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# Occurrence and distribution of *Ambylomma americanum* as determined by passive surveillance in Ontario, Canada (1999–2016)

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ABSTRACT

The lone star tick, *Amblyomma americanum*, is spreading northward from its historical stronghold in the southeastern United States. As a vector and biting pest, public and veterinary health officials must remain vigilant of the lone star tick's expanding range. We use ticks submitted to Public Health Ontario Laboratory (1999–2016) to describe the spatial and temporal dynamics of *A. americanum* in Ontario, Canada, as well as submitter demographics. We identified 847 *A. americanum* submissions during the surveillance period, with 773 (91.3%) non-travel-related and 74 (8.7%) travel-related submissions. Annual *A. americanum* submissions increased over the surveillance period. Approximately 91% of non-travel-related submissions were adult ticks and 9% were nymphs. The highest submission rates were from individuals living in the Eastern and South West regions of the province. Adult specimens were primarily submitted from May through July and nymphs from March through September. Higher numbers of submissions were from young children (< 10 years) and older adults (55–74 years), with equal proportions of male and female submitters. The majority of travel-related submissions were from travellers returning from the southeastern United States (i.e., Florida, North Carolina, South Carolina, Tennessee, Texas). *Amblyomma americanum* distribution is scattered in Ontario and submissions are likely the consequence of ongoing detection of adventive specimens. Further tick dragging is required to confirm the presence of established lone star tick populations in the province. Given the relatively rapid expansion of blacklegged ticks, *Ixodes scapularis*, populations in Ontario, we expect climate change to facilitate the range of expansion of *A. americanum* into the province. We propose an algorithm for identifying *A. americanum*-risk areas, which will aid public and veterinary health officials when assessing the risks posed by lone star ticks.

## 1. Introduction

The lone star tick, *Amblyomma americanum*, occurs in North America from approximately Maine to Florida in the east and from Nebraska to northwestern Mexico in the west (Childs and Paddock, 2003; Centers for Disease Control and Prevention, CDC, 2011). The first documentation of a lone star tick in Canada was from Aweme, Manitoba in 1912 (Gregson, 1956). In Canada, lone star ticks have been reported from Alberta, British Columbia, Manitoba, Newfoundland, Nova Scotia, Ontario and Quebec, although regular reports are typically from southern Ontario (Lindquist et al., 2016). For states bordering Ontario, lone star tick range includes Ohio, Pennsylvania and New York, with established populations concentrated in the southern portions of these states (Mean and White, 1997; CDC, 2011; Christenson et al., 2017). The range of *A. americanum* has expanded northward and westward from its historical range in the southeastern United States over the last several decades, especially where climate, habitat and hosts are amenable to tick survival and reproduction (Springer et al., 2014;

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Dahlgren et al., 2016; Monzón et al., 2016; Sonenshine, 2018). As with the blacklegged tick, *Ixodes scapularis*, climate change is contributing to the expanding range of the lone star tick (Springer et al., 2015; Ludwig et al., 2016; Clow et al., 2017; Sonenshine, 2018). Considering the expanding range of lone star ticks and the impacts of pathogens associated with these ticks, Ontario public and veterinary health officials need to remain vigilant of incursions and monitor for established populations.

*Amblyomma americanum* is a three-host tick that prefers deciduous forests with dense undergrowth, where they actively seek and blood feed on a variety of hosts. Most commonly associated with white-tailed deer and wild turkeys, *A. americanum*, depending on stage, feed on a wide range of large mammals (e.g., cattle, horses, humans), small and medium-sized animals (e.g., cats, coyotes, dogs, foxes, opossums, rabbits, racoons, rodents, squirrels) and birds (e.g., gallinaceous and passerine birds) (Childs and Paddock, 2003; Allan et al., 2010). Besides being a biting pest, *A. americanum* is a vector of several pathogens, including *Cytauxzoon felis* (agent of feline cytauxzoonosis), *Ehrlichia chaffeensis* (human ehrlichiosis), *E. ewingii* (human/canine ehrlichiosis), Panola Mountain *Ehrlichia* sp. (human/animal ehrlichiosis), *Francisella tularensis* (human/animal tularemia) and *Theileria cervi* (deer theileriosis) (Samuel and Trainer, 1970; Waldrup et al., 1992; Goddard and Varela-Stokes, 2009; Reichard et al., 2009). Additional pathogens have been detected in *A. americanum*, including: rickettsial agents *Rickettsia rickettsii*, *R. amblyommatis*, *R. montanensis* and *R. parkeri*; the spirochete *Borrelia lonestari*; and Heartland virus (HRTV) (family *Phenuiviridae*, genus *Phlebovirus*); however, the role of lone star ticks in transmission of these pathogens is not well described or is unknown (Smith et al., 2010; Fritzen et al., 2014; Godsey et al., 2016). In addition, these ticks are associated with galactose-alpha-1,3-galactose (alpha-gal) allergy or mammalian meat allergy and Southern Tick-Associated Rash Illness (STARI) in humans (Feder et al., 2011; Wolver et al., 2013). While historically considered a nuisance pest, *A. americanum* is increasingly associated with tick-borne diseases.

To determine the public and veterinary health risks posed by *A. americanum*, we assess their occurrence and distribution in Ontario. In addition, we describe the spatial and temporal dynamics of *A. americanum* submitted through passive tick surveillance from 1999 through 2016, describe submitter demographics and propose an algorithm for identifying *A. americanum*-risk areas.

