Aversion of the invasive Asian longhorned tick to the white-footed mouse, the dominant reservoir of tick-borne pathogens in the U.S.A.: Asian longhorned tick aversion

Article *in* Medical and Veterinary Entomology · April 2020

DOI: 10.1111/mve.12441

CITATIONS
0

READS
82

3 authors:

Isobel Ronai
Columbia University
15 PUBLICATIONS **93** CITATIONS
SEE PROFILE

Danielle M Tufts
Columbia University
134 PUBLICATIONS **300** CITATIONS
SEE PROFILE

Maria Diuk-Wasser
Columbia University
159 PUBLICATIONS **3,160** CITATIONS
SEE PROFILE

Medical and Veterinary Entomology

Medical and Veterinary Entomology (2020), doi: 10.1111/mve.12441

SHORT COMMUNICATION

Aversion of the invasive Asian longhorned tick to the white-footed mouse, the dominant reservoir of tick-borne pathogens in the U.S.A.

I. RONAI†, D. M. TUFTS† and M. A. DIUK-WASSER

Department of Ecology, Evolution, & Environmental Biology, Columbia University, New York, NY, U.S.A.

Abstract. The Asian longhorned tick (*Haemaphysalis longicornis*) was reported for the first time in the U.S.A. in 2017 and has now spread across 12 states. The potential of this invasive tick vector to transmit pathogens will be determined through its association to hosts, such as the white-footed mouse (*Peromyscus leucopus*), which is the primary reservoir for the causative agent of Lyme disease (*Borrelia burgdorferi*) and other zoonotic pathogens. Larval *H. longicornis* were placed on *P. leucopus*; 65% of the larvae ($n = 40$) moved off the host within a short period of time, and none engorged. By contrast, larval blacklegged ticks (*Ixodes scapularis*) did not move from where they were placed in the ear of the mouse. A laboratory behavioural assay was then conducted to assess the interaction of *H. longicornis* with the hair of potential mammalian host species in the U.S.A. *H. longicornis* larvae were significantly less likely to enter the hair zone of *P. leucopus* and humans compared to the hair of domestic cats, domestic dogs and white-tailed deer. This study identifies a tick–host interaction behaviour, which can be quantified in a laboratory assay to predict tick–host associations and provides insights into how ticks select a host.

Key words. *Canis lupus familiaris*, *Felis catus*, *Homo sapiens*, *Odocoileus virginianus*, acquired tick resistance, ALT, blacklegged tick, host immunity, host-seeking, ixodidae.

Introduction

The Asian longhorned tick (*Haemaphysalis longicornis*) transmits numerous human pathogens and is a highly invasive tick species (Hoogstraal *et al.*, 1968; Heath, 2016; Beard *et al.*, 2018). In the U.S.A., this species was reported for the first time in 2017 (Rainey *et al.*, 2018), although archival evidence suggests *H. longicornis* has been present in the U.S.A. since 2010 (United States Department of Agriculture, 2019). Currently, *H. longicornis* has been detected in 12 states: Arkansas, Connecticut, Delaware, Kentucky, Maryland, New Jersey, New York, North Carolina, Pennsylvania, Tennessee, Virginia, and West Virginia (Beard *et al.*, 2018; The Centers for Disease Control and Prevention, 2019). Modelling studies indicate that this species has the potential to spread throughout the majority of the U.S.A. (Rochlin, 2018).

As *H. longicornis* establishes and spreads to new ecosystems, it encounters new host communities. The host bloodmeal is critical not only for vector survival and reproduction but also for the pathogens to be acquired and transmitted. In the U.S.A., the non-domestic mammalian host community for ticks includes: small mammals (such as white-footed mice and other rodents); medium mammals (such as racoons and opossum); and large mammals (such as deer) (LoGiudice *et al.*, 2003). The host species of high public health significance is the white-footed mouse (*Peromyscus leucopus*), the primary vertebrate reservoir host for zoonotic pathogens, such as the causative agent of Lyme disease (*Borrelia burgdorferi*) (LoGiudice *et al.*, 2003). However, all life stages of *H. longicornis* have shown limited association with small rodents compared to medium- and large-sized mammals, in both its native and invasive ranges (Hoogstraal *et al.*, 1968; Kim *et al.*, 2006; Heath, 2016; Tufts *et al.*, 2019).

