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Original article

Tick infestations of wildlife and companion animals in Ontario, Canada, with detection of human pathogens in *Ixodes scapularis* ticks

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ABSTRACT

The growing risk of transmission of tick-borne zoonotic pathogens to humans in Ontario, Canada, warrants investigations into regional tick distribution, tick burdens of local peridomestic animals, and prevalence of tick-borne pathogens. The objectives of this study were to investigate the geographic distribution and magnitude of tick infestations in opportunistically sampled mammalian wildlife and companion animals (i.e., dogs) in southern Ontario and to test these ticks for evidence of zoonotic tick-borne pathogens. Ticks collected from wildlife carcasses, live-trapped wildlife and companion animals (2015–2016), as well as wildlife diagnostic cases (2011–2013), were identified to species and life stage. *Ixodes scapularis* ticks were tested by real-time PCR for *Anaplasma phagocytophilum, Babesia microti, Borrelia miyamotoi* and *Borrelia burgdorferi* sensu stricto (s.s.). *Amblyomma americanum* ticks were tested for *Ehrlichia chaffeensis*. A total of 1687 ticks of six species were collected from 334 animals, including 224 raccoons (n = 1381 ticks) and 50 dogs (n = 67 ticks). The most common tick species collected from parasitized raccoons were *Ixodes texanus* (n = 666 ticks) and *Dermacentor variabilis* (n = 600 ticks), which were removed from 58.5% (median: 2 ticks; range: 1–36) and 49.1% (median: 2 ticks; range: 1–64) of raccoons, respectively. Of *I. scapularis* tested, 9.3% (4/43) were positive for *Bo. burgdorferi* s.s. and 2.3% (1/43) for *A. phagocytophilum*. These results reveal that numerous tick species parasitize common, peridomestic wildlife and that at least two zoonotic, tick-borne pathogens circulate in southern Ontario. Host-tick vector-pathogen dynamics should continue to be monitored in the face of global climate change, landscape alterations and expanding human populations.

1. Introduction

Hard ticks (Acari: Ixodidae) are vectors of numerous zoonotic pathogens with transmission cycles that involve wildlife and companion animal hosts (Dantas-Torres et al., 2012). These hosts often live in close proximity to humans, contributing to public health risk and associated economic burden (Jongejan and Uilenberg, 2009). Further, the geographic distribution of many tick-borne pathogens is expanding northward in the northern hemisphere due to increasingly favorable environments for ticks and their hosts (Clow et al., 2017b). This ongoing expansion has been associated with rising incidence of human tick-borne disease in northern latitudes and led to mounting concern that tick-borne pathogens will pose an even greater public health threat in the future (Estrada-Peña and de la Fuente, 2014; Ogden et al., 2006a).

Global climate change is likely contributing to shifts in the eco-epidemiology of vector-borne pathogens, including increased vulnerability of regions such as southern Ontario, Canada to the influx and potential establishment of tick vectors (Estrada-Peña and de la Fuente, 2014). For example, the northward expansion of *Ixodes scapularis* ticks has led to a higher incidence of Lyme disease, caused by *Borrelia burgdorferi* sensu stricto (s.s.), among humans in Ontario (Bouchard et al., 2015; Ogden et al., 2006a). *Ixodes scapularis* may also transmit a variety of other zoonotic pathogens, including *Anaplasma*

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phagocytophilum, Borrelia miyamotoi, and *Babesia microti* (Lindquist et al., 2016). However, few studies have assessed the geographic distribution and prevalence of tick-borne pathogens in southern Ontario, Canada to identify potentially important regional tick vectors and vertebrate reservoir hosts (Clow et al., 2016a; Nelder et al., 2014; Werden et al., 2015).

The present study was carried out to assess common, peridomestic mammalian wildlife and to a lesser extent, companion animals (i.e., dogs; *Canis lupus familiaris*) for tick infestations in southern Ontario. Our objectives were to: 1) investigate the geographic distribution and tick burdens of parasitized mammalian wildlife (e.g., raccoons, striped skunks, and groundhogs) and dogs; and 2) test *I. scapularis* ticks for evidence of zoonotic tick-borne pathogens, including *A. phagocytophilum, Bo. burgdorferi* s.s., *Bo. miyamotoi* and *Ba. microti*; and *Amblyomma americanum* ticks for *Ehrlichia chaffeensis*.