## 2. Materials and methods

### 2.1. Study location

Ontario, Canada is located in the Great Lakes region of North America, with a population of 13.5 million. During the timeframe of data collection, 36 public health units (PHUs) administered public health services including facets of Ontario's passive tick surveillance program (Table A.1). PHUs are further organized into seven health regions: Central East (DUR, HKP, PEE, PTC, SMD, YRK), Central West (BRN, HAL, HAM, HDN, NIA, WAT, WDG), Eastern (EOH, HPE, KFL, LGL, OTT, REN), North East (ALG, NPS, PQP, SUD, TSK), North West (NWR, THB), South West (CHK, ELG, GBO, HUR, LAM, MSL, OXF, PDH, WEC) and Toronto (TOR).

### 2.2. Passive tick surveillance and data

Ontario's passive tick surveillance program has been described previously (Public Health Ontario, PHO, 2016a, b; Nelder et al., 2014). The Public Health Ontario Laboratory (PHOL) identifies ticks submitted by the public through health care professionals (e.g., clinics, hospitals) or PHUs, using standard identification keys (Cooley and Kohls, 1944; Clifford, 1961; Keirans and Litwak, 1989; Durden and Keirans, 1996; Keirans and Durden, 1998). A submitter is a member of the public from whom a tick was collected, or a person for whom a submission was made on their behalf (e.g., a parent submits a tick they found crawling or feeding on their child).

Data captured by the passive tick surveillance program include the submitter's city of residence, submitter age and sex, date of submission and travel information. Information on attachment or engorgement status of submitted ticks was not available. In addition, data include the species of tick identified, with corresponding stage (larva, nymph, adult) and sex of adult tick specimens. Submitters bring in ticks for identification to various settings (e.g., PHU offices, clinics, hospitals); data for submission setting type was available for ticks submitted from 2010 through 2016. We used the submitter's city of residence to aggregate data to the PHU-level. If city of residence data were missing, we used the location of PHU or healthcare facility responsible for the tick submission. Exact location of exposure or tick acquisition is usually unknown for submissions and, for the purpose of this research, we assumed the most likely exposure location was near the submitter's home or, more broadly, in vicinity of submitter's city of residence (Falco and Fish, 1998; Stafford, 2007). We used the date of submission to PHOL as the closest available date for tick exposure. Travel information was available for only a portion of the study period (2010–2016). In fall 2009, PHOL sent a letter to PHUs and health care professionals indicating that it would no longer accept ticks from companion animals for identification; therefore, data prior to 2010 possibly include specimens from companion animals. Several PHUs in southeastern Ontario that had established endemic areas of Lyme disease risk ceased accepting tick submissions in 2014 (EOH, KFL, LGL), potentially impacting subsequent detections of *A. americanum* (Nelder et al., 2014).

To determine if a submitter made multiple non-travel-related submissions (multiple submission event), first we matched submissions by common date of submission, then by common submitter age, sex and location of residence. We identify a submission cluster in a PHU, potentially indicative of an established population, when additional tick submissions occur before and after a multiple submission event.

### 2.3. Statistical analyses and mapping

We calculated the rate of *A. americanum* submissions per 100,000 population for all variables. We used population data and estimates from Statistics Canada via IntelliHEALTH Ontario to calculate submission rates (extracted 1 February 2017). Pearson correlations were used to assess the linear association of variables (e.g., number of ticks submitted by month or PHU, travel versus non-travel-related submissions), using Excel v14.0 (Microsoft, 2010).

We assessed the influence of year (1999–2016; 1999–2013, submissions halted in several PHUs after 2013), health region, submitter age and sex, season, tick life stage and tick sex on the overall rate of non-travel-related submissions (outcome) using univariable Poisson regression. We also assessed the influence of year and PHU on the rate of non-travel-related submissions for each health region (outcome) using Poisson regression.

We carried forward variables to multivariable modelling that were significantly associated with the overall rate of non-travel-related submissions, based on a liberal *p*-value of 0.2. Prior to model building, we assessed for potential confounding and interactions. To generate the multivariable model, each variable along with any potential confounders and interaction terms were added in a stepwise manner, combining forwards and backwards model building.

For all regression models (univariable and multivariable), we used PHU population for that year as the "exposure" variable, which created a rate of tick submissions based on the population at risk. We used Pearson's goodness of fit test to assess for overdispersion and overall model fit. If overdispersion was evident, we employed negative binominal regression. A significance level of $\alpha = 0.05$ was used for all statistical analyses. We used STATA v14.2 (StataCorp, 2015) for regression modelling. We created the submission rate map using Esri ArcGIS v10.3 (Esri, 2014), using quartiles for classifying PHUs.

### 2.4. Developing an algorithm for identifying A. americanum-risk areas

To develop an algorithm for identifying *A. americanum*-risk areas or established populations, we use existing definitions as a starting point and then refine these for the Ontario context by applying passive tick surveillance data. In 1997, the CDC (in-text personal communication) defined United States established populations as "six or more specimens confirmed from an area" (Means and White, 1997). Recently, Springer et al. (2014) classified lone star ticks at the county level: "established if six or more ticks, or two or more life stages, were recorded during a specified time period." Springer et al. (2015) updated their previous criteria with a temporal component: "*A. americanum* was categorized as 'established' in a county if six or more ticks, or two or more life stages, were associated with one or more collection records in a single year." Dahlgren et al. (2016) used a similar definition at the county level, with no temporal aspect: "established, if at least six ticks from one life stage or at least two life stages were found." Definitions for established populations are consistent with accepted criteria used for assessment of blacklegged tick populations (Dennis et al., 1998; Eisen et al., 2016). For United States definitions, total submissions include ticks submitted through passive surveillance only or via a combination of passive and active surveillance techniques; however, for Ontario we explicitly include an active surveillance component.