Correspondence: Danielle M. Tufts, Columbia University, 1200 Amsterdam Ave, New York, NY 10027, U.S.A. Tel.: 559-250-4430; fax: 212-854-8188. E-mail: dt2503@columbia.edu

†These authors contributed equally to this work.

The host association of the larval life stage is the most epidemiologically significant; if *H. longicornis* larvae do not feed on *P. leucopus*, the possibility of infecting their next host (such as humans) during the nymphal stage is minimized.

Here, the interaction of the invasive *H. longicornis* larvae with *P. leucopus* and other potential mammalian host species commonly encountered in the U.S.A. is investigated, including humans. The behaviour of *H. longicornis* is also compared to that of the native blacklegged tick (*Ixodes scapularis*), the main vector of *B. burgdorferi*, and at least six other human pathogens in the U.S.A. (Petersen *et al.*, 2019).

Materials and Methods

Ticks

During fieldwork on Staten Island (New York, USA) in August 2018, engorged *H. longicornis* adult females were collected from a white-tailed deer (*Odocoileus virginianus*) (Tufts *et al.*, 2019). A subset of these adult females were tested for pathogens and found to be negative. Three females were maintained in individual vials in an incubator (21 °C, 95–100% humidity, and light: dark cycle was 16:8 h to simulate summer conditions) and allowed to lay eggs. Larvae emerged from the egg masses 4 months later. The *I. scapularis* larvae were obtained from a laboratory-reared colony through the NIH Biodefense and Emerging Infections Research Resources Repository (NIAID, NIH: *I. scapularis* larvae, NR-44115). These larvae were maintained in the same incubator (21 °C, 95–100% humidity, and light: dark cycle was 16:8 h to simulate summer conditions) and were used in the study within 6 months from emergence.

Behavioural assessment of responses to live white-footed mouse host

Ten *H. longicornis* ($n = 4$ replicates) or 10 *I. scapularis* larvae ($n = 3$ replicates) were placed in the ear canal of an anaesthetised mouse ($n = 4$, two replicates per mouse, one per ear). The behaviour of the ticks in the ear canal of the anaesthetised mouse was observed every 30 s for 15 min, and the duration of time the ticks took to: (a) move off the ear of the mouse and (b) drop off the mouse was noted. To investigate whether the remaining *H. longicornis* would feed to repletion, they were left on the mice for 3 days. Individual mice were housed in single cages positioned over water. The mouse cages were inspected daily for any engorged larvae, and the number of recovered larvae was recorded. All animal procedures were in accordance with guidelines approved by the Columbia University Institutional Animal Care and Use Committee (IACUC), protocol number: AC-AAAY2450.

Behavioural arena assay of interaction with potential native hosts

Hair was removed from: three frozen white-footed mice (*P. leucopus*) from the field; two cats (*Felis catus*) that were domestic pets; one dog (*Canis lupus familiaris*) that was a domestic pet; multiple white-tailed deer from the field; and one human (*Homo sapiens*). None of the animals were treated with flea or tick repellent. Human hair was obtained from the head of one of the researchers (DMT) and was not dyed or treated with any chemicals and was not shampooed for 2 days before being obtained. Open Petri dishes (150 mm × 15 mm) were used as behavioural arenas, and the dish was divided into two areas (the hair zone and non-hair zone divided by a centre line) (Fig. 1). The hair was arranged in a layer inside the hair zone, and a new Petri dish was used for each hair treatment to prevent scent cross-contamination. At time zero, *H. longicornis* or *I. scapularis* larvae ($n = 10$, three replicates per tick species per hair treatment) were placed on the centre line. Any tick that moved to the rim of the Petri dish was relocated with a brush to the base of the dish directly below the rim. The behavioural arenas were observed in a laboratory room that was maintained at a constant temperature, humidity and light level.

