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Tick infestation effects on haemoglobin levels of deer mice (*Peromyscus maniculatus*)

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Research Article

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Abstract

Deer mice (*Peromyscus maniculatus*) are hosts to ixodid ticks as well as the associated tick-borne pathogens they can spread. As the ranges of black-legged ticks (*Ixodes scapularis*) and American dog ticks (*Dermacentor variabilis*) expand northwards, naïve host populations of deer mice are likely to become infested by ticks and experience the physiological effects that ticks can have on them *via* blood-feeding. The prevalence of these haematophagous ticks can affect the haemoglobin levels of the mice they infest. Haemoglobin levels were compared and analysed in deer mice populations at three different sites with varying tick exposure. These results suggested that without confounding effects, the abundance of black-legged and American dog ticks on individual mice had a significant negative effect on the hosts' haemoglobin levels, but only in an area with high tick infestation. This was seen across the average haemoglobin levels between populations, where there was a significant difference between the source population with the longest established tick populations and the source population where neither black-legged nor American dog ticks were prevalent. As the ticks' ranges expand and they become more abundant, it is important to understand how their prevalence and intensity can alter host physiology, potentially affecting their own range expansion and the spread of the diseases they may carry.

Introduction

Blood-feeding by haematophagous ectoparasites, such as hard-bodied ticks (Acari: Ixodidae), is required to reach subsequent life stages (Arsnoe *et al.*, 2015), and can have severe negative effects on the physiology and fitness of their vertebrate hosts (Dryden and Gaafar, 1991; Carleton, 2008; Pfäffle *et al.*, 2009; Bordes *et al.*, 2007; Godinho *et al.*, 2013; Hersh *et al.*, 2014; Jones *et al.*, 2019). These effects include reduced haemoglobin levels (Carleton, 2008), regenerative anaemia (Pfäffle *et al.*, 2009) and mortality related to blood loss (Jones *et al.*, 2019).

The relationship between haematophagous parasites and the effects of their blood-feeding on their hosts is not clear (Kutzer and Armitage, 2016; Papkou *et al.*, 2016). Several black-legged ticks (*Ixodes scapularis*) can feed from white-footed mice (*Peromyscus leucopus*) at once or in succession due to exposed skin lesions that allow ticks to infest the same host despite a strong inflammatory response at the attachment sites that can increase host resistance (Anderson *et al.*, 2017). Yet, insufficient immune responses could result in mice being vulnerable to other bacterial infections (Rosales *et al.*, 1999; Dlugosz *et al.*, 2014).

Black-legged ticks and American dog ticks (*Dermacentor variabilis*) tend to feed on *Peromyscus* mice at immature stages (Ostfeld *et al.*, 1996; Sonenshine, 2018), and have been documented sharing the same areas on a host (Morshed *et al.*, 2003; Shaw *et al.*, 2003; Gómez-Rodríguez *et al.*, 2015). Deer mice (*Peromyscus maniculatus*) are competent vectors of several tick-borne pathogens, including *Borrelia burgdorferi* and *Anaplasma phagocytophilum* (Rand *et al.*, 1993; Larson *et al.*, 2018). However, because ticks feed on mice for a short period of time, it is difficult to measure any long-term impacts that they may have on host fitness.

Other ectoparasites that feed on *Peromyscus* mice for long periods of time (i.e. fleas, mites) are consistently affecting the vulnerability of their host' immune response (Mize *et al.*, 2011), reducing the amount of iron in the blood due to constant blood loss (O'Brien *et al.*, 2003), which could cause an insufficient production of haemoglobin (Judy and Price, 1958; Andrews, 1997). Iron-deficient black rats (*Rattus rattus*) were found to be susceptible to infections of the pathogen *Salmonella typhimurium* (Baggs and Miller, 1973) and botfly infections can decrease haemoglobin levels in deer mice, potentially reducing their capacity for thermogenesis and aerobic performance (Wilde *et al.*, 2018). Investigating how ectoparasite feeding habits influence the haematology of deer mouse hosts – with and without the presence of ticks – will allow us to better understand how black-legged and American dog ticks may impact host populations as these ticks move northward (Leighton *et al.*, 2012).

The objective of this study was to determine if ticks have a significant effect on the haematology of deer mice by examining how tick prevalence and intensity influence host haemoglobin levels. Here, we focused on the relationship between haemoglobin levels in deer mice and ticks in general, as well as individual species: black-legged ticks and American dog ticks. It was hypothesized that: (1) if mice are parasitized by a greater intensity of ticks, then they will have

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lower haemoglobin levels compared to mice infested with ticks at low intensities because more blood-feeding is occurring at one time and (2) Deer mice that live in areas where ticks are prevalent should have lower haemoglobin levels than deer mice living in unestablished areas because they are being affected by blood-feeding. Individual mice that are infested with higher abundances of ticks are expected to have lower haemoglobin levels compared to a mouse with low tick abundance as more ticks are feeding from the same source at one time. Furthermore, mice that inhabit areas where ticks are prevalent and at high intensities are expected to have lower haemoglobin levels than mice that are ecologically naïve to these parasites. Examining the differences between naïve and experienced mouse populations as well as variation among individual mice can bridge the gap in understanding tick effects on a host to help make better predictions on how haematology can affect the ecology of *Peromyscus*.

