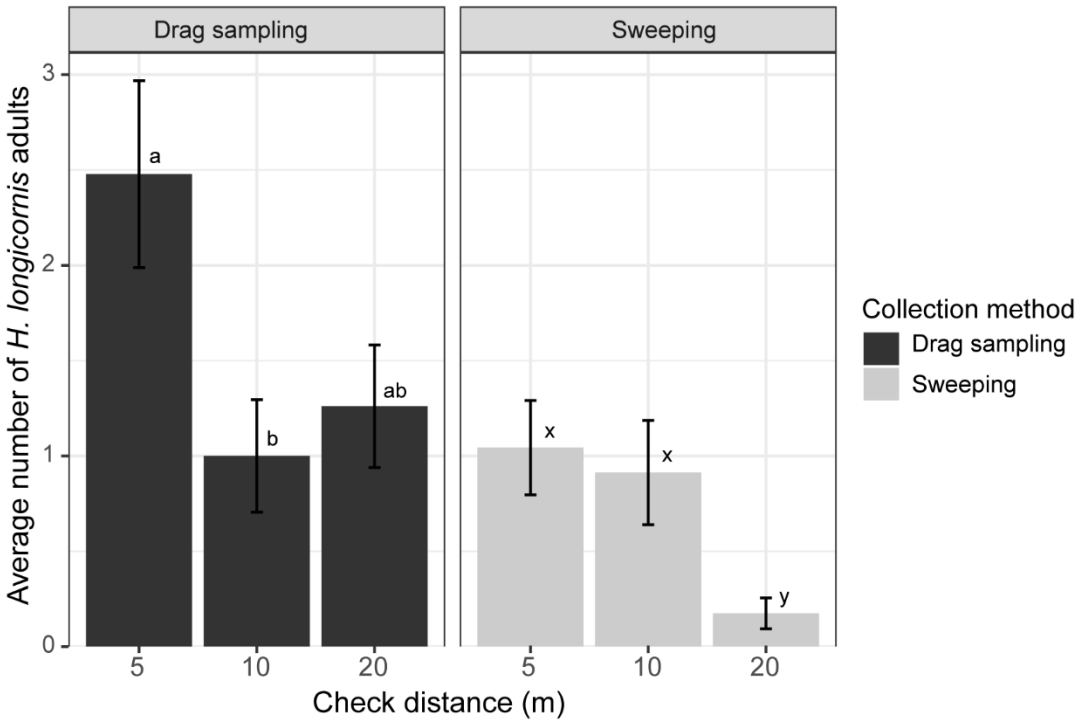


Figure 6: Average number of adult *H. longicornis* collected per check distance per collection method