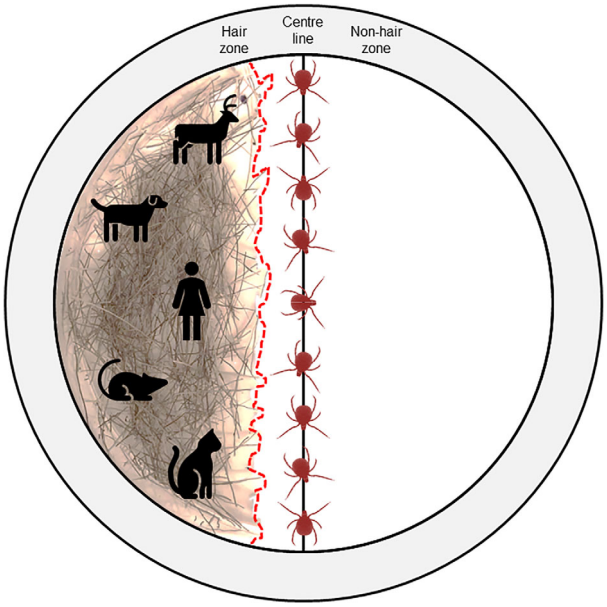

Fig. 1. Diagram of the behavioural assay arena. Petri dishes (150 mm × 15 mm) were divided into two zones with a centre line: hair zone and non-hair zone. The host hair (white-footed mouse, cat, dog, white-tailed deer, and human) was placed in the hair zone and formed an irregular hair interface (dashed line). At the start of the behavioural assay, *Haemaphysalis longicornis* or *Ixodes scapularis* larvae ($n = 10$) were placed on the centre line and assays were replicated three times for each hair treatment. [Colour figure can be viewed at wileyonlinelibrary.com].

To assess the behaviour of the ticks when encountering host hair, each trial of the behavioural assay was video recorded for 5 min. The videos were analysed by two double-blinded observers. The main behavioural response of the ticks was an interaction with the hair interface (come within 1 mm of the hair or touch the hair), which is the edge of the hair zone (dotted line in Fig. 1). The number of times the ticks interacted with the hair interface was counted, and the frequency of interactions per tick per minute was reported. Note that a tick sometimes

interacted with the hair interface multiple times. The outcome of the interaction was then recorded, and the tick either: (a) entered the hair zone (Video S1) or (b) turned away from the hair interface (Video S2).

Statistical analysis

All statistical analyses were conducted using R software. The effect of tick species and host hair treatment on the number of times ticks interacted with the hair interface was examined using a non-parametric Kruskal-Wallis test. The resulting outcome (entered the hair zone or turned away at the hair interface), given an interaction, was assessed using a generalized linear mixed model (GLMM) fit by maximum likelihood (Laplace approximation) with observer and replicate as random effects (R package lme4). A GLMM model was used to compare the interaction behaviour between the two species of tick, and then separate analyses were performed for each tick species to compare the probability of entering the hair zone of different hosts to that of the white-footed mouse (reference category). Finally, a Student's *t*-test was used to compare the time individual *H. longicornis* and *I. scapularis* ticks spent in the hair zone.

Results

Within a 15-min timeframe after placement on white-footed mice, 67.5% of the *H. longicornis* ($n = 40$) moved from the site of placement inside the mouse ear canal, whereas 0% of the *I. scapularis* ($n = 30$) moved (Fig. S1A). In addition, 55% of the *H. longicornis* ($n = 40$) dropped off the mice, whereas 0% of *I. scapularis* ($n = 30$) dropped off (Fig. S1B). Before the mice were relocated to a cage, an additional four *H. longicornis* dropped off; therefore, 65% of *H. longicornis* ($n = 40$) dropped off the mice. For the remaining *H. longicornis* on the mice ($n = 14$), none were ever observed to have attached, and no engorged larvae were recovered in the subsequent days. This finding is in marked contrast to the authors' past experience with larval *I. scapularis*, which readily engorge following attachment to *P. leucopus*.