### 2.5. Ethics statement and data availability

This manuscript reports on routine surveillance activities, and therefore approval from the research ethics committee was not required. Information about PHO's data request process is available online at https://www.publichealthontario.ca/en/About/Pages/data.aspx.

## 3. Results

We identified 847 *A. americanum* from 1999 through 2016, including 773 (91.3%) non-travel-related and 74 (8.7%) travel-related submissions (Fig. 1). The median number of submissions per year from 1999 through 2004 was 24 (inter quartile range, IQR = 20.5–46.0), increasing to 38 from 2005 through 2010 (IQR = 34.0–64.0) and 70.5 from 2011 through 2016 (IQR = 63.5–85.0). Submissions from 2010 through 2016 (n = 468) were made through various settings: clinics/health practitioner offices (n = 188), PHU offices (n = 178), hospitals (n = 98), private laboratories (n = 3) and other (n = 1).

### 3.1. Non-travel-related submissions

90.8% (702/773) of non-travel-related submissions were adult specimens and 9.2% (71/773) were nymphs; 438 (62.4%) of adult ticks were female and 264 (37.6%) were male (Table 1). There was a significantly higher rate of adult versus nymph submissions (incidence rate ratio, IRR = 1.38; 95% confidence intervals, CI, 1.08–1.77; $p = 0.009$), based on univariable analysis.

Adult specimens were primarily submitted from May through July and nymphs from March through September (Fig. 2, Table 1), with a higher rate of submissions in spring (IRR = 2.20; 1.21–3.99; $p = 0.01$) and summer (IRR = 2.04; 1.11–3.74; $p = 0.02$) compared to fall, based on univariable analysis. The median number of non-travel-related submissions per year from 1999 through 2004 was 24 (IQR = 18.8–44.0), increasing to 35 during the 2005–2010 period (IQR = 26.5–54.0) and 62.5 during the 2011–2016 period (IQR = 47.5–70.0).

The highest non-travel-related submission rates included TSK (2.79 per 100,000; n = 2 tick submissions), HUR (1.68 per 100,000; n = 9), PDH (1.29 per 100,000; n = 13), ELG (1.13 per 100,000; n = 7), CHK (0.93 per 100,000; n = 10) and OXF (0.92 per 100,000; n = 18), with no submissions from NWR and THB (Fig. 3). In the Central East region, submission rates were higher in HKP and PTC, and lower in PEE, compared to the referent DUR (for consistency, referent PHUs were chosen alphabetically; Table 2). In Central West, there were lower submission rates from HAL, HAM, NIA and WAT, compared to BRN. In the Eastern region, there was a lower submission rate from OTT, compared to EOH. In the South West region, there were lower submission rates from MSL and WEC, compared to CHK.

Sex of submitter was available for 88.5% (684/773) of non-travel-related submissions, with 50.4% (345/684) from females and 49.6% (339/684) from males (Table 1). Age was available for 85.5% (661/773) of submitters and the mean age was 45.8 ± 0.93 years. Relatively higher numbers of submissions were from young children (< 10 years) and older adults (55–74 years). No significant differences were detected in the rate of submissions by sex ($p > 0.5$) or by age category ($p = 0.7$).

The explanatory variables of year (up to 2013), season, health region, tick life stage and tick sex were included in multivariable modelling. Health region showed evidence of confounding with all the above variables and was the only variable that remained significant ($p < 0.05$) in the multivariable model.

We identified 25 instances of multiple submissions by a given individual, involving 44 adult and 11 nymph specimens. Multiple

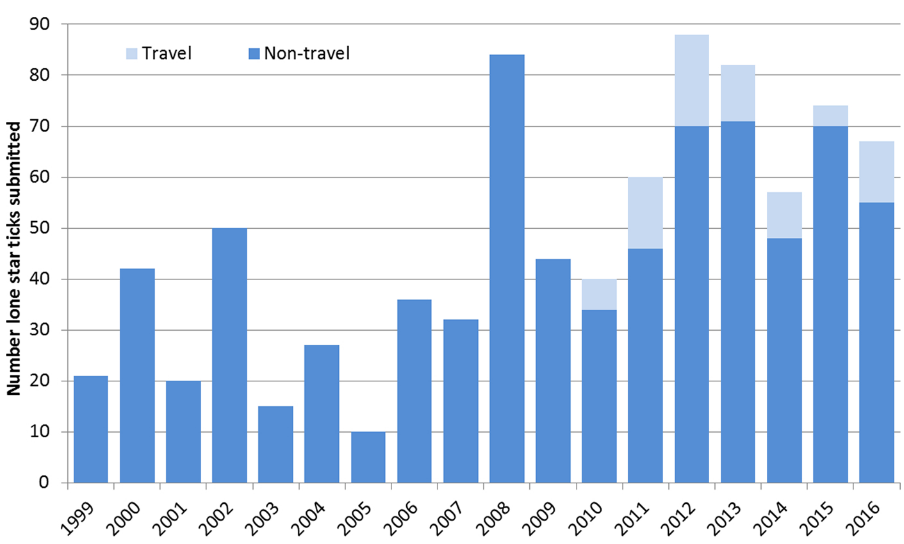


**Fig. 1.** Travel-related and non-travel-related *A. americanum* submissions by year: Ontario, Canada (1999–2016)[a].
[a]Includes travel-related (n = 773) and non-travel-related (n = 74) related submissions. Travel history information was not formally reported for submissions from 1999 through 2009; therefore, all submissions for this period are assumed non-travel-related submissions.