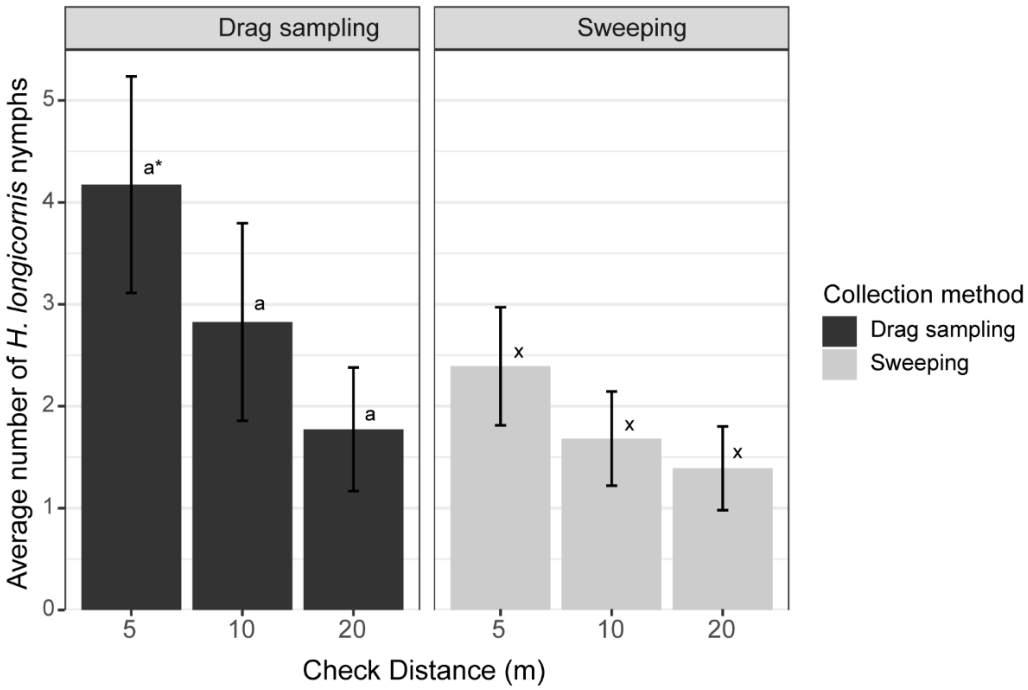


Figure 7: Average number of *H. longicornis* nymphs collected per check distance. *significance between 5 m and 20 m was trending (p = 0.06)

Comparison of body sizes

We also compared the morphology of *H. longicornis* specimens sampled in Yonkers, NY, to parthenogenetic and bisexual races from its native range, China (Table 2). Populations of larvae and adults from New York had a similar body size to parthenogenetic *H. longicornis* from its native range. Nymphs were smaller than parthenogenetic populations and more comparable to bisexual populations.

Table 2: Comparison of body sizes of populations of *H. longicornis* in Yonkers, NY to populations of parthenogenetic (Sichuan, China) and bisexual races (Hebei, China) reported by Chen et al. 2012

	Yonkers, NY (mm)		**Parthenogenetic** (mm)		**Bisexual** (mm)	
	Length Mean ± SEM (range)	Width Mean ± SEM (range)	Length	Width	Length	Width
Larva	**0.67 ± 0.00** (0.62-0.71)	**0.48 ± 0.00** (0.43-0.52)	0.67 ± 0.00 (0.65-0.70)	0.50 ± 0.01 (0.45-0.52)	0.63 ± 0.01 (0.60-0.65)	0.45 ± 0.01 (0.41-0.46)
Nymph	**1.53 ± 0.01** (1.32- 1.68)	**0.96 ± 0.01** (0.81-1.14)	1.68 ± 0.03 (1.61-1.79)	1.03 ± 0.01 (0.97-1.08)	1.55 ± 0.04 (1.36-1.74)	0.92 ± 0.02 (0.84-1.03)
Adult (female)	**2.95 ± 0.01** (2.34-3.62)	**1.80 ± 0.01** (1.40-2.10)	3.11 ± 0.08 (2.39-3.99)	1.71 ± 0.04 (1.36-2.04)	2.68 ± 0.09 (2.16-3.17)	1.47 ± 0.03 (1.16-2.58)

Sample sizes for Yonkers, NY: Larvae = 57; Nymphs =105; Adults (females) = 254.

DISCUSSION

Effective collection methods are essential to determine tick density and seasonal activity, to evaluate the risk of human exposure, and to obtain representative samples for monitoring the spread of pathogens. Standardized methods are also important for easier comparison between studies. The effectiveness of collection method varies by tick species, life stages, and landscape features (Ginsberg and Ewing 1989; Carroll and Schmidtmann 1992; Falco and Fish 1992; Chong et al. 2013). Studies comparing collection techniques for *H. longicornis* in the US is lacking. To fill the knowledge gap, our study compared the effectiveness of collection methods commonly used for native ticks in the northeast US to collect the newly introduced invasive tick, *H. longicornis*. We assessed efficacies of three methods (drag sampling, sweeping, and CO_2

traps) and three check distances (5 m, 10 m, and 20 m) for the collection of adult and nymph *H. longicornis*.

Drag sampling recovered higher number of ticks of both life stages. On average drag sampling collected 2.2 times more adults and 1.6 times more nymphs than sweeping. The reasons drag sampling outperformed sweeping and CO_2 trap methods are likely connected to features of the methods, and the habitat types sampled in our study. The size of the collection fabric for drag sampling was double the size that of sweeping. The per m^2 density of ticks were not compared for the above collection methods to avoid the need for extrapolation and to preserve the practicality of recommending either sampling method to practitioners. Each method also differs in the nature of disturbance created during sampling. Unlike sweeping, where collection fabric is swept adjacent to the sampler, a sampler walks directly in front of the fabric during drag sampling. This disturbance may be sensed by the tick, stimulating them to attach to the approaching fabric, or, on the contrary, causing ticks to drop off. Further research is required to warrant this idea. We observed distinct differences in the abundance of *H. longicornis* at our two field sites, Armonk and Yonkers, which we attribute to the ecology, origin, and dispersal of initial tick populations in these two sites. Drag sampling proved most effective in Yonkers, which is a grassy, open space public trail, however, the effectiveness of each method depends on habitat types (Carroll and Schmidtmann 1992; Schulze et al. 1997). There are use-cases for which sweeping method and CO_2 traps may be better suited, such as in habitats with dense vegetation. Moreover, host availability may also influence the effectiveness of collection methods, though research on this topic is scarce. During field collection of *H. longicornis* at a dairy farm, the author observed that drag sampling and CO_2 trap methods in paddocks yielded

surprisingly few tick specimens compared to the size of populations attached to the cows (Appendix II).