H. longicornis and *I. scapularis* had a similar frequency of interactions with all of the hair treatment interfaces (Kruskal-Wallis: $\chi^2_1 = 0.367$, $P = 0.5448$; Fig. S2). There was also no significant effect of hair treatments across the two species of tick (Kruskal-Wallis: $\chi^2_4 = 4.283$, $P = 0.3691$; Fig. S2). Therefore, *H. longicornis* interacted as frequently with the hair treatments as *I. scapularis*.

An observation was made as follows: when a tick interacted with the hair interface, it raised its front legs (the location of the sensory Haller's organ (Carr *et al.*, 2017)) and waved them. After each interaction, the tick either entered the host hair zone or turned away from the hair interface. The outcome of the interaction with the hair interface was used as a behavioural metric.

After an interaction with the hair interface, *H. longicornis* larvae were significantly less likely to enter the host hair zone compared to *I. scapularis* larvae (GLMM, $P = 0.0365$, Fig. 2, Table 1). The behaviour within each tick species was then analysed. *H. longicornis* larvae were significantly more likely to enter the hair zone of cats, dogs, or white-tailed deer than the hair zone of white-footed mice ($P = 0.0095$, $P = 0.0261$ and $P = 0.0039$, respectively; Fig. 2A, Table 1). In addition, *H. longicornis* larvae were as likely to enter the hair zone of humans as the hair zone of white-footed mice ($P = 0.1645$, Fig. 2A, Table 1). *I. scapularis* larvae were significantly more likely to enter the hair zone of white-footed mice than the hair zone of white-tailed deer or humans ($P = 0.0447$ and $P = 0.0021$, respectively; Fig. 2B, Table 1). In addition, *I. scapularis* larvae were as likely to enter the hair zone of white-footed mice as the hair zone of cats or dogs ($P = 0.3415$ and $P = 0.4094$, respectively; Fig. 2B, Table 1). It was also found that *H. longicornis* larvae spent significantly less time within the hair zone of each hair treatment compared to *I. scapularis* larvae ($P = 0.0040$).

Discussion

Host-seeking *H. longicornis* larvae were observed to exhibit aversion to the hair of white-footed mice. This tick species actively avoids the hair of the white-footed mice as more than 50% of them quickly dropped off the mice. This newly invasive tick is therefore unlikely to select the white-footed mouse as a host in the natural environment of the U.S.A. The findings of this laboratory-based study help explain why the recent U.S.A. passive and active field studies of *H. longicornis* did not find *H. longicornis* of any life stage on white-footed mice despite collection of host-seeking *H. longicornis* ticks in the same regions (Tufts *et al.*, 2019; United States Department of Agriculture, 2019). The aversion of *H. longicornis* to the white-footed mouse and humans reduces the likelihood of this tick becoming an important vector of zoonotic pathogens for which white-footed mice are the main reservoir host in the U.S.A. (such as *B. burgdorferi*, *Babesia microti* and *Anaplasma phagocytophilum*; Petersen *et al.*, 2019). Furthermore, *H. longicornis* are unable to maintain *B. burgdorferi* transstadially after feeding on infected *Mus musculus* when confined in feeding capsules (Breuner *et al.*, 2019).