2. Materials and methods

2.1. Tick collection from animals

Ticks were opportunistically collected from wildlife carcasses collected in Ontario, Canada and donated to the Canadian Wildlife Health Cooperative (CWHC) from May-December of 2015 and 2016; archived ticks from previous CWHC diagnostic cases from 2011 to 2013 were also included. Carcasses were examined for ticks over a 5-min period with a focus on less densely haired regions (e.g., inguinal and axillary areas, and ventrum, pinnae) as well as the face. Ticks opportunistically collected from dogs at seven veterinary clinics within a 7 km radius of Guelph, Ontario from May 2015 to May 2017 also were contributed to the study.

In addition, ticks were collected from live-trapped (and released) wildlife during June-October 2016 in Guelph, Hamilton and Peterborough, Ontario. Wild mammals were trapped using Tomahawk traps (sizes 106 and 108; Tomahawk Live Trap Co. Tomahawk, WI, USA). Raccoon (*Procyon lotor*) and striped skunk (*Mephitis mephitis*) trapping was conducted by the Ontario Ministry Natural Resources and Forestry (OMNRF). Baited (anchovies, cat food) traps were set in the evening and checked the following dawn. Raccoons and skunks were anesthetized with medetomidine (40–50 u g/kg) and tiletamine (1–2 mg/kg) with atipamezole hydrochloride (0.2-0.25 mg/kg) as a reversal agent. Baited (fruit, oats, peanut butter) traps for groundhogs (*Marmota monax*) and eastern gray squirrels (*Sciurus carolinensis*) were set before first light each morning and checked at mid morning. Groundhogs and gray squirrels were anesthetized within an induction chamber filled with 1–3% isoflurane gas (Baxter Corporation, Mississauga, Ontario, Canada). Anesthetized animals were weighed with a Pesola scale (Pesola AG, Chaltenbodenstrasse, Schindellegi, Switzerland) and identified with ear tags (raccoons and striped skunks; National Band and Tag Co. Newport, KY, USA) or subcutaneously injected pit tags (groundhogs and gray squirrels; Biomark, ID, USA). Each animal was examined for ticks over a 3-min period as described above for carcasses. Animals were then recovered from anesthesia and released at the point of capture.

All wildlife trapping and handling was approved by the Animal Care and Use Committee (University of Guelph AUP#3471 and OMNRF AUP#358) in compliance with National Institutes of Health guidelines for the care and use of laboratory animals.

2.2. Tick storage and identification

Ticks collected from wildlife were placed in cryovials and stored at 4 °C until identification (within 48 h), then transferred to −80 °C. Ticks from dogs and archived CWHC diagnostic cases were placed into 70% ethanol and maintained at room temperature. Ticks were morphologically identified to species, life stage, and sex using a dissecting microscope and taxonomic key (Lindquist et al., 2016). Specimen damage precluded the sex determination of a small proportion of adult ticks.

2.3. Pathogen detection

Ticks removed from the same host animal were sorted into pools of up to 10 conspecific adults or up to 25 nymphs (Dupuis et al., 2013). DNA was extracted by using QIAGEN DNeasy 96 tissue kits (Qiagen Inc., Mississauga, Canada).

Amblyomma americanum ticks were tested for *E. chaffeensis* using real-time polymerase chain reaction (PCR) targeting the 16S ribosomal RNA (rRNA) gene as previously described (Loftis et al., 2003). *Ixodes scapularis* ticks were screened by a duplex real-time PCR assay that targeted the 23S rRNA gene of *Borrelia* spp. (including all members of the *Bo. burgdorferi* sensu lato complex) and the *msp2* gene of *A. phagocytophilum* (Courtney et al., 2004). Samples that tested positive for *Borrelia* spp. were subjected to a confirmatory *ospA* real-time PCR assay for *Bo. burgdorferi* s.s. and a *glpQ* real-time PCR assay for *Bo. miyamotoi* (Dibernardo et al., 2014; Ullmann et al., 2005). In addition, DNA extracts from *I. scapularis* ticks were tested for *Ba. microti* by a real-time PCR assay targeting the *cctη* gene (Nakajima et al., 2008). All real-time PCR testing was performed at the National Microbiology Laboratory (Public Health Agency of Canada, Winnipeg, Manitoba, Canada) as previously described (Dibernardo et al., 2014).

2.4. Data and statistical analysis

Data for each animal from which ticks were collected included: date of death (carcass) or tick removal (live animals), host species, number of ticks removed, geographic coordinates of the location where found (wildlife) or town center of the veterinary clinic (dogs) and reported travel history outside of Ontario (dogs). In addition, for wild mammals, sex and age (i.e., immature < 1 year and adult ≥1 year) were recorded; the latter was determined by the animals' weight.