Materials and methods

Field methods

Three sites in Ontario, Canada were visited for data and specimen collection from May to August 2019. The most southern site, Long Point Provincial Park (LP; 42.5817° N, −80.3952° W) is an area where both black-legged and American dog tick populations have been long-established (Watson and Anderson, 1976; Lindsay *et al.*, 1991). The other two sites, Queen Elizabeth II Wildlands Provincial Park (QEW; 44.7534° N, −78.7844° W) and Algonquin Provincial Park (AP; 45.3402° N, −78.2618° W) are areas with no established populations of black-legged ticks, although QEW has known established American dog tick populations (Minigan *et al.*, 2018; P. Careless, QEW Park Biologist, personal communication). Neither black-legged ticks nor American dog ticks have colonized AP (Public Health Ontario, 2019). The sites chosen for this experiment convey the progression of the geographic expansion of black-legged ticks and American dog ticks in Ontario from both its southern and eastern populations (Hamer *et al.*, 2014; Clow *et al.*, 2016, 2017; Minigan *et al.*, 2018), and are areas within the geographical habitat range of deer mice; Bedford and Hoekstra, 2015). Traplines were placed in similar forest types across sites to maintain a consistent or similar habitat, although sites were from different ecozones (Crins *et al.*, 2009).

At each site, three traplines at least 0.3 km apart from each other were set up with twenty Longworth traps (Penlon Ltd., Oxford, UK) set per line. Traplines were 90 m long and had two Longworth traps 10 m apart from each other every 10 m (Fryxell *et al.*, 1998; Falls *et al.*, 2007). Traps were baited with water-soaked sunflower seeds and were set half an hour before sunset (2000 h-2100 h). Traps were checked half an hour before sunrise (0430 h-0530 h) after the mice had been active through the night (Clark and Durden, 2002). The traps were set 5 days each week. They were checked 3 days consecutively, with a 1-day break, and then checked for two more consecutive days to reduce the amount of stress mice may have from repeated captivity or trap response behaviour (Nichols *et al.*, 1984). To account for a change in seasonality, and the difference in parasite assemblages that occurs within the spring-summer months, sampling alternated between the three sites in ascending latitude, spending 1 week at each site, three times, for a total of 3 weeks ('sessions') per site. Sessions were divided by early (May–June), mid (June–July) and late (July–August) summer. By alternating sites within the season, it was possible to account for the different assemblages that may be occurring within populations, as temperatures change, and specific parasites occur in different quantities.

Mice caught in traps were removed and weighed using a Pesola® scale (±0.1 g). Age was determined by body mass, where mice that were 15 g or less were considered juveniles, while mice greater than 15 g were considered adults (Banfield, 1974; Schmidt *et al.*, 2019). Sex and reproductive status were determined visually. Enlarged testes in males and perforated vagina and the presence of nipples in females indicated reproductive individuals (Gaitan and Millien, 2016). No pregnant females were included in this study. The age of ticks was determined by the number of legs the tick had, where the larva has six legs, and nymphs have eight (Lindquist *et al.*, 2016).

Upon capture, each mouse was examined for 60 seconds for a sampling of ectoparasites (Patterson *et al.*, 2013). Individual arthropod specimens were combed off the host using a louse comb sterilized with ethanol (Hawlena *et al.*, 2006; Patterson *et al.*, 2013) or plucked off, using sterilized tweezers (Bobbie *et al.*, 2016). These specimens were then placed in a collection vial with 80% ethanol (Krogmann and Holstein, 2010). Tick identification was completed using guides (Lindquist *et al.*, 2016; Dubie *et al.*, 2017), and were later confirmed by Dr Robbin Lindsay from the National Microbiology Laboratory (Winnipeg, Manitoba, Canada).

Captured mice were placed in a 50 mL Falcon conical centrifuge tube with a hole in the tip to allow for the mouse to breathe. By pushing gently above the tibia, the right hind leg of the mouse could be extended out of the tube and shaved using an electric trimmer (Parasuraman *et al.*, 2010). Needles (20G) were used with a 1 mL syringe in accordance with the Standard Operating Procedure for Laurentian University and general principles of laboratory blood collection (Parasuraman *et al.*, 2010). Blood samples consisted of 0.007 mL of blood/g of the mouse' body mass taken from the saphenous vein in the shaved hind leg of the mouse *via* needled syringe (Randolph, 1980; National Research Council Institute for Laboratory Animal Research, 1996; PREDICT One Health Consortium, 2016). On site, the blood samples were placed in a microcuvette specialized for the handheld haemoglobin analyser to test for haemoglobin concentrations: HemoCue Hb 201+ analyser (HemoCue AB, Ängelholm, Sweden; Tufts *et al.*, 2013; Weldon *et al.*, 2015). Results were provided immediately by the analyser and recorded.

It was necessary to shave the legs of the mice to acquire blood samples and so the shaved areas were also used as markers on the mice to indicate that they were already sampled to prevent sampling an individual more than once (Powell and Proulx, 2003). Although it is common for small mammals to be given ear tags for identification purposes in field studies (Harper and Austad, 2001; Hersh *et al.*, 2014; Torre *et al.*, 2016; Buchholz and Dick, 2017), Ostfeld *et al.* (1993) found that this method will increase tick infestations on the host' ears. It should be noted that the exposed shaved legs of the mice in this study were never observed to have ticks or bite marks in this specific area, implying shaving the legs of the mice did not result in the same bias as ear tags do in attracting ticks. Instead, because their right legs were shaved, it was clear which mice were recaptures when returning to each site every 3 weeks, as the fur on these mice had not fully regrown. These mice were documented as recaptures and were not resampled. The legs of these recaptures were re-shaved, however, so that upon returning to the site in the next cycle, they could be clearly identified again.