Why has *H. longicornis* evolved an aversion to small rodents such as the white-footed mouse? A possible explanation for this aversion is that feeding on mice reduces the fitness of *H. longicornis*. When *H. longicornis* feed on mice, they detach early, have a prolonged duration for feeding, have impaired engorgement, have low egg clutch sizes, and have high moulting death (Kovář, 2004). House mice have an immunological response via antibody production to *H. longicornis*, and after one exposure, acquired tick resistance develops (Matsuda *et al.*, 1985; Kovář, 2004). Alternatively, when *H. longicornis* feed on mice, they might have an increased chance of being dislodged, injured, or killed due to the mice excessively grooming.

This study's findings that larval *H. longicornis* are more likely to enter the hair zone of medium- and large-sized mammals, are consistent with field studies of *H. longicornis* (Tufts *et al.*, 2019; United States Department of Agriculture, 2019). Medium-sized mammals have intermediate competence

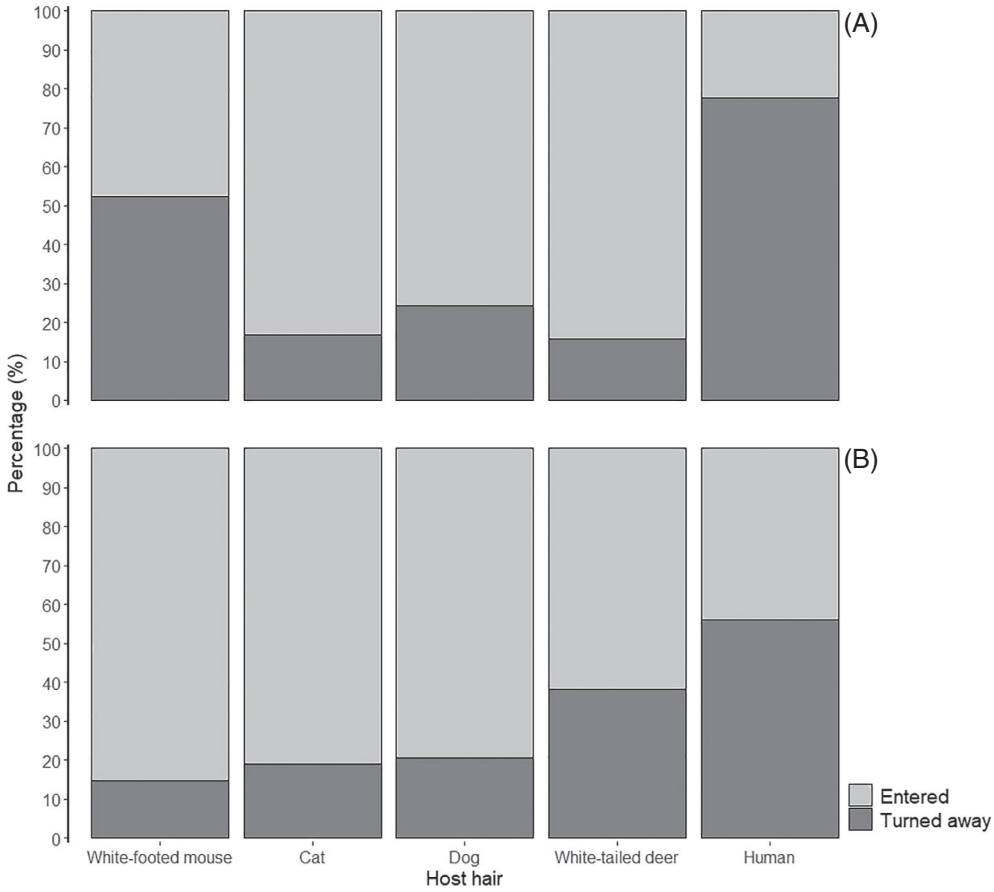

Fig. 2. The percentage of ticks that, on interaction with the interface of the host hair (white-footed mouse, cat, dog, white-tailed deer, and human), either entered the hair zone or turned away from the interface for (A) *Haemaphysalis longicornis* larvae and (B) *Ixodes scapularis* larvae.

Table 1. GLMM of *Haemaphysalis longicornis* larvae and *Ixodes scapularis* larvae.