CO_2 baited traps have been reported effective in collecting other native ticks of the US, *Amblyomma americanum* Linnaeus, 1758 (Kinzer et al. 1990; Schulze et al. 1997), *I. dammini* (=*I. scapularis*) (Falco and Fish1992), *Amblyomma cajennense* Fabricius, 1787 (Guedes et al. 2012), and *Ornithodoros parkeri* Cooley, 1936 (Miles 1968). In this study, CO_2 traps were generally ineffective. *H. longicornis* was attracted to CO_2 traps in the field, but it lost interest quickly and exited the trap. We observed that most *H. longicornis* attracted to a trap exited the fabric within 10 min. However, some were retained near the reservoir for > 30 min, probably anaesthetized by their closer proximity to CO_2 (Norval et al. 1989). We conclude the inefficacy of CO_2 traps likely reflects the *H. longicornis* biology, rather than the tick density at our site, as reported for *I. ricinus* Linnaeus, 1758, where CO_2 traps outperformed drag sampling method at high density sites, and were comparable at low tick density (Gray 1985). A few different methods were evaluated for improving the retention of *H. longicornis* around CO_2 reservoirs, including water and oil moats, tanglefoot brush-on glue, and various insecticide-treated cloths (Appendix I). If ticks fell for the retention traps, oil moats and brush-on glue were most effective. However, ticks appeared to sense the traps and mostly evaded any contact. CO_2 traps required significantly less collection time, so, with an effective way to retain specimens around the CO_2 reservoirs, this method may become an efficient *H. longicornis* monitoring tool.

Tick drop-off and behavior of *H. longicornis* may explain the superiority of 5 m to 10 m or 20 m, given the correlation between check distance and the tick drop-off rate described for *I. scapularis* (Borgmann-Winter and Allen 2019). Five-meter check distance, which experienced the least amount of disturbance, recovered more ticks on average than longer check distances.

Anecdotally, we observed that *H. longicornis* adults are more sensitive to disturbance than native tick, *I. scapularis*, dropping off at any external disturbance. It indicates a possibility of higher drop-off rate for *H. longicornis*, which should be confirmed in future studies.

Based on these findings, tick surveillance and control programs that have resources (budget, time, and staff) available are encouraged to employ 5 m check distance for collecting *H. longicornis*. However, if the resources are not available, implement the next shorter check distance. In deciding which method to implement, programs need to consider the resources at their disposal, landscape/habitat type of collection sites, and compare pros and cons of the methods. The advantages of using drag sampling is that the larger fabric (i) is more readily kept in constant contact with the ground and (ii) spans a larger surface area than sweeps. The disadvantage of drag sampling is that it is difficult to implement this method in dense brush and vegetation. In contrast, the benefits of sweeping are (i) it can be used in narrow spaces and thick vegetation, (ii) scanning time is shortened due to smaller fabric size, and (iii) side sweeping allows for the sampler to avoid brambles and exposure to ticks. However, because the fabric is small and light, even the smallest twig can flip the edges, which requires constant fixing/flattening of the fabric. This could be fixed by adding weight to the sweep fabrics. Additional research is warranted to assess trade-offs between resources (time) and the effectiveness of collecting representative samples of *H. longicornis* from various commonly surveilled habitats.

Altogether, our study provides findings crucial for effective collection of *H. longicornis* and describes the ecology and biology of the populations in the northeast US, which influence the tick's surveillance, survival, and distribution.

APPENDIX I: *Haemaphysalis longicornis* videos and micrographs

In addition to collecting *H. longicornis* ticks for the comparison of collection methods and check distances, the ticks' field behavior was recorded, and stacked images were taken of all life stages and important body parts. The videos and micrographs can be accessed at Cornell University's repository, eCommons, by following the link https://ecommons.cornell.edu/handle/1813/66885/recent-submissions. Below are short descriptions of each video (1-7) and picture (8-13) that are listed on eCommons page.