**Table 1**
Non-travel-related *A. americanum* submission rates and univariable analyses by explanatory variable: Ontario, Canada (1999–2016).[a]

| Explanatory variable | Category | Number submitted | Rate per 100,000 population (SD) | Incidence rate ratio | 95% confidence interval | *p*-value | Likelihood ratio test of alpha = 0, *p*-value value |
|---|---|---|---|---|---|---|---|
| Year (1999–2016) (n = 773) | 1999–2004 | 175 | 0.40 (0.39) | Referent | | | |
| | 2005–2010 | 239 | 0.39 (0.32) | 0.97 | 0.77–1.23 | 0.8 | < 0.001 |
| | 2011–2016 | 359 | 0.40 (0.35) | 0.96 | 0.78–1.20 | 0.8 | |
| Year (1999–2013) (n = 600)[b,c] | 1999–2003 | 148 | 0.40 (0.41) | Referent | | | |
| | 2004–2008 | 189 | 0.40 (0.33) | 1.02 | 0.82–1.26 | 0.8 | – |
| | 2009–2013 | 263 | 0.43 (0.33) | 1.17 | 0.96–1.44 | 0.1 | |
| Season (n = 773)[c] | Fall (Oct–Dec) | 11 | 0.29 (0.31) | Referent | | | |
| | Winter (Jan–Mar) | 37 | 0.29 (0.27) | 1.67 | 0.85–3.27 | 0.1 | – |
| | **Spring (Apr–Jun)** | **526** | **0.40 (0.35)** | **2.20** | **1.21–3.99** | **0.01** | |
| | **Summer (Jul–Sep)** | **199** | **0.41 (0.46)** | **2.04** | **1.11–3.74** | **0.02** | |
| Public health region[c] | Toronto | 92 | 0.04 (0.002) | Referent | | | |
| | **Eastern** | **316** | **0.58 (0.35)** | **9.04** | **6.93–11.80** | **< 0.001** | – |
| | **Central East** | **159** | **0.25 (0.21)** | **4.07** | **3.15–5.27** | **< 0.001** | |
| | **Central West** | **179** | **0.32 (0.22)** | **6.73** | **5.24–8.66** | **< 0.001** | |
| | **North East** | **27** | **0.89 (0.57)** | **19.79** | **12.89–30.39** | **< 0.001** | |
| | **South West** | **184** | **0.63 (0.42)** | **10.88** | **8.47–13.97** | **< 0.001** | |
| Submitter age (n = 661) | < 19 | 123 | 0.35 (0.33) | Referent | | | |
| | 19–35 | 78 | 0.40 (0.36) | 0.94 | 0.65–1.32 | 0.7 | < 0.001 |
| | 36–65 | 317 | 0.39 (0.38) | 1.05 | 0.82–1.34 | 0.7 | |
| | > 65 | 143 | 0.40 (0.32) | 1.10 | 0.82–2.47 | 0.5 | |
| Submitter sex (n = 684) | Female | 345 | 0.40 (0.37) | Referent | | | |
| | Male | 339 | 0.40 (0.34) | 0.96 | 0.80–1.15 | 0.7 | < 0.001 |
| Tick life stage (n = 773)[c] | Nymph | 71 | 0.34 (0.30) | Referent | | | |
| | **Adult** | **702** | **0.40 (0.35)** | **1.38** | **1.08–1.77** | **0.009** | – |
| Tick sex (n = 702)[c] | Nymph | 71 | 0.34 (0.35) | Referent | | | |
| | Male | 264 | 0.36 (0.31) | 1.28 | 0.94–1.77 | 0.1 | – |
| | **Female** | **438** | **0.43 (0.38)** | **1.46** | **1.08–1.98** | **0.01** | |

Bolded categories and values represent significant differences compared to referent.
[a] Univariable analysis was based on Poisson or negative binomial regression with population of that public health unit for the corresponding year as the exposure variable. We excluded the North West region as no ticks were submitted from that health region.
[b] 2014–2016 excluded, as passive submissions to PHU offices ceased in 2014 (i.e., EOH, KFL, LGL).
[c] Poisson regression.

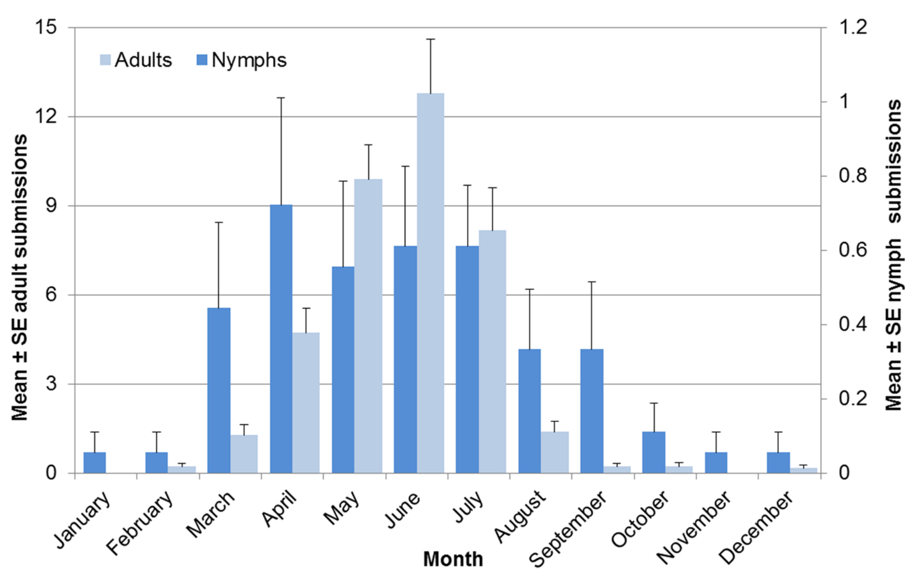


**Fig. 2.** Mean non-travel-related *A. americanum* submissions by stage and month: Ontario, Canada (1999–2016)[a].
[a]Includes all non-travel-related submissions (n = 773).