Statistical methods

Prevalence and mean intensity of ectoparasites were completed using QPweb (Reiczigel *et al.*, 2019), where prevalence refers to the percentage of mice in a population with one or more ectoparasites (Bush *et al.*, 1997). For this study, mean intensity refers to

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Table 1. Descriptive results including mean haemoglobin levels per site and prevalence and mean intensity (average abundance) of ectoparasites found on deer mice (*Peromyscus maniculatus*) across all sites and per site

	LP ($n = 20$)		QEW ($n = 7$)		AP ($n = 17$)		All sites ($n = 44$)	
Mean haemoglobin levels	14.89 g dL^{-1} ±1.97		16.49 g dL^{-1} ±1.33		16.55 g dL^{-1} ±1.47		15.78 g dL^{-1} ±1.86	
Species	IS	DV	IS	DV	IS	DV	IS	DV
Total number of ticks	28	102	0	9	0	0	37	111
Prevalence (%)	85.0	95.0	0.0	28.6	0.0	0.0	37.8	48.9
Mean tick intensity (min–max)	1.40 (0–4)	5.10 (0–19)	N/A	0.86 (0–4)	N/A	N/A	0.63 (0–4)	2.45 (0–19)
Nymph prevalence (%)	1.5	5.4	0.0	11.1	0.0	0.0	1.4	5.75

Numbers in parentheses indicate the sample size of hosts for each column. LP, Long Point Provincial Park; QEW, Queen Elizabeth II Wildlands Provincial Park; AP, Algonquin Provincial Park; IS, black-legged ticks (*Ixodes scapularis*) abundance; DV, American dog ticks (*Dermacentor variabilis*) abundance.

the mean number of ectoparasites found across mice in a population, whereas abundance refers to the number of ectoparasites found on individuals (Bush *et al.*, 1997). An abundance of ticks was determined by the number of parasites per host (Rózsa *et al.*, 2000). Prevalence was examined as parasite species presence/absence per individual host. A total of 44 individual deer mice haemoglobin measurements were included in analyses among all sites. This included haemoglobin measurements from 16 adult female mice and three juvenile female mice, 20 adult male mice and five juvenile male mice.

Statistical analyses were conducted in the R environment (R core Team 2019) *r: a language and environment for statistical computing*. r foundation for statistical computing, vienna, austria. https://www.r-project.org/. [v3.6.1]) using base R and the package 'ggplot2' (Wickham, 2016 [v3.2.1]) for figures. To determine differences in haemoglobin levels among source populations, a one-way ANOVA and Tukey' Honest Significance test were conducted, where haemoglobin levels were the response variable, and source population (LP, QEW, AP) was the predictor. The effect of differences in tick prevalence on mouse population haemoglobin levels was measured by comparing mean haemoglobin levels between population sources and across all individuals (LP + QEW + AP) when (1) all mice were included, (2) only mice infested with ticks were included, or (3) only mice not infested with ticks were included. Standard deviations of these means were also included. One-way ANOVAs were conducted for all individuals, and for each source population site comparing haemoglobin levels and tick prevalence (both black-legged and American dog tick together).

Due to the small sample size ($n = 44$), host age, sex and sexual reproduction were not included as predictor variables (Bouchard *et al.*, 2011). The predictor variables included in three statistical models were: (1) interaction term between general tick abundance per host and source population (LP, QEW, AP), tick nymph presence, and the session when each site was visited, (2) an interaction term between an abundance of American dog ticks per host and source population, an interaction term between an abundance of black-legged ticks per host and source population, tick nymph presence and session and (3) abundance of American dog ticks per host, an abundance of black-legged ticks per host, an interaction term between abundances of black-legged ticks and American dog ticks, and tick nymph presence. Since two tick species were involved in the analyses and their distributions varied, it was important to consider their interactions together and separately to determine any effects on deer mice haemoglobin levels.

The package 'tidyverse' (Wickham *et al.*, 2019 [v1.3.0]) was used to test multicollinearity between independent variables *via* variance inflation factors (VIF). All explanatory variables considered in analyses had VIF values <3 (Harrison *et al.*, 2018; Frost, 2019). Models were fitted as generalized linear models (GLMs) with a Gaussian distribution using a continuous variable as the response variable (haemoglobin levels). Shapiro–Wilk's tests showed that haemoglobin levels were distributed normally across individuals ($P = 0.09$). All other parametric assumptions were met *via* Breusch–Pagan test per model (for homoscedasticity; Breusch and Pagan, 1979), and residual *vs* fitted plots (for linearity between the response variable and each predictor). No model had a leverage hat value >1.

Pearson' correlation formula was used to determine correlation coefficients between all predictor variables included in the final models and haemoglobin levels to determine effect sizes (Weber *et al.*, 2016). Black-legged ticks and American dog ticks were weakly correlated (0.31). The source population was moderately to highly correlated with general tick, black-legged tick and American dog tick abundances ($r = -0.76$, $r = -0.64$ and -0.54, respectively). To avoid multicollinearity between these variables, Models 1 and 2 included an interaction term between tick abundances and source population.