Treatment	*Haemaphysalis longicornis*			*Ixodes scapularis*		
	n	Odds ratio	*P*-value	*n*	Odds ratio	*P*-value
White-footed mouse	37	1	—	33	1	—
Cat	23	5.0901	0.0096∗∗	8	0.5537	0.3415
Dog	41	3.1535	0.0261∗	34	0.5915	0.4094
White-tailed deer	22	6.9331	0.0039∗∗	22	0.2830	0.0447∗
Human	29	0.4616	0.1645	25	0.1381	0.0021∗∗

The number of individual larval interactions (*n*) with the host hair interface (white-footed mouse, cat, dog, white-tailed deer, and human), calculated odds ratio and *P*-value. Each treatment was compared to the white-footed mouse (reference).
$*P < 0.05$.
$**P < 0.01$.

for tick-borne pathogens such as *B. burgdorferi* (LoGiudice *et al.*, 2003), and it is currently unknown whether medium-sized mammals can serve as a source of pathogens for *H. longicornis* in the U.S.A. In addition, larval *H. longicornis* were found to have an aversion for human hair. Notably, there are only two cases so far reported of *H. longicornis* biting a human in the U.S.A. (United States Department of Agriculture, 2019).

On physical contact with a passing potential host, a tick must either climb onto the host or ignore it and subsequently feed on this host or not. How different tick species select their host is currently not well understood (Carr *et al.*, 2017). Host stimuli such as body heat and carbon dioxide are not species specific and are likely unhelpful for host selection. This study has identified that ticks have a unique tick–host interaction behaviour (enter or turn away at the hair interface), which suggests that they utilize a species-specific property of the animal hair to select a host. After a tick climbs onto the host, it then must either bite and feed or not; the properties of the host that drive these behaviours are currently unknown.

In conclusion, this study finds that the newly invasive *H. longicornis* has an aversion to the white-footed mouse, the dominant reservoir of tick-borne pathogens in the U.S.A. *H. longicornis* also has an aversion to humans, which decreases human risk for tick bites. Pathogen transmission studies therefore need to consider not only attraction of a vector to a host but also host aversion. Furthermore, the behavioural assay the authors have developed, which utilizes host hair, could provide a measure of potential tick–host associations that do not yet occur in nature, such as newly invasive ticks or ticks expanding their geographic range.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1. Percentage of *Haemaphysalis longicornis* ($n = 40$) or *Ixodes scapularis* ($n = 30$) larvae that remained after placement (time 0) on the (A) ear of the mouse and (B) mouse body.

Fig. S2. Number of interactions a larval tick (*Haemaphysalis longicornis* or *Ixodes scapularis*, $n = 30$) made with the interface of the host hair (white-footed mouse, cat, dog, white-tailed deer and human) per trial.

Movie S1. *Haemaphysalis longicornis* interacting with the hair interface and entering into the hair zone.

Movie S2. *Haemaphysalis longicornis* interacting with the hair interface and turning away from the interface.

Acknowledgements

The authors thank Kevin Zhao and Daniel Mathisson for assisting with video analysis and Pilar Fernandez for advice on data analysis. They thank Thomas Hart who provided the deer hair and the pets (Venus, Luna, and Lucy) who contributed their hair. In addition, they thank the anonymous reviewers for their constructive comments that improved the manuscript. The authors declare that they have no conflicts of interest.

This work was supported by *an Australian Government Endeavour Research Fellowship to I.R. and* the Centers for Disease Control and Prevention (Cooperative Agreement Number U01CK000509-01). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the Centers for Disease Control and Prevention or the Department of Health and Human Services.

References

Beard, C.B., Occi, J., Bonilla, D.L., Egizi, A.M., Fonseca, D.M., Mertins, J.W., *et al.* (2018) Multistate infestation with the exotic disease–vector tick *Haemaphysalis longicornis*—United States, August 2017–September 2018. *MMWR. Morbidity and Mortality Weekly Report* 67.