The prevalence, median, and exact 95% confidence intervals of tick-borne pathogens for each host species were estimated using STATA14® Intercooled (StataCorp, College Station, TX, USA).

3. Results

A total of 1687 ticks, comprised of six species, were collected from wildlife and companion animals in southern Ontario. This included 1580 ticks from wildlife and dogs collected from May 2015 to May 2017, and 107 ticks from archived CWHC wildlife diagnostic cases collected from 2011 to 2013. The most common tick species was *Ixodes texanus* (n = 686; 40.7%), followed by *Dermacentor variabilis* (n = 666; 39.5%), *I. cookei* (n = 275; 16.3%), *I. scapularis* (n = 43; 2.5%), *I. marxi* (n = 15; 0.9%) and *Amblyomma americanum* (n = 1; 0.1%). One tick was not taxonomically identified (Table 1). The majority of ticks was comprised of females (n = 1081; 64.1%), followed by males (n = 369; 21.9%), non-sexed adults (n = 126; 7.5%), nymphs (n = 108; 6.4%) and larvae (n = 3; 0.2%).

Raccoons (n = 224) and dogs (n = 50) were the most commonly examined tick-source animals. Most of the ticks collected from raccoons consisted of *I. texanus* (n = 666 ticks) and *D. variabilis* (n = 600). At least one individual of each of these tick species was removed from 58.5% (median: 2 ticks; range: 1–36 ticks) and 49.1% (median: 2 ticks; range: 1–64 ticks) of parasitized raccoons, respectively. Less commonly examined parasitized species included the striped skunk (n = 32) and groundhog (n = 12), for which *I. cookei* was the most common tick observed (Table 1).

Among *I. scapularis* tested, 9.3% (4/43; 95% CI: 2.6–22.1%) were positive for *Bo. burgdorferi* s.s. and 2.3% (1/43; 95% CI: 0.1–12.3%) for *A. phagocytophilum*. The *Bo. burgdorferi* s.s.-positive ticks were collected from a coyote and three domestic dogs with no reported history of travel outside of Ontario. The coyote was collected in the city of Clifford, Wellington County, in southwestern Ontario. One dog was

Table 1
Tick species and numbers collected from 334 parasitized wildlife and dogs by species from May 2015 to May 2017 and in archived tick samples collected from wildlife in southern Ontario, Canada from 2011 to 2013.