Results

Across hosts, a total of 111 American dog ticks (16 of which were nymphs) and 28 black-legged ticks (two of which were nymphs; Table 1) were found. The average population sizes for each site across sessions (early, mid and late summer) were determined by the Lincoln–Peterson index (Grimm *et al.*, 2014): LP = 28 mice, QEW = 18 mice, AP = 11 mice (Table A1). LP had the greatest intensity and prevalence of both focal tick species, while AP had neither species present.

Three general linear models were considered to analyse predictor variables that may be affecting the response variable – deer mouse haemoglobin levels. Across the models, the abundance of ticks (both species measured together and individually) were significantly correlated with deer mouse haemoglobin levels ($P < 0.05$; Tables 2–4), but only in LP ($P < 0.05$; Tables 2 and 3, Fig. 1). In general, the abundance of black-legged ticks and American dog ticks were also found to be significantly negatively correlated with haemoglobin levels in deer mice ($P = 0.002$ and $P = 0.007$, respectively), but their interaction was not ($P = 0.521$; Table 4; Figure 2). Pearson correlation coefficients between haemoglobin levels and tick species (both tick species together, black-legged ticks and American dog ticks separately) were moderately and negatively correlated ($r = -0.49$, -0.51, -0.5, respectively; Table A2), suggesting moderate effect size (Weber *et al.*, 2016). The prevalence of nymphs was also found to significantly affect haemoglobin levels in Model 2 and Model 3, ($P = 0.025$; Table 3 and $P = 0.033$; Table 4), but not Model 1 ($P = 0.292$; Table 2, Fig. 3). This is likely due to the Pearson correlation value for

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Table 2. Results of the general linear model on the effects of session when sample was collected (early, mid, late summer), nymph presence (Y/N) and the interaction between tick abundance (Ticks) and source population on haemoglobin levels in deer mice (*Peromyscus maniculatus*)

Predictors	Estimate	Standard error	*T* value	Pr (>|*t*|)
Intercept	17.498	0.972	18.000	$<2\times10^{-16}$ ***
Session	−0.454	0.374	−1.215	0.232
Nymph presence (Y)	0.857	0.802	1.069	0.292
Ticks: Source population (AP)	−0.093	1.842	−0.050	0.960
Ticks: Source population (LP)	−0.171	0.048	−3.551	0.001 **
Ticks: Source population (QEW)	0.013	0.393	0.033	0.974

The symbols '**' and '***' indicate *P* values <0.01 and <0.001, respectively.
Null deviance: 148.48 on 43 degrees of freedom. Residual deviance: 105.58 on 38 degrees of freedom. LP, Long Point Provincial Park; QEW, Queen Elizabeth II Wildlands Provincial Park; AP, Algonquin Provincial Park.

Table 3. Results of the general linear model on the effects of session when sample was collected (early, mid, late summer), nymph presence (Y/N) and the interactions between black-legged tick and American dog tick abundances independently and source population on haemoglobin levels in deer mice (*Peromyscus maniculatus*)

Predictors	Estimate	Standard error	*T* value	Pr (>|*t*|)
Intercept	16.761	0.769	21.788	$<2\times10^{-16}$ ***
Session	−0.117	0.307	−0.378	0.707
Nymph presence	1.575	0.674	2.338	0.025 *
IS: Source population (AP)	N/A	N/A	N/A	N/A
IS: Source population (LP)	−0.835	0.234	−3.571	0.001 **
IS: Source population (QEW)	N/A	N/A	N/A	N/A
DV: Source population (AP)	N/A	N/A	N/A	N/A
DV: Source population (LP)	−0.222	0.059	−3.774	0.001 **
DV: Source population (QEW)	−0.075	0.338	−0.221	0.826

The symbols '*', '**' and '***' indicate *P* values <0.05, <0.01 and <0.001, respectively.
Null deviance: 148.48 on 43 degrees of freedom. Residual deviance: 77.67 on 38 degrees of freedom. IS, black-legged ticks (*Ixodes scapularis*) abundance; and DV, American dog ticks (*Dermacentor variabilis*) abundance. LP, Long Point Provincial Park; QEW, Queen Elizabeth II Wildlands Provincial Park; AP, Algonquin Provincial Park.

nymphs, which was both negatively and weakly correlated with haemoglobin (−0.14; Table A2), suggesting a small effect size (Weber *et al.*, 2016). For all models, the relationship between deer mice haemoglobin levels and the predictor field session (LP, QEW, AP) had *P* values >0.05 (Tables 2 and 3), suggesting that this variable had no significant effect on haemoglobin levels.

An ANOVA testing the differences in haemoglobin levels across sites showed that there was a significant difference in haemoglobin levels among the sampled locations ($P = 0.01$). A Tukey' honest significance test showed that AP haemoglobin levels were significantly higher than those at LP (P adj = 0.01), and the differences between LP and QEW haemoglobin levels and between QEW and AP were not significant (P adj = 0.09 and P adj = 0.99, respectively; Figure 4). These differences are further explained by the differences in haemoglobin levels due to tick prevalence. ANOVA results showed a significant difference when ticks were present *vs* absent on a host across sites ($P = 0.012$), although within sites, this difference was not significant: QEW ($P = 0.492$), and AP ($P = 0.814$; Table 4). Since LP mice were all infested, tick prevalence could not be compared, however, LP mice did have the lowest mean haemoglobin levels overall (Table 4).