Breuner, N.E., Ford, S.L., Hojgaard, A. *et al.* (2019) Failure of the Asian longhorned tick, *Haemaphysalis longicornis*, to serve as an experimental vector of the Lyme disease spirochete, *Borrelia burgdorferi* sensu stricto. *Ticks and Tick-Borne Diseases*, **11**,101311.

Carr, A.L., Mitchell, R. III, Dhammi, A., Bissinger, B.W., Sonenshine, D.E. & Roe, R.M. (2017) Tick Haller's organ, a new paradigm for arthropod olfaction: How ticks differ from insects. *International Journal of Molecular Sciences*, **18**, 1563.

Heath, A.C.G. (2016) Biology, ecology and distribution of the tick, *Haemaphysalis longicornis* Neumann (Acari: Ixodidae) in New Zealand. *New Zealand Veterinary Journal*, **64**, 10–20.

Hoogstraal, H., Roberts, F.H., Kohls, G.M. & Tipton, V.J. (1968) Review of *Haemaphysalis* (*Kaiseriana*) *longicornis* Neumann (resurrected) of Australia, New Zealand, New Caledonia, Fiji, Japan, Korea, and northeastern China and USSR, and its parthenogenetic and bisexual populations (Ixodoidea, Ixodidae). *The Journal of Parasitology*, **54**, 1197–1213.

Kim, C.-M., Yi, Y.-H., Yu, D.-H. *et al.* (2006) Tick-borne rickettsial pathogens in ticks and small mammals in Korea. *Applied and Environmental Microbiology*, **72**, 5766–5776.

Kovář, L. (2004) Tick saliva in anti-tick immunity and pathogen transmission. *Folia Microbiologica*, **49**, 327–336.

LoGiudice, K., Ostfeld, R.S., Schmidt, K.A. & Keesing, F. (2003) The ecology of infectious disease: Effects of host diversity and community composition on Lyme disease risk. *Proceedings of the National Academy of Sciences*, **100**, 567–571.

Matsuda, H., Fukui, K., Kiso, Y. & Kitamura, Y. (1985) Inability of genetically mast cell-deficient W/W^v mice to acquire resistance against larval *Haemaphysalis longicornis* ticks. *The Journal of Parasitology*, **71**, 443–448.

Petersen, L.R., Beard, C.B. & Visser, S.N. (2019) Combatting the increasing threat of vector-borne disease in the United States with a national vector-borne disease prevention and control system. *The American Journal of Tropical Medicine and Hygiene*, **100**, 242–245.

Rainey, T., Occi, J.L., Robbins, R.G. & Egizi, A. (2018) Discovery of *Haemaphysalis longicornis* (Ixodida: Ixodidae) parasitizing a sheep in New Jersey, United States. *Journal of Medical Entomology*, **55**, 757–759.

Rochlin, I. (2018) Modeling the Asian Longhorned Tick (Acari: Ixodidae) suitable habitat in North America. *Journal of Medical Entomology*, **56**, 384–391.

The Centers for Disease Control and Prevention (2019) What you need to know about Asian longhorned ticks—A new tick in the United States. https://www.cdc.gov/ticks/longhorned-tick/index.html [accessed June 2019].

Tufts, D.M., VanAcker, M.C., Fernandez, M.P., DeNicola, A., Egizi, A. & Diuk-Wasser, M.A. (2019) Distribution, host-seeking phenology, and host and habitat associations of *Haemaphysalis longicornis* ticks, Staten Island, New York, USA. *Emerging Infectious Diseases*, **25**, 792–796.

United States Department of Agriculture. (2019) National *Haemaphysalis longicornis* (Asian longhorned tick) situation report. http://www.aphis.usda.gov/animal_health/animal_diseases/tick/downloads/longhorned-tick-sitrep.pdf [accessed June 2019].

Accepted 10 March 2020