1. **Interaction between Asian longhorned tick (*Haemaphysalis longicornis*) and a human host**

   During field collections, *H. longicornis* ticks did not readily latch onto a human host, unlike *I. scapularis*. Instead, they avoided and crawled around the author's fingers when placed in their path. So, to further test the behavior, the author gathered and held *H. longicornis* adults in her palm for a few minutes, thinking maybe the ticks would latch on and feed if they were forced. However, as seen in the video, once the palm was opened, the ticks crawled away as fast as they could and found the closest edge to jump off. Such extreme dislike for human host is peculiar and interesting.

2. **Use of permethrin to trap Asian longhorned tick (*Haemaphysalis longicornis*)**

   On the first day of CO_2 trap deployment, *H. longicornis* were observed to visit the dry-ice reservoir, but quickly lost interest and crawled away (within 30 min). So, a few different solutions to retain the attracted ticks around the traps were evaluated. A small piece of corduroy cloth was treated with permethrin and placed on top of an untreated 1 m^2 corduroy cloth. Dry ice reservoir (styrofoam boxes) were then placed on the treated cloth.

Visible in the video, few ticks that walked on the treated cloth appeared disorientated, but did not die on the spot, so they were able to crawl away.

3. **Use of tanglefoot, brush on sticky coating, to trap Asian longhorned tick (*Haemaphysalis longicornis*)**

   This video shows the effectiveness of tanglefoot, brush-on glue, for retaining ticks around CO_2 reservoir. The observation was conducted in a lab space. *H. longicornis* evaded stepping on the surface with glue; they walked around it to reach the dry-ice reservoir on the other side. When they were intentionally placed on the glue surface, the ticks struggled and stayed stuck, but some of them, within 20 min, were able to detach their legs from the glue and crawl away.

4. **Can a vegetable oil moat trap Asian longhorned tick (*Haemaphysalis longicornis*)?**

   Of all the solutions tried, an oil moat appeared to be the most promising at containing *H. longicornis* around dry-ice reservoirs. Once in the oil, ticks lost traction with their legs and were stuck, floating in the oil. However, that was only successful if the ticks crawled into the moat. Frequently, the ticks crawled around the moat or crawled on a rim of the moat to reach the dry-ice reservoir that was placed on the other side.

5. **Does the Asian longhorned tick (*Haemaphysalis longicornis*) drown in water?**

   H. longicornis might drown in water if they are held in a closed container for a long time. But, for the purpose of this project, using a water moat (where container would have open top) was ineffective. Adult ticks were able to successfully and without any inconvenience crawl at the bottom of a water moat and crawl out.

6. **Questing Asian long-horned tick (*Haemaphysalis longicornis*), adult**

   As seen in the video, which was recorded at a normal speed, *H. longicornis* is very fast compared to native US ticks that were collected from the sites, namely, *I. scapularis* and *D. variabilis.*

7. **Reaction of deer tick (*Ixodes scapularis*) and Asian longhorned tick (*Haemaphysalis longicornis*) to a human presence**

   This video compares the reactions of *I. scapularis* and *H. longicornis* when they sense a human presence. *I. scapularis* accelerated towards the human host, whereas, *H. longicornis* avoided any contact with a human, even when prodded.

8. **Haller's organ of an Asian longhorned tick (*Haemaphysalis longicornis*)**

   The Haller's organ is a concave structure found on the front legs of ticks. It is used to detect environmental factors such as heat, CO_2, and humidity. Therefore, the ticks wave their front legs and use their Haller's organ to locate a host, for blood meal.

9. **Asian longhorned tick (*Haemaphysalis longicornis*) nymph**

The picture below is a ventral view of a *H. longicornis* nymph with the tick's defining features such as laterally extended palps.

10. **Asian longhorned tick (*Haemaphysalis longicornis*) larva**

Following is a full body picture of a *H. longicornis* larva with three pairs of legs and short palps with laterally extended second segment.

11. Anal groove of an Asian longhorned tick (*Haemaphysalis longicornis*)

Anal groove is used to separate *Ixodes* from other metastriate tick genera. For instance, the shape of the anal groove of *H. longicornis* is distinct from that of *I. scapularis* (seen in the pictures below).