Discussion

Tick abundance

It was found that the abundance of black-legged ticks and American dog ticks negatively affected haemoglobin levels in individual hosts, though more specifically these abundances affected hosts in LP. The average haemoglobin measurement for non-infested mice in this study was 16.46 g dL^{-1}. This is similar to the results in Wilde *et al.*'s (2018) study comparing haemoglobin levels of deer mice that are infested and not infested with botflies, where the average haemoglobin measurement for hosts not infected with botflies (*Cuterebra* sp.) was 15.79 g dL^{-1}. However, the average haemoglobin level for mice with botfly infestations in Wilde *et al.*'s study (12.54 g dL^{-1}) was lower than the results for mice in this study (15.17 g dL^{-1}), suggesting other (endo)parasites may have greater negative effects on their hosts. However, it is important to note that haemoglobin variation can be affected by several different variables, such as altitude (Storz *et al.*, 2007), age (Wilde *et al.*, 2018) and dehydration (Kim *et al.*, 2017).

The prevalence and intensity of ticks has been shown to have a negative effect on haemoglobin levels in several mammals. Two studies found that cattle calves infested with ticks had substantially lower haemoglobin levels compared to their non-infested counterparts (Rahman *et al.*, 2009; Kaur *et al.*, 2017). Similarly, a study on moose calves (*Alces alces*) that were moderately to severely infested with winter ticks (*Dermacentor albipictus*) had high mortality rates (Jones *et al.*, 2019). Results from this study also show that both black-legged tick and American dog tick abundance affect haemoglobin levels in individual deer mice, which could be detrimental to their physiology. In European hedgehogs (*Erinaceus europaeus*), the blood-feeding activity of two tick species of the genus *Ixodes* (*I. ricinus* and *I. hexagonus*)

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Table 4. Results of the general linear model on the effects of nymph presence (Y/N), black-legged tick abundance, American dog tick abundance and the interaction between black-legged tick and American dog tick abundances on haemoglobin levels in deer mice (*Peromyscus maniculatus*)

Predictors	Estimate	Standard error	*T* value	Pr (>|*t*|)
Intercept	16.561	0.276	59.987	$<2\times10^{-16}$ ***
Nymph presence	1.444	0.654	2.209	0.023 *
IS	−0.957	0.290	−3.301	0.002 **
DV	−0.270	0.095	−2.859	0.007 **
IS:DV	0.055	0.085	0.647	0.521

The symbols '*', '**' and '***' indicate *P* values <0.05, <0.01 and <0.001, respectively. Null deviance: 148.48 on 43 degrees of freedom. Residual deviance: 77.43 on 39 degrees of freedom. IS, black-legged ticks (*Ixodes scapularis*) abundance; and DV, American dog ticks (*Dermacentor variabilis*) abundance. LP, Long Point Provincial Park; QEW, Queen Elizabeth II Wildlands Provincial Park; AP, Algonquin Provincial Park.

resulted in lower haemoglobin levels and led to regenerative anaemia in several individuals (Pfäffle *et al.*, 2009). Gaitan and Millien (2016) reported that white-footed mice infested with higher intensities of black-legged ticks tended to have lower movement rates than mice hosting fewer ticks, suggesting that higher infestation rates incapacitate these mice in some way. This may be related to lower haemoglobin levels, which can have a negative effect on oxygen transport (Storz *et al.*, 2007) and thermogenesis (Wilde *et al.*, 2018).

These studies suggest that the negative impacts of parasitism by black-legged ticks and American dog ticks could be detrimental, although not necessarily fatal. Hersh *et al.* (2014) found that larval tick burdens do not affect the survival of *Peromyscus* hosts, and most of the ticks found in LP and QEW were found at the larval stage. Since larval ticks cannot transmit pathogens, but rather acquire them during this life stage (and then pass them on as nymphs; Huang *et al.*, 2019), Hersh *et al.*' (2014) study suggests that it is not the tick burden alone which is affecting their hosts.

Black-legged ticks were only found in LP, therefore only LP mice have been seen to have their haemoglobin levels altered by this tick species, although it is possible that other mouse populations could see similar effects if they encountered black-legged ticks. The prevalence and intensity of American dog ticks at LP was also much higher relative to QEW and so it could be that tick parasite loads are only negatively affecting their hosts at high infestation rates. The interaction between the focal tick species in this study was not found to be significant. This suggests that there are no confounding effects occurring when both species are infesting a host.

Moreover, despite both species having a significant negative effect on haemoglobin levels in mice, the abundances of black-legged ticks and American dog ticks varied greatly. Infections can play a role in the haematology of the host, reducing blood cell counts, which can result in anaemia (Westblade *et al.*, 2017). Some infections can affect a host' haematology, such as *Babesia* protozoans that invade mature red blood cells and directly affect haemoglobin concentrations (Borggraefe *et al.*, 2006). Black-legged ticks can transmit *Babesia* protozoans to their *Peromyscus* hosts, but American dog ticks cannot (Westblade *et al.*, 2017), so it may be possible that black-legged ticks are indirectly affecting haemoglobin levels in hosts rather than directly by transmitting pathogens that affect the blood. A recent study found that *Babesia odocoilei* was identified in black-legged ticks at Long Point (Milnes *et al.*, 2019), so it is possible this may be occurring in the focal host species, but this variable was not examined in this analysis.