	Number of hosts with ticks (%; median;[a] range)	Number of ticks (%; 95% CI)	Adults (%; 95% CI)			Nymphs (%)
			Female	Male	Unknown	
Raccoon (*Procyon lotor*; n = 224); parasitized by 1381 total ticks						
Ixodes texanus	131 (58.5; 2; 1–36)	666 (48.2; 45.6–50.9)	590 (88.6; 85.9–90.9)	13 (2.0; 1.0–3.3)	46 (6.9; 5.1–9.1)	17 (2.6; 1.5–4.1)
Dermacentor variabilis	110 (49.1; 2; 1–64)	600 (43.4; 40.8–46.1)	278 (46.3; 42.3–50.4)	322 (53.7; 49.6–57.7)	0	0
I. cookei	20 (8.9; 1; 1–44)	100 (7.2; 5.9–8.7)	40 (40.0; 30.3–50.3)	0	45 (45.0; 35.0–55.3)	15 (15.0; 8.6–23.5)
I. marxi	3 (1.3; 5; 1–7)	13 (0.9; 0.5–1.6)	4 (30.8; 9.1–61.4)	0	6 (46.2; 19.2–74.9)	3 (23.1; 5.0–53.8)
I. scapularis	1 (0.4; 2; NA)	2 (0.1; 0.0–0.5)	2 (100.0; 15.8–1.0)	0	0	0
Dog (*Canis lupus familiaris*; n = 50); parasitized by 67 total ticks						
D. variabilis	24 (48.0; 1; 1–4)	37 (55.2; 42.6–67.4)	22 (59.5; 42.1–75.2)	15 (40.5; 24.8–57.9)	0	0
I. scapularis	25 (50.0; 1; 1–3)	27 (40.3; 28.5–53.0)	23 (85.2; 66.3–95.8)	3 (11.1; 2.4–29.2)	0	1 (3.7; 0.1.–19.0)
I. cookei	2 (4.0; 1; 1–1)	2 (3.0; 0.4–10.4)	1 (50.0; 1.3–98.7)	0	0	1 (50.0; 1.3–98.7)
Amblyomma americanum	1 (2.0; 1; NA)	1 (1.5; 0.0–8.0)	1 (100.0; 2.5–1.0)	0	0	0
Striped skunk (*Mephitis mephitis*; n = 32); parasitized by 118 total ticks						
I. cookei	26 (81.3; 1; 1–16)	92 (78.0; 69.4–85.1)	46 (50.0; 39.4–60.6)	0	5 (5.4; 1.8–12.2)	41 (44.6; 34.2–55.3)
I. texanus	2 (6.3; NA; 3–17)	20 (16.9; 10.7–25.0)	3 (15.0; 3.2–37.9)	0	17 (85.0; 62.1–96.8)	0
D. variabilis	3 (9.4; 1; 1–3)	5 (4.2; 1.4–9.6)	3 (60.0; 14.7–94.7)	2 (40.0; 5.3–85.3)	0	0
I. marxi	1 (3.1; NA; NA)	1 (0.8; 0.0–4.6)	1 (100.0; 2.5–1.0)	0	0	0
Groundhog (*Marmota monax*; n = 12)						
I. cookei	12 (100.0; 2; 1–11)	37 (100.0; 0.0–90.5)	17 (45.9; 29.5–63.1)	0	6 (16.2; 6.2–32.0)	14 (37.8; 22.5–55.2)
All other wildlife (n = 16)[b]						
I. cookei	8 (50.0; 4; 1–20)	41 (50.6; 39.3–61.9)	27 (65.9; 49.4–79.9)	2 (4.9; 0.6–16.5)	0	12 (29.3; 16.1–45.5)
D. variabilis	2 (12.5; NA; 1–23)	24 (29.6; 20.0–40.8)	16 (66.7; 44.7–84.4)	5 (20.8; 7.1–42.2)	0	3 (12.5; 2.7–32.4)
I. scapularis	6 (37.5; 1; 1–8)	14 (17.3; 9.8–27.3)	7 (50.0; 23.0–77.0)	7 (50.0; 23.0–77.0)	0	0
I. marxi	1 (6.3; 1; NA)	1 (1.2; 0.0–6.7)	0	0	0	1 (100.0; 2.5–1.0)
Unknown	1 (6.3; 1; NA)	1 (1.2; 0.0–6.7)	0	0	1 (100.0; 2.5–1.0)	0
Total	379	1687[c]	1081 (64.1; 61.7-66.4)	369 (21.9; 19.9-23.9)	126 (7.5; 6.3-8.8)	108 (6.4; 5.3-7.7)

CI: confidence interval.
NA: not applicable.
[a] Median number of ticks per parasitized animal.
[b] All other wildlife includes species with n < 5 (parasitized host animals): fisher (*Martes pennanti*; n = 3), red fox (*Vulpes vulpes*; n = 3), porcupine (*Erethizon dorsatum*; n = 2), beaver (*Castor canadensis*; n = 1), black bear (*Ursus americanus*; n = 1), Canada goose (*Branta canadensis*; n = 1), domestic cat (*Felis catus*; n = 1), red squirrel (*Sciurus vulgaris*; n = 1) and Virginia opossum (*Didelphis virginiana*; n = 1).
[c] The total number of ticks includes three *I. cookei* larvae collected from a groundhog.

from Lyndhurst, United Counties of Leeds and Grenville, eastern Ontario, and two were from Guelph, Wellington County, in southwestern Ontario. The *A. phagocytophilum*-positive tick was collected from a red fox (*Vulpes vulpes*) in Marysville, Frontenac County, eastern Ontario (Fig. 1). All 43 *I. scapularis* ticks tested negative for *Bo. miyamotoi* and *Ba. microti*. The single *A. americanum* tick collected was from a dog in Rockwood, Ontario and tested negative for *E. chaffeensis*.

4. Discussion

Monitoring for zoonotic tick-borne pathogens is important, not only for public health surveillance, but to help elucidate ongoing changes in geographic distribution and potential interactions among ticks, hosts, and pathogens. These interactions are complicated by dynamic and diverse environments that are continuously subjected to climatic and landscape changes. A better understanding of this complex interplay is useful in predicting patterns of pathogen emergence. The development of targeted tick-borne pathogen surveillance strategies and interpretation of the data generated depend upon knowledge of pathogen transmission cycles that may involve numerous mammalian vertebrate hosts and tick life stages (Dantas-Torres et al., 2012). The present study provides information on ticks, wildlife, and zoonotic, tick-borne pathogens in southern Ontario, Canada, a region susceptible to climate-change associated vector incursions and vector-borne disease spread (Ogden et al., 2006a).