H. longicornis

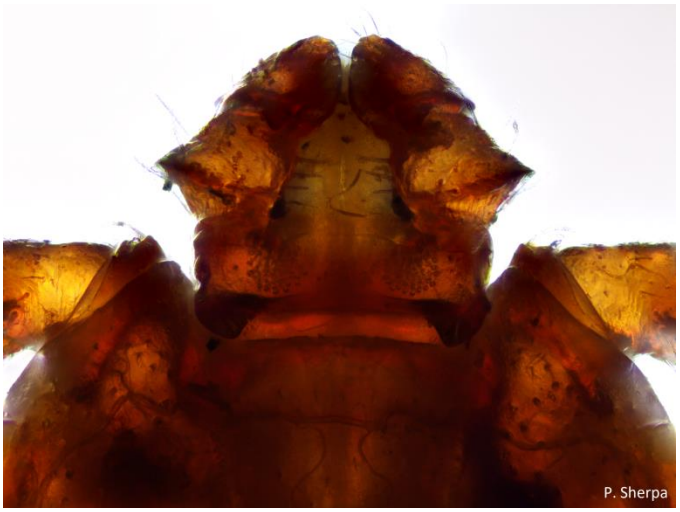
I. scapularis

12. Ventral view of an adult, female Asian longhorned tick (*Haemaphysalis longicornis*)

The following picture shows the defining features of adult *H. longicornis* tick's anterior body, namely, hypostome, laterally extended second segment of the palps, and spurs on coxae.

13. Asian longhorned tick (*Haemaphysalis longicornis*) adult, female

The following picture shows the anterior, dorsal view of an adult female *H. longicornis*.



APPENDIX II: Study of *Haemaphysalis longicornis* in Port Waikato, North Island, New Zealand

INTRODUCTION

From mid-December 2019 to mid-January 2020, the New Zealand cattle tick, *Haemaphysalis longicornis*, was collected from pastures and dairy cows at an 8000-acre farm located in Port-Waikato, North Island, New Zealand (37°29ˈ46.8″S, 174˚45ˈ43.3″E) (Figure a1). *H. longicornis* transmits a protozoan parasite, *Theileria orientalis* (Ikeda) that causes *Theileria*-associated bovine anemia (TABA) among New Zealand cattle (Lawrence et al. 2016). In addition, *T. orientalis* infection causes other clinical signs such as lethargy and anorexia (McFadden et al. 2011). In some cases, it causes abortion, stillbirth, and death leading to loss of cattle and damage to dairy production (Vink et al. 2016). The farm had itself lost about fifty dairy cows to *T. orientalis* infection in the last five years (farm manager, pers. comm., 4 January 2020).

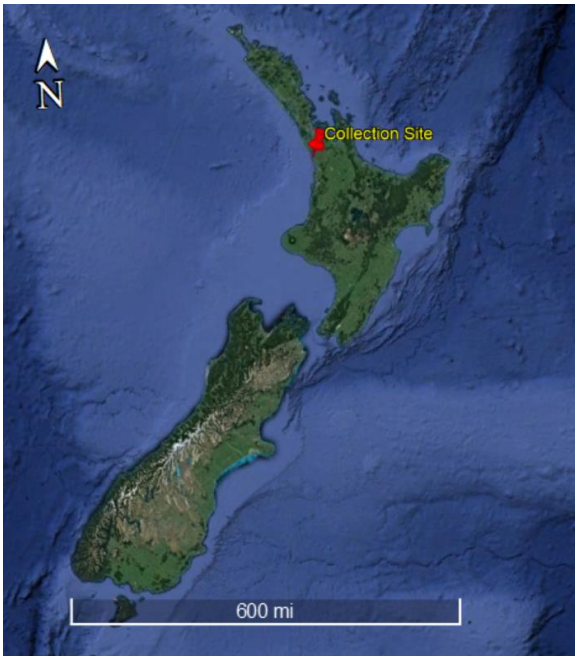


Figure a1: Location of the field collection site

Existence and proliferation of the tick population on the farm plays an important role in the distribution of TABA among naïve herds of cattle (Lawrence et al. 2016; Heath 2016). *H. longicornis* was first recorded in New Zealand in 1911 and quickly established a viable population (Heath 2016). From 1929, the distribution of *H. longicornis* extended to areas in the South Island, moving from the more widely infested North Island (Health 2016). Determination of a simple and effective method for collecting ticks in the field would be immensely useful for informing control measures for the vector and the pathogen at the farm and regional level. Historically, most *H. longicornis* surveillance in New Zealand has been *ad hoc* and based on collection of ticks directly from animal hosts, so the usefulness of other, environmental surveillance methods in collecting the tick is unclear and untested. The main goal of this short-term project was to compare the effectiveness of drag sampling and CO_2 trap methods as alternative tools for *H. longicornis* collection, in its established invasive range. The work was conducted in collaboration with Kevin Lawrence, a senior lecturer in cattle medicine at Massey University, and the farm.