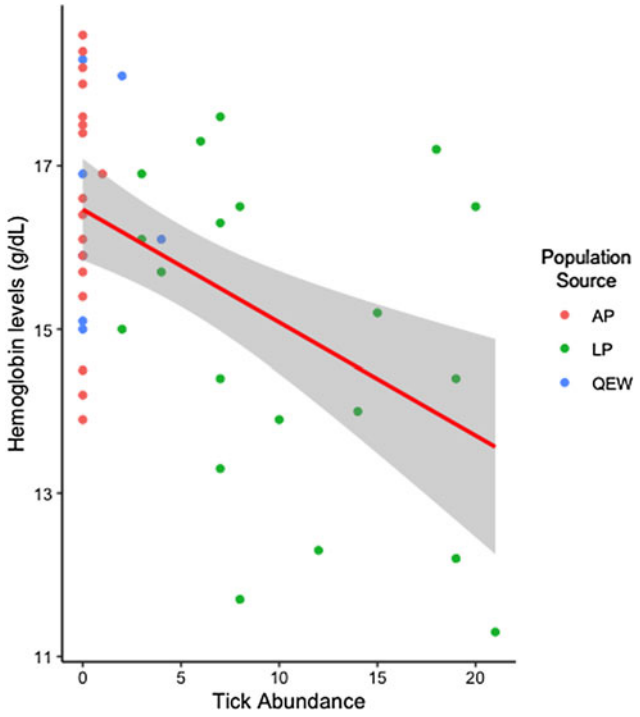

Fig. 1. Generalized linear regression results depicting the effect of general tick abundance on haemoglobin levels in deer mice (*Peromyscus maniculatus*; $n = 44$) when the source population is specified. Each point indicates an individual host. Shaded area indicates the 95% confidence interval. In this model, tick abundance was found to be significantly affecting haemoglobin levels at LP (Long Point) only (*P* value <0.01; Table 2).

Nymph prevalence

Nymph ticks were shown to have a significant effect in the investigated models. Since nymph ticks are larger than larval ticks and can feed from hosts for longer periods of time (Estrada-Peña & de la Fuente, 2014; Kocan *et al.*, 2015), they should consume more blood than their larval counterparts. Nymphs are also more likely to spread diseases, further affecting their host' physiology (Lindquist *et al.*, 2016). The larger blood meals and potential effects nymphs have on their host' physiology could explain how the prevalence of nymphs negatively affected haemoglobin levels. Yet, most nymphs in this study were American dog ticks, with only two black-legged nymphs counted. Few studies have compared the differences in blood meal volume between tick species in general, but Koch and Sauer (1984) found that adult female wood ticks ingested greater volumes compared to black-legged ticks. Although there has not been any comparison between larval and nymph ticks of these species, wood ticks tend to be larger than black-legged ticks when unfed and engorged (Lindquist *et al.*, 2016). It is thus possible that nymph wood ticks can acquire greater blood volumes than nymph black-legged ticks, though further testing is required.

Haemoglobin variation across sites and tick prevalence

It was expected that the variation in tick species prevalence between deer mouse populations would affect haemoglobin levels, such that deer mice from a population where ticks have not established would have higher haemoglobin levels than mice from a population where ticks have established. Within sites, it was expected that there would be a difference in haemoglobin levels depending on the tick species intensities. It was found that haemoglobin levels significantly differed across sites, but not within sites. Haemoglobin levels differed significantly between

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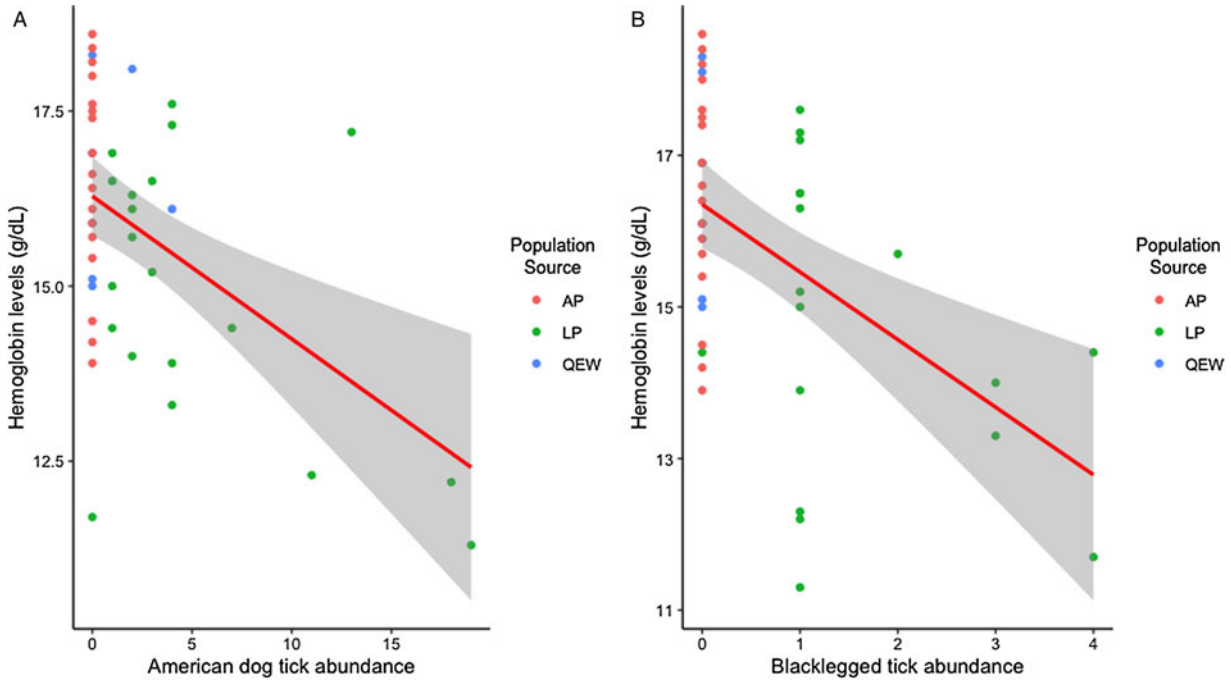