The present study revealed that *I. scapularis* ticks collected from wildlife and companion animals in southern Ontario had evidence of *Bo. burgdorferi* s.s. and less commonly, *A. phagocytophilum*. The former is the causative agent of Lyme disease and the latter is the cause of human granulocytic anaplasmosis; both pathogens represent an emerging public health threat in Ontario and neighboring regions.

Raccoons were the most common species with ticks in the present study, followed by dogs, striped skunks and groundhogs. In addition, raccoons generally had the heaviest tick burdens, which included multiple tick species. Both raccoons and skunks are widely distributed and highly adapted wildlife species that successfully exploit both urban and rural environments shared by humans (Bondo et al., 2015). These traits also led to a bias toward raccoons as the most commonly sampled wildlife species in the present study.

Passive surveillance, which often relies on opportunistic sample collection, is a valuable tool for monitoring ticks and zoonotic, tick-borne pathogens (Nelder et al., 2014; Ogden et al., 2006b). For example, a recent analysis of samples submitted by members of the Ontario public illustrates the range expansion of *I. scapularis* in the province and highlights the increasing prevalence and non-uniform distribution of *Bo. burgdorferi* s.s. (Nelder et al., 2014). In addition, *A. phagocytophilum* was recently detected in *I. scapularis* in the Thousand Islands region of Ontario (Werden et al., 2015) as well as in a small percentage of ticks removed from humans in Ontario (0.3%; n = 14,369) (Nelder et al., 2014). Similarly, drag sampling for *I. scapularis* in Michigan revealed an expanding tick population as well as evidence of both *Bo. burgdorferi* s.s. and *A. phagocytophilum* (Hamer