METHODS

During the collection period, the farm grazed approximately 15,000 sheep; 1,300 beef cows; and 700 dairy cows. Sheep and beef cows grazed in the same paddocks, whereas, dairy cows grazed in paddocks at a different part of the property.

Drag sampling: A 1 m^2 double-sided corduroy cloth was used for drags. The procedure for sample collection was the same as the collections conducted in Westchester county, NY. Various locations on the farm were sampled including paddocks where dairy cows, dairy calves, sheep, or beef cows were actively grazing, and paddocks where the cattle had not been grazed for at least a month (Figure a2). Each time, a 100 m distance was sampled, and the fabric was

checked for ticks every 10 m. Collections were conducted between December 20, 2019 and January 16, 2020. Field collections were performed on dry days between the hours of 9:00 and 16:00. Wind speed picked up during the afternoons rendering field sampling difficult. Ticks were collected in glass vials filled with 70% ethanol, using fine-tipped forceps.

Figure a2: Drag sampling on a roadside grassy patch, adjacent to dairy-calf paddocks

CO_2 traps: Over the course of two weeks of warm and dry weather, CO_2 traps were deployed at thirteen sites, spread across different parts of the farm. Two traps were set per site and placed at least 20 m apart. As described earlier, styrofoam boxes, with holes drilled on each side, were filled with pelleted dry ice and placed on a white fabric, in the field. The lids were taken off for a better airflow and dispersion of CO_2. One of the fabrics was previously used during tick collection from dairy cows, so it had dung spatter and barn odors, whereas, the other was not scented. In the field, the fabrics were held down using hot water filled bottles, which added heat around the dry ice reservoirs and kept fabrics flat on the ground (Figure a3). Traps were checked every 20-30 min and ticks were collected and stored in glass vials with 70%

ethanol. During that time interval, surrounding areas were sampled using drags; at least 50 m distance was maintained between CO_2 traps and the drag sampling areas.

Figure a3: CO_2 trap deployed at a paddock that had been recently grazed by cows

Collection from cows: The dairy cows were divided into two herds of approximately 350 each, with one herd being mostly younger cows and the other with mixed age group. The herds were rotationally grazed in separate paddocks and were milked twice a day. In the morning, the cows were brought to the shed, from their night paddock, and milked between 4:30 to 7:00 hrs. Then they were herded to their pre-planned day paddock. Similarly, after the afternoon milking that occurred between the hours of 15:00 and 17:00 hrs., the cows were either returned to their paddock from the previous night or to a new night paddock. Cows usually stayed in the same paddock for a couple of nights, after which, they were rotated to a new, un-grazed paddock.

Engorged *H. longicornis* were collected from the dairy cows during the milking times. The barn had a metal platform, behind the milking carousel, which provided a safe distance and space for collecting ticks from the udder, escutcheon, and tail head of individual cows (Figure a4). In addition, the cows were briefly scanned, as they left the milking shed, to check if ticks could be observed on other parts of the cows' bodies. Engorged ticks were collected, the total

numbers from individual cows were counted, recorded, and the specimens were stored in plastic vials and jars. Attempts were made to collect unfed ticks but collecting recently attached ticks from cows on a moving carousel was impossible without hurting the cows. So, the presence of unfed ticks was recorded without removing them.

Figure a4: Collecting engorged ticks from dairy cows

Data analysis: Relative abundance (total number of ticks/total number of hosts (infested + uninfested)) and mean intensity (total number of ticks on a sample of hosts/total number of infested hosts) were calculated for the engorged *H. longicornis* collected from the cows as in Heath et al. (1987). Average number of engorged ticks collected per sampling time and day was analyzed using R. Statistical significance of the differences were not calculated because of the small sample size for each category.

RESULTS

Drag sampling: Two adults, two nymphs, and 251 larvae were collected by sampling more than 2000 m from different parts of the farm. All larvae were collected during the second week of January.