Fig. 2. Generalized linear regression results depicting the effects of black-legged and American dog tick abundances on haemoglobin levels in deer mice (*Peromyscus maniculatus*; $n = 44$) when source population is specified. Each point indicates an individual host. Shaded area indicates the 95% confidence interval. (A) American dog tick abundance is significantly affecting haemoglobin levels (P value <0.01) as is (B) black-legged tick abundance (P value <0.01; Table 3).

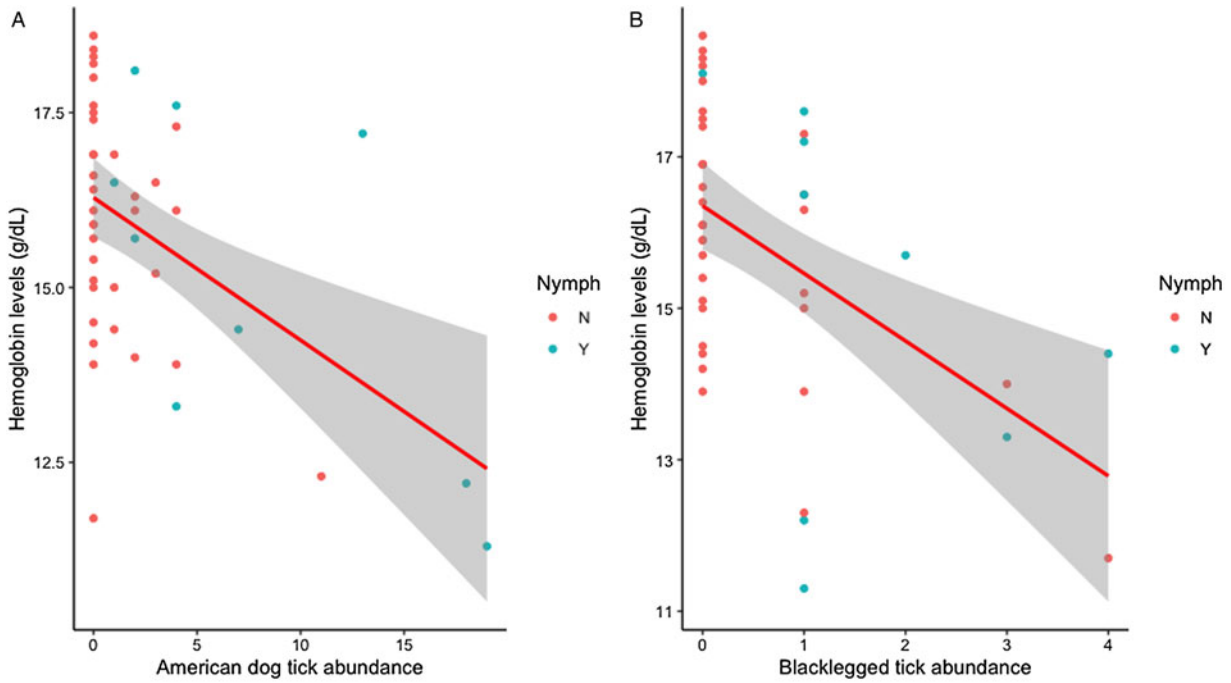

Fig. 3. Generalized linear regression results depicting the effects of black-legged and American dog tick abundances on haemoglobin levels in deer mice (*Peromyscus maniculatus*; $n = 44$) when nymph presence is specified on the host. Each point indicates an individual host. The shaded area indicates the 95% confidence interval. (A) American dog tick abundance is significantly affecting haemoglobin levels (P value <0.01) as is (B) black-legged tick abundance (P value <0.01; Table 4), but not their interaction. Nymph presence was also found to be significantly affecting haemoglobin levels (P value <0.01; Table 4).

LP, which had the highest tick intensity and greatest tick prevalence, and AP, which did not have an established American dog tick or black-legged tick population Table 5.