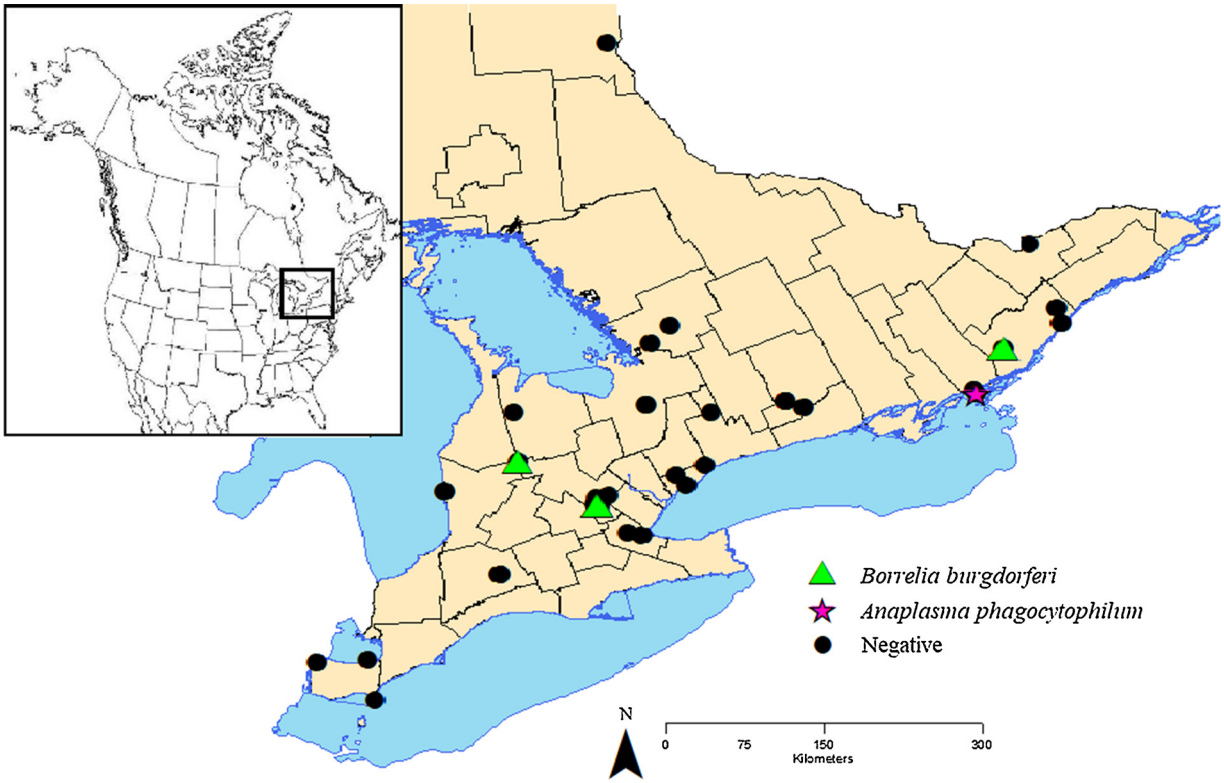

Fig. 1. Geographic distribution of tested *Ixodes scapularis* and *Amblyomma americanum* ticks opportunistically collected from free-ranging wildlife and dogs in southern Ontario, Canada between May 2015 and May 2017. *Ixodes scapularis* ticks (n = 43) were tested by real-time PCR for *Anaplasma phagocytophilum*, *Babesia microti*, *Borrelia miyamotoi*, *Bo. burgdorferi* sensu stricto, and *Amblyomma americanum* (n = 1) was tested by real-time PCR for *Ehrlichia chaffeensis*.

et al., 2007). The expanding range of *I. scapularis* and the zoonotic pathogens they transmit is more concerning because as a generalist feeder, *I. scapularis* infests a range of hosts that share common habitats, including humans and companion animals (Bouchard et al., 2015).

Ixodes texanus was the most common tick species collected from raccoons and was observed on nearly half of raccoons sampled. This tick species was also observed on a high percentage (~70%) of raccoons recently surveyed in the Ontario neighbor state of Michigan in the United States (Hamer et al., 2010). Information on *I. texanus* life history and distribution in Canada is scarce, and their role as a vector of tick-borne pathogens is not well defined (Lindquist et al., 2016). However, *I. texanus* ticks were observed on raccoons that tested seropositive for *Babesia lotori* and *Ehrlichia* spp. in the eastern U.S. and a small proportion of *I. texanus* collected from a southern flying squirrel (*Glaucomys volans*) and raccoon in Michigan tested positive for *Bo. burgdorferi* (Anderson et al., 1981; Dugan et al., 2005; Hamer et al., 2010). These results suggest the need to further investigate the role of *I. texanus* as a potential vector of zoonotic pathogens.

Dermacentor variabilis was the second most common tick species observed on raccoons in the present study. In addition to raccoons, *D. variabilis* ticks were also removed from dogs, striped skunks, coyotes and a Virginia opossum (*Didelphis virginiana*). These results support the prior observation that *D. variabilis* ticks have a broad host range (Lindquist et al., 2016). The observation of *I. cookei*, *I. marxi*, and *I. texanus* on striped skunks and *I. cookei* on groundhogs is also consistent with previous reports (Bishopp and Trembley, 1945; Ko, 1971; Lindquist et al., 2016). The infrequent collection of *A. americanum* in surveillance programs in Ontario, as well as from a dog in the present study, suggests that this species is not currently established in the province (Nelder et al., 2014; Scott et al., 2016). However, it has been detected in states bordering Ontario, including Michigan, where it was the fifth most common species submitted in a 12-year (1985–1996) passive surveillance study, and in New York State, where the range of *A. americanum* has expanded into northern and western regions (Means and White, 1997; Walker et al., 1998).

Wildlife are increasingly used in passive surveillance for pathogens of zoonotic and agricultural importance. However, this practice may include inherent limitations, such as lack of systematic sample collection over time and space. Also, the use of wildlife carcasses in tick-borne pathogen surveillance may lead to falsely low detection rates because ticks may drop off carcasses prior to necropsy, especially as the postmortem to sampling interval increases. Finally, adult ticks may be an over-represented life stage based on larger size and thus increased visibility.

Results from the present study provide further evidence of the presence of tick-borne pathogens in southern Ontario and outline the tick species commonly found on various peridomestic wildlife hosts within a heavily human-populated area. The detection of ticks on a variety of mammalian hosts justifies the inclusion of multiple wild and domestic animal species in surveillance to improve detection and track changes in the geographic distributions of targeted tick species and associated zoonotic, tick-borne pathogens. The detection of *Bo. burgdorferi* s.s. and *A. phagocytophilum* highlights the importance of continued tick surveillance and the promotion of public health awareness in southern Canada and other regions of similar latitudes that are sensitive to increased temperatures associated with global climate change.

Conflict of interest

The authors declare no conflict of interest.

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