CO_2 traps: A total of 14 adults and two nymphs were collected using CO_2 traps. Collection of mostly adults in December and January reflects the phenology trend reported for *H. longicornis* from New Zealand (Heath et al. 1987, 2016). Of the total ticks,13 adults and one

nymph were collected from the trap with the cow-scented fabric. This observation suggests the possibility of combining host scent and CO_2 to increase the probability of collecting *H. longicornis*. Similarly, most ticks (10 adults and 2 nymphs) were collected from a small patch grassy area which had never been grazed (Figure a2). Only 4 adults were collected from the paddocks with the presence of calves and cows. This indicates that CO_2 traps might be less effective in attracting ticks when there are live hosts readily available in the same paddock. In the paddocks with hosts, it is possible that most ticks are already on hosts, leaving few ticks to be collected by other methods. More research is warranted to confirm these observations.

Cows: Ticks were mostly collected from udders, escutcheon and sometimes from the vulva and the tail head (Figure a5). The site of attachment and feeding could be explained by the ticks' choice based on factors such as vascularization and temperature (Heath et al. 1987).

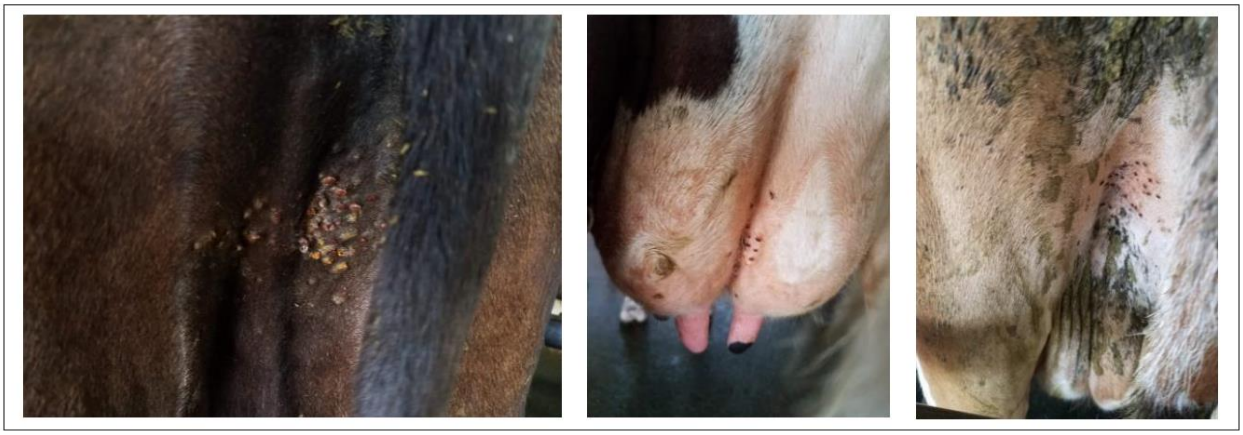


Figure a5: Tick attachment sites on dairy cows

A total of 888 engorged *H. longicornis* were collected from 3,582 individual cows. The cow with the highest number of engorged ticks had 20, attached on the udder and the escutcheon. Mean intensity (MI) and relative abundance (RA) per herd and time (morning versus afternoon) are presented (Table a1). RA of engorged ticks was higher in the morning for both herds and herd 1 had more tick per infested cow (MI) in the morning.

Table a1: Relative abundance and mean intensity of engorged ticks on the dairy cows. Herd 1 had the younger cows, whereas herd 2 had a mixed age group

	Morning		Afternoon	
	Herd 1	Herd 2	Herd 1	Herd 2
RA	0.38	0.07	0.002	0.009
MI	2.58	1.66	1.25	1.4

The above trend for MI and RA remained the same even after removing data for December 24th and 25th, 2019, which yielded a very high number of engorged ticks (Figure a6).

Even when engorged ticks were not present, approximately 42-50% of cows in herd one and 63-69% in herd two were infested with un-engorged ticks on any given sampling day and time. Among that infested population, 12-37% of the cows had clusters of more than six ticks at a time. Consistently greater infestation in herd 1 might suggest a high mobility among the cows in the group; herd 1 consisted of younger cows, whereas herd 2 had mixed age group (farm manager, pers. comm., 1 January 2020). The impact from difference in pastures might be minimal because both herds were grazed in paddocks that were in proximity, with similar landscape and vegetation.