As the global climate warms and more habitat becomes suitable for ixodid tick species, the geographic ranges of these species continue to expand and establish, infesting host populations previously naïve to such parasites (Leighton *et al.*, 2012; Clow *et al.*, 2016, 2017; Minigan *et al.*, 2018). Little is known about the effect of tick infestations on naïve mammal host populations (such as AP mice who have not been exposed to either focal tick species), despite the geographic expansion of these pathogen vectors (Rand *et al.*, 1993; Larson *et al.*, 2018; Sonenshine, 2018). In general, naïve hosts are especially vulnerable to initial parasitic interactions (Brockhurst *et al.*, 2007), yet hosts that have prior exposure to

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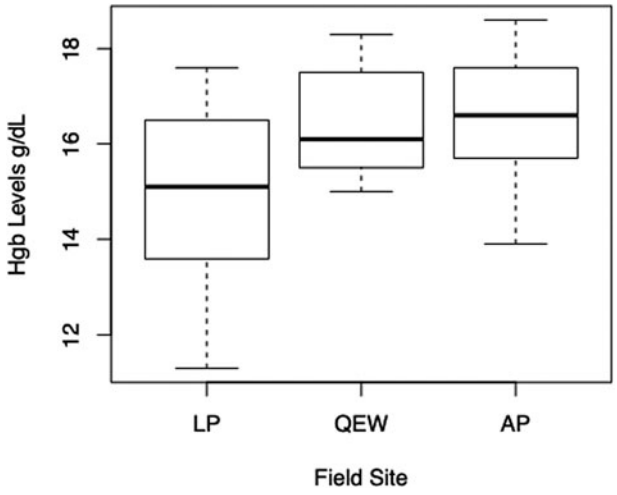

Fig. 4. Deer mouse (*Peromyscus maniculatus*) haemoglobin levels across each site (LP: $n = 20$. QEW: $n = 7$, AP: $n = 17$). ANOVA results show a significant difference across sites ($P = 0.01$). Based on a Tukey' Honest Significance test the comparative differences between sites are LP-QEW (P adj = 0.09), LP-AP (P adj = 0.01) and QEW-AP (P adj = 0.99). LP, Long Point Provincial Park; QEW, Queen Elizabeth II Wildlands Provincial Park; AP, Algonquin Provincial Park.

Table 5. Mean haemoglobin levels from each site including all deer mice (*Peromyscus maniculatus*) hosts, only hosts infested with ticks and only hosts not infested with ticks

Sites	Mean Hgb levels (g dL^{-1})	Mean Hgb levels with tick infested mice only (g dL^{-1})	Mean Hgb levels with non-infested mice only (g dL^{-1})	Pr(>*F*)
All Sites	15.78 ±1.86	15.17 ±2.00	16.46 ±1.46	0.012*
LP	14.89 ±1.97	14.89 ±1.97	N/A	N/A
QEW	16.49 ±1.33	17.1 ±1.41	16.24 ±1.38	0.492
AP	16.55 ±1.47	16.90*	16.53 ±1.52	0.814

The symbols '*', '**' and '***' indicate P values <0.05, <0.01 and <0.001, respectively. P values given are one-way ANOVA results between haemoglobin levels of deer mice infested with ticks *vs* mice without ticks. LP, Long Point Provincial Park; QEW, Queen Elizabeth II Wildlands Provincial Park; AP, Algonquin Provincial Park. It should be noted for AP, only one tick was documented at this site; was not a focal species.

ectoparasites tend to be more resistant than hosts that have not been exposed to them, due to their adaptive responses that have led to stronger immune defences (Kennedy, 2010; Jones *et al.*, 2015).

Within sites, there was no significant effect of ticks on haemoglobin levels. However, the sample sizes across sites were imbalanced, and QEW had a small sample size ($n = 8$), with only two infested hosts, while the one individual infested with a tick in AP, was not infested with a focal species. Nonetheless, these results suggest that in general, tick abundance can reduce a host' haemoglobin level and that tick intensity affects haematology. Within a population, however, haemoglobin levels are not significantly different between infested mice and non-infested mice. It is possible that by having prior exposure (or lacking exposure) to these ectoparasites in a population, the immune defences within the population are similar (Nédélec *et al.*, 2016). This could result in non-significant differences in haemoglobin levels, regardless of whether ticks are prevalent on individual hosts in that population.

Conclusion

Overall, haemoglobin levels varied depending on tick abundance across individuals and prevalence across populations. The most significant difference was between a population with a high infestation rate and a population with little to no infestation. Given that the baseline haemoglobin level for non-infested mice did not differ across populations, it is plausible that low infestation rates do not negatively affect the haemoglobin of deer mice. Tick abundance appeared to contribute most to haemoglobin differences in LP, where tick infestation was highest. When examining the effects of both tick species together, black-legged ticks only and wood ticks only, haemoglobin levels declined significantly when there were greater intensities of ticks on an individual. Despite black-legged ticks being found at lower intensities than wood ticks, both species significantly affected haemoglobin levels. These results should be considered as both focal tick species expand their geographic ranges and are introduced into new host populations (such as AP). The susceptibility to tick infestations and their effects has the potential to alter ecosystem processes that can ultimately affect other host species as zoonotic pathogens are transmitted from mice to ticks to other host species at higher trophic levels, including humans.

Data. The data that support the findings of this study are available from the corresponding author upon request.

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Author contributions.
EF conceived and designed the study, conducted data gathering and performed statistical analyses. EF wrote the manuscript with supervision, reviewing and support from ASH.

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Conflict of interest. The authors declare there are no conflicts of interest.

Ethical standards. All methods in this study were approved by the Animal Care Committee (ACC) at Laurentian University, file number 6017269.

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