submissions primarily occurred from April through June (42 of 55 specimens). Three submissions included more than two ticks: DUR (April 2014; 2 females, 1 nymph); WAT (March 2011; 3 nymphs); and WEC (April 2013; 2 females, 1 male). We detected several clusters of lone star tick submissions, potentially indicative of risk-areas or established populations. One cluster was centered in WEC (April 2013); submissions did not include nymphs and annual tick counts were ≤6 (n = 4 ticks in 2011; 6 in 2012; 5 in 2013; 4 in 2014). A second cluster was centered in SMD (April 2015); submissions did not include nymphs and annual tick counts were ≤5 (3 in 2013; 5 in 2014; 4 in 2015; 5 in

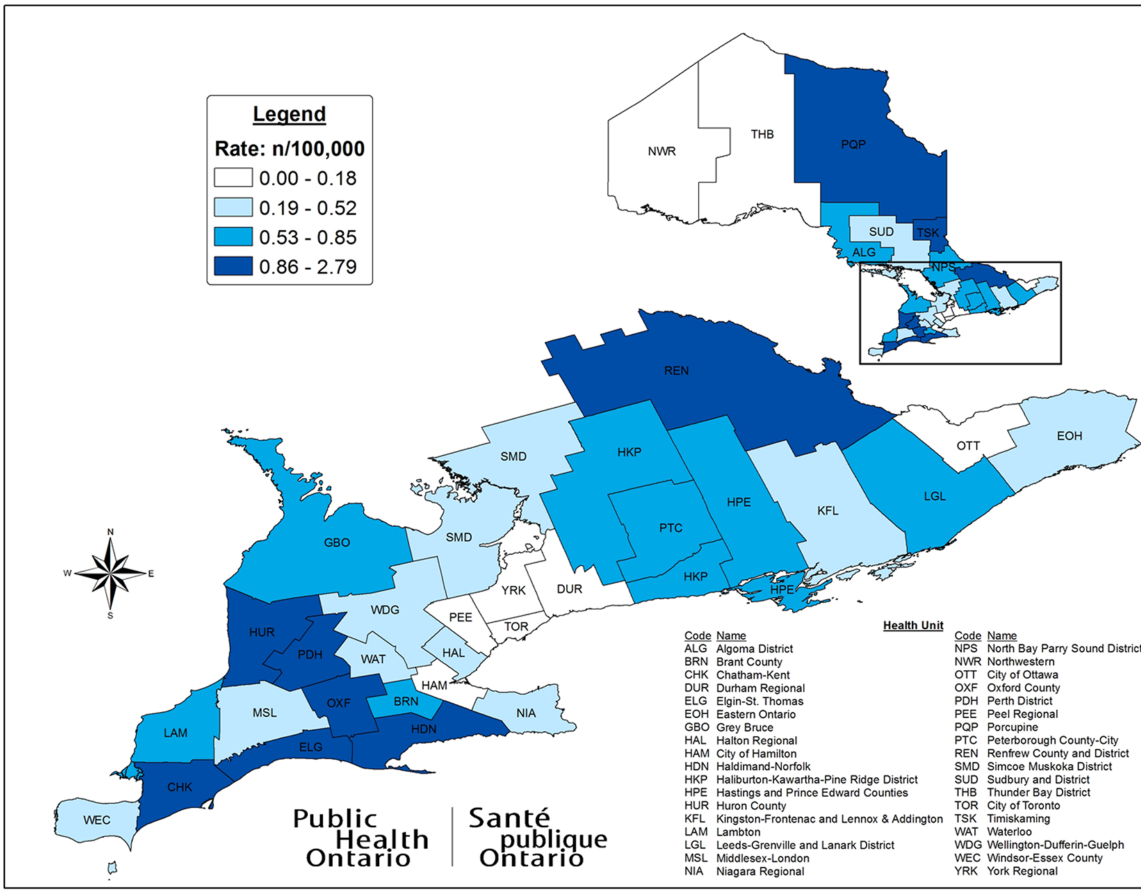


**Fig. 3.** Non-travel-related *A. americanum* submission rates by public health unit: Ontario, Canada (1999–2016)[a,b].
[a]Submissions are based on the public health unit where the submitter resides, and do not necessarily reflect the location of where the tick was acquired.
[b]PHU, number submitted, submission rate per 100,000 population (SD). ALG, 5, 0.85 (0.02); BRN, 10, 0.73 (0.03); CHK, 10, 0.93 (0.02); DUR, 27, 0.16 (0.01); ELG, 7, 1.13 (0.03); EOH, 7, 0.49 (0.01); GBO, 29, 0.62 (0.01); HAL, 35, 0.22 (0.03); HAM, 21, 0.18 (0.01); HDN, 15, 0.91 (0.01); HKP, 19, 0.56 (0.01); HPE, 22, 0.61 (0.01); HUR, 9, 1.68 (0.03); KFL, 49, 0.52 (0.01); LAM, 17, 0.76 (0.01); LGL, 26, 0.59 (0.002); MSL, 22, 0.22 (0.01); NIA, 37, 0.22 (0.004); NPS, 12, 0.78 (0.004); NWR, 0, 0; OTT, 22, 0.11 (0.005); OXF, 18, 0.92 (0.03); PDH, 13, 1.29 (0.01); PEE, 32, 0.08 (0.01); PQP, 1, 1.16 (0); PTC, 15, 0.73 (0.02); REN, 6, 0.96 (0.02); SMD, 45, 0.19 (0.01); SUD, 7, 0.50 (0.002); TOR, 92, 0.04 (0.002); THB, 0, 0; TSK, 2, 2.79 (0); WAT, 32, 0.19 (0.01); WDG, 29, 0.37 (0.02); WEC, 59, 0.25 (0.005); YRK, 21, 0.11 (0.02).