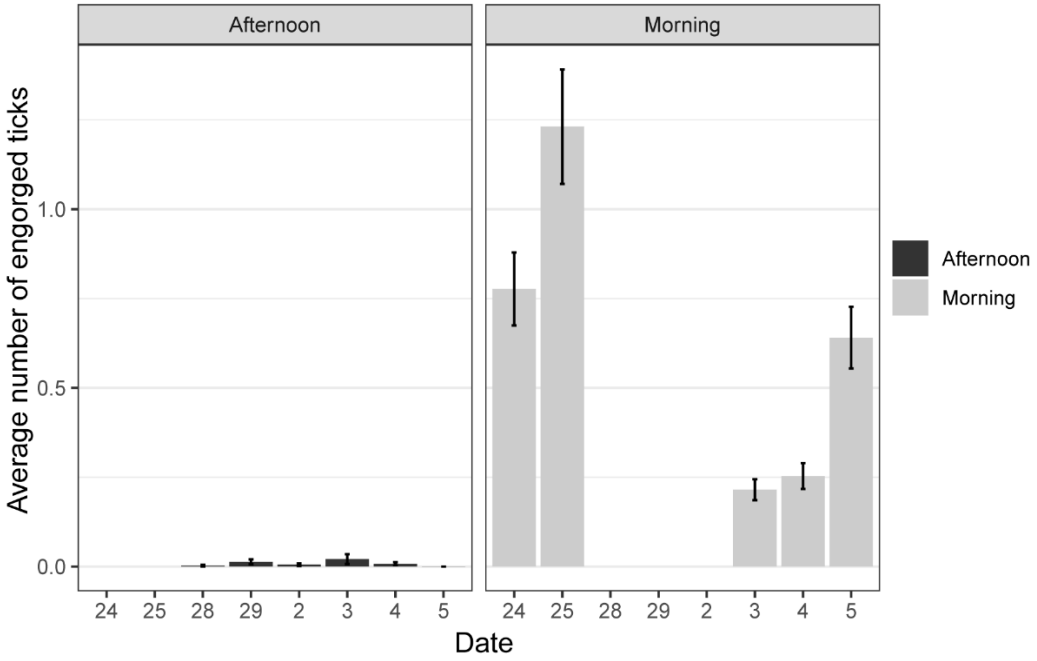


Figure a6: Average number of engorged ticks per collection date (late December 2019 to early January 2020)

CONCLUSIONS

Analysis of the data from this project indicates that collection from cows was the most effective method followed by CO_2 traps and drag sampling for collecting *H. longicornis* in North Island, New Zealand. When high numbers of ticks were observed and collected from dairy cows, follow-up field collections, using drag sampling and CO_2 trapping methods, were conducted in the same paddocks that the cows had recently grazed. Surprisingly, these two methods barely collected any ticks from the paddocks. This suggests that questing ticks respond to a wide range of stimuli, which may include host movement, scent, carbon dioxide, and temperature, making CO_2 traps less successful in the presence of vertebrate hosts. Or, simply, that most ticks attach to hosts, resulting in low numbers left in the environment. CO_2 traps can however be an effective collection method in areas where live hosts are scarce.

Cows were infested with engorged ticks in the mornings, but those ticks were gone by the afternoon. This suggests that engorged ticks were dropping off, in paddocks, between the morning and the afternoon milking times. A similar trend was observed with *Rhipicephalus microplus* (formerly *Boophilus microplus*) ticks, where only few fully engorged ticks were found on cows during the daytime (Wharton and Utech 1970). The authors reported that the ticks engorged at a slow pace until they reached a certain size, then the engorgement progressed rapidly. On cows that spent nights in yards (like the farm cows that grazed in pastures), the final engorgement of *R. microplus* ticks started after dark and peak number of engorged ticks were observed at dawn (Wharton and Utech 1970).

Cows were followed to their paddocks after morning milking, to check if ticks were dropping off on the way to the pasture, but very few engorged ticks were found on dirt path. External environmental factors such as photoperiod, temperature, and host activity might provide

cues for engorged ticks to drop off in grassy paddocks, which are suitable habitats for egg laying (Oliver 1989; Heath 2016).

The farm practice of moving cows between day and night paddocks might be aiding in the dissemination and maintenance of the tick population around the farm. Therefore, it is necessary to consider the impacts of such practices when thinking about control or eradication measures for the ticks on the farm.

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