2016). A third cluster was centered in KFL (May 2011); submissions included nymphs (n = 2; 2011) and annual tick counts were ≤7 (7 in 2011; 6 in 2012; 7 in 2013). We identified additional PHUs with multiple submission events throughout southern Ontario; however, the events lacked additional retrospective and prospective submissions.

### 3.2. Travel-related submissions

15.8% (74/468) of submitters reported travel outside of Canada (2010–2016) (Fig. 1, Table A.2). 82.4% (61/74) of travel-related submissions were adult specimens and 17.6% (13/74) were nymphs. 62.3% (38/61) of adult ticks were male and 37.7% (23/61) were female.

Travellers submitted ticks from April through June (adults: 52/61 or 85.2%; nymphs: 8/13 or 61.5%). Travel-related submissions by month were correlated with non-travel-related submissions by month ($r^2 = 0.71$; $p = 0.01$). The median number of travel-related submissions per year was 11 (IQR = 6.0–14.0).

Travel-related submissions were most common from SMD (n = 9) and YRK (n = 8) (Table A.2). Travel-related submissions by PHU were not correlated with non-travel-related submissions by PHU ($r^2 = 0.28$; $p = 0.1$). Submitter travel destinations included the United States (n = 67), Belize (n = 2), Peru (n = 2) and one each from Costa Rica, Mexico and Panama. United States-related-travel submissions were most common from Florida (n = 15), South Carolina (n = 8), Tennessee (n = 7), North Carolina (n = 6) and Texas (n = 6).

Sex of submitter was available for 87.8% (65/74) of travel-related submissions, with 40.0% (26/65) of submissions from females. Age was available for 93.2% (69/74) of submitters and the mean age was 55.3 ± 1.91 years; there was no significant difference in age of female (54.8 ± 3.31 years) and male submitters (56.4 ± 2.39 years) ($F_{(1 63)} = 0.14$; $p = 0.7$).

### 3.3. Proposed algorithm for identifying A. americanum-risk areas

We propose an algorithm for identifying *A. americanum*-risk areas in Ontario (Fig. 4). Taking a strict dichotomous approach to monitoring *A. americanum*, as in our proposed algorithm, is not ideal under all circumstances. Rather, we view the algorithm as a basic step in lone star tick surveillance, where the assumption is that lone star tick densities are low in the environment. For example, if tick dragging produces large numbers of specimens (e.g., > 50) in a location, then a PHU may want to immediately declare an *A. americanum*-risk area and communicate this risk to the public. In addition, the detection of a high density of ticks in an area, through alternate active or passive means (e.g., large numbers identified through veterinarians, examination of hunter-killed deer) could potentially expedite the declaration of a risk area.

**Table 2**
Univariable analyses for non-travel-related *A. americanum* submission rates by year and health region: Ontario, Canada (1999–2016).

| Health region | Explanatory variable[a] | Category | Incidence rate ratio | 95% confidence interval | *p*-value |
|---|---|---|---|---|---|
| Central East (n = 159) | Year | 1999–2004 | Referent | | |
| | | 2005–2010 | 0.90 | 0.59–1.38 | 0.6 |
| | | 2011–2016 | 0.98 | 0.66–1.46 | 0.9 |
| | PHU | DUR | Referent | | |
| | | **HKP** | **3.50** | **1.94–6.30** | **< 0.001** |
| | | **PEE** | **0.51** | **0.30–0.84** | **0.009** |
| | | **PTC** | **4.55** | **2.42–8.56** | **< 0.001** |
| | | SMD | 1.21 | 0.75–1.94 | 0.4 |
| | | YRK | 0.64 | 0.36–1.14 | 0.1 |
| Central West (n = 179) | Year | 1999–2004 | Referent | | |
| | | 2005–2010 | 0.91 | 0.62–1.34 | 0.6 |
| | | 2011–2016 | 0.74 | 0.52–1.06 | 0.1 |
| | PHU | BRN | Referent | | |
| | | **HAL** | **0.29** | **0.14–0.58** | **0.001** |
| | | **HAM** | **0.25** | **0.12–0.53** | **< 0.001** |
| | | HDN | 1.23 | 0.55–2.75 | 0.6 |
| | | **NIA** | **0.31** | **0.15–0.62** | **0.001** |
| | | **WAT** | **0.26** | **0.13–0.54** | **< 0.001** |
| | | WDG | 0.51 | 0.25–1.04 | 0.06 |
| Eastern (n = 119)[b] | Year | 1999–2003 | Referent | | |
| | | 2004–2008 | 0.71 | 0.40–1.26 | 0.2 |
| | | 2009–2013 | 0.67 | 0.40–1.13 | 0.1 |
| | PHU | EOH | Referent | | |
| | | HPE | 1.23 | 0.45–3.34 | 0.7 |
| | | KFL | 1.04 | 0.41–2.62 | 0.9 |
| | | LGL | 1.19 | 0.46–3.12 | 0.7 |
| | | **OTT** | **0.23** | **0.08–0.60** | **0.003** |
| | | REN | 1.96 | 0.57–6.76 | 0.3 |
| North East (n = 27) | Year | 1999–2004 | Referent | | |
| | | 2005–2010 | 0.82 | 0.23–2.89 | 0.7 |
| | | 2011–2016 | 0.85 | 0.34–2.16 | 0.7 |
| | PHU | ALG | Referent | | |
| | | NPS | 0.92 | 0.32–2.60 | 0.9 |
| | | PQP | 1.36 | 0.16–11.68 | 0.8 |
| | | SUD | 0.59 | 0.19–1.85 | 0.4 |
| | | TSK | 3.27 | 0.63–16.83 | 0.2 |
| South West (n = 184) | Year | 1999–2004 | Referent | | |
| | | 2005–2010 | 0.97 | 0.64–1.47 | 0.9 |
| | | 2011–2016 | 1.13 | 0.78–1.63 | 0.5 |
| | PHU | CHK | Referent | | |
| | | ELG | 1.22 | 0.46–3.19 | 0.7 |
| | | GBO | 0.66 | 0.32–1.36 | 0.3 |
| | | HUR | 1.80 | 0.73–4.43 | 0.2 |
| | | LAM | 0.82 | 0.37–1.78 | 0.6 |
| | | **MSL** | **0.24** | **0.11–0.51** | **< 0.001** |
| | | OXF | 0.99 | 0.46–2.14 | > 0.9 |
| | | PDH | 1.39 | 0.61–3.16 | 0.4 |
| | | **WEC** | **0.27** | **0.14–0.52** | **< 0.01** |
| Toronto (n = 92)[c] | Year | 1999–2004 | Referent | | |
| | | 2005–2010 | 0.97 | 0.56–1.70 | 0.9 |
| | | 2011–2016 | 0.91 | 0.55–1.53 | 0.7 |

PHU, public health unit. Bolded categories and values represent significant differences compared to referent.
[a] Univariable analysis was based on Poisson regression with population (for the corresponding sex and age category in that public health unit) as the exposure variable. We excluded the North West region because there were no tick submissions from that health region.
[b] 2014–2016 excluded, as passive submissions to public health unit offices ceased in 2014 (i.e., EOH, KFL, LGL).
[c] Toronto is both a health region and a public health unit, so we only explored year.

## 4. Discussion

Currently, there is no evidence for the presence of established *A. americanum* populations in Ontario; however, continued passive and active surveillance is warranted to assess public and veterinary health risks. While *A. americanum* is not yet a permanent resident of Ontario, animals and humans are still at risk of lone star tick bites and infection from associated pathogens. Annual *A. americanum* submissions increased over the surveillance period and will likely continue to increase. From 1967 through 1977, the Ontario public submitted an average of less than one lone star tick per year to public health (Scholten, 1977). In the northern United States, researchers have noted nominal increases in lone star tick submissions from Maine (1989–2006), Michigan (1985–1996) and New York (1988–1996); however, these studies were not contemporaneous to our study, making comparisons difficult (Means and White, 1997; Walker et al., 1998; Rand et al., 2007). Nonetheless, average annual submissions of *A. americanum* are higher in Ontario (47 per year; 1999–2016), compared to Maine (≈6 per year), Michigan (≈19 per year) and New York (≈27 per year) (Means and White, 1997; Walker et al., 1998; Rand et al., 2007). In an *A. americanum*-established area such as Monmouth County, New Jersey, passive submissions have increased from just over 100 in 2006 to over 450 in 2015 (average ≈ 200 per year) (Egizi et al., 2017). Encountering lone star ticks in Ontario is gradually becoming more common, but anticipating where there is increased risk of exposure remains difficult to predict due to their scattered distribution.

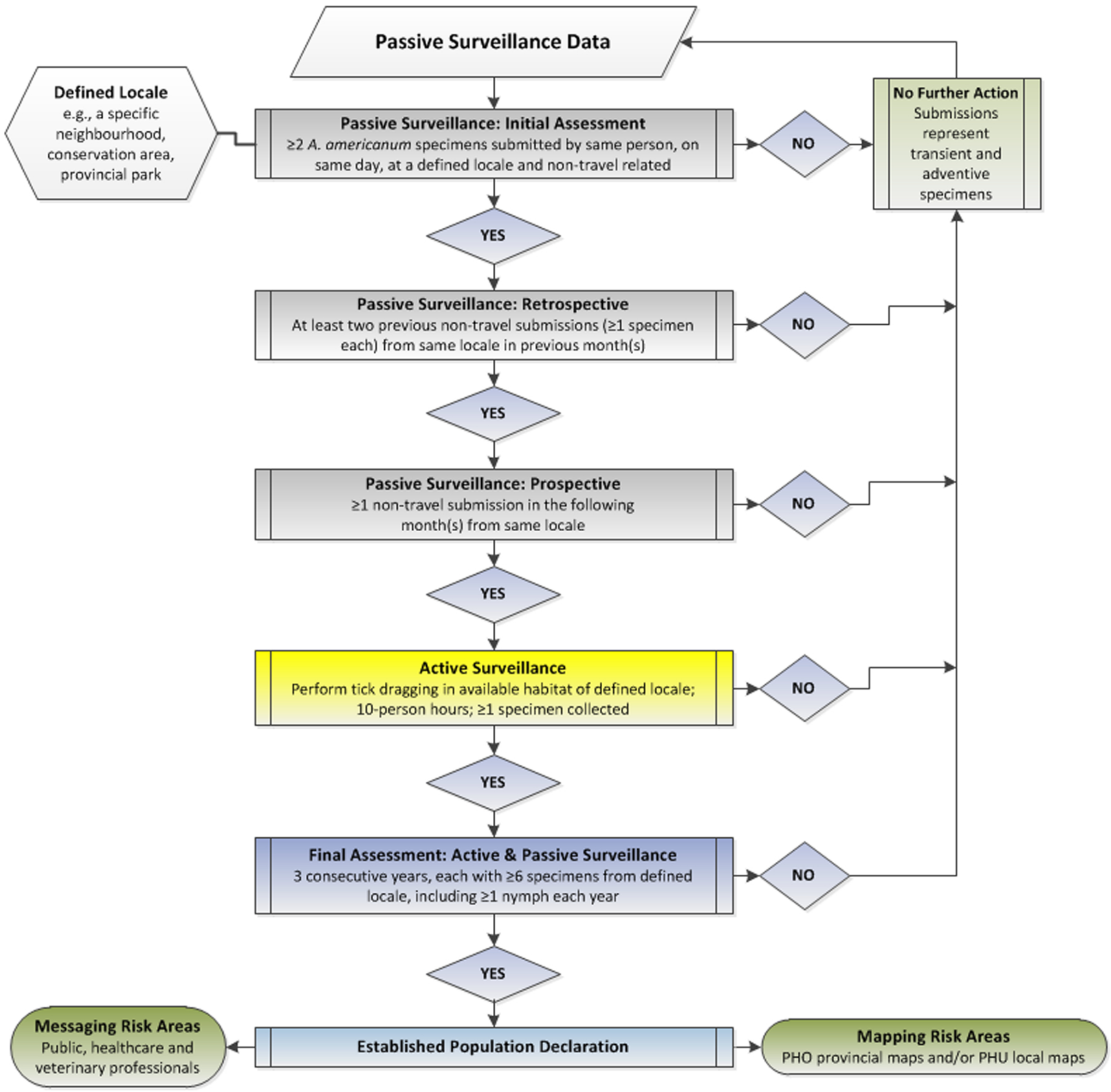


**Fig. 4.** Proposed algorithm for identifying *A. americanum*-risk areas in Ontario, Canada[a].
[a]Tick dragging should occur in the late morning and early afternoon during May, June and July. Tick dragging should be performed in deciduous forests with undergrowth.

*Amblyomma americanum* submissions are widely distributed in southern Ontario, with increased submission rates noted for several regions, specifically the North East, Eastern and South West regions. We expected a relatively higher submission rate from the Eastern region of the province, a region dominated by deciduous forest (preferred habitat of the lone star tick); however, the higher submission rate in the South West was unexpected. The South West region is dominated by agricultural land interspersed with small pockets of forest; therefore, habitat alone cannot explain the distribution we see with lone star tick submissions in Ontario, at least at the scale of this study. Ludwig et al. (2016) noted that the Montréal Region of Québec, Canada, is suitable for lone star tick establishment; thus, we assume similar and suitable habitat exists in portions of southern Ontario. The high rate in the North East region is most likely a reflection of very low population density. Rate of submissions per 100,000 was chosen for the passive surveillance data because we know submissions can be highly influenced by population density (Nelder et al., 2014). However, it is only a proxy measure and may be skewed in areas where population density is very low or very high. Explanations for the observed lone star tick distribution in Ontario will require further hypothesis testing, but avenues of investigation include variations in host densities, weather variables and habitat types. The scattered distribution of *A. americanum* likely reflects the adventitious nature of the species in Ontario.

The seasonality of *A. americanum* submissions in Ontario is similar to the seasonality reported from areas where the tick is established (Kollars et al., 2000). In Ontario, adult tick submissions start to increase in April, then peak from May through July, with fewer submissions after July. Seasonality is less discernable for nymphs due to the lower numbers submitted in Ontario; however, submissions increase in March and remain relatively higher through September. Peak activity of *A. americanum* ticks in Ontario is similar to activity in Missouri, with higher adult activity from May through July and higher nymphal activity higher from May through August (Kollars et al., 2000). Simulations of seasonality, modelled for *A. americanum* in Montréal, Québec, reflect a similar seasonality as we report for Ontario, but with nymphs peaking slightly later than adults in Montréal (Ludwig et al., 2016). The seasonality of adventitious lone star ticks reported here is similar to the seasonality of populations in the United States.

The demographics of *A. americanum* submitters are similar to those reported for blacklegged ticks. The age of submitters are similarly bimodal in both species, with higher submission rates from those less than 10 and those 55–74 years of age (Nelder et al., 2014). The average age of submitter for lone star ticks is approximately 46 years, compared to 41 years for blacklegged tick submitters. The distribution of lone star tick submissions by sex of submitter was equal for men vs. women, in contrast to *I. scapularis* where more submissions are from men (Nelder et al., 2014). The similar submitter demographics for lone star and blacklegged ticks point to a public locally exposed to ticks at similar rates independent of tick species, which may indicate a shared habitat for the two tick species.

The northward expansion of *A. americanum* populations is commonly attributed to the re-introduction and population growth of their primary hosts (i.e., white-tailed deer, wild turkeys), coupled with increased temperatures (Felz et al., 1996; McShea et al., 1997; Childs and Paddock, 2003). White-tailed deer and wild turkey are relatively abundant in southern Ontario; however, correlating tick and host abundance is difficult because PHU-level data are not available for hosts (Ontario Ministry of Natural Resources, 2007; Ontario Ministry of Natural Resources and Forestry, 2017). Other factors contributing to the northward expansion of lone star ticks include adaptive evolution (high genetic diversity increasing the tick's ability to exploit more environmental conditions) and land use changes (farmland succession to secondary growth forest) (Springer et al., 2015; Dahlgren et al., 2016; Monzón et al., 2016; Sonenshine, 2018). Research indicates established populations of *A. americanum* in western New York (1988–1996), yet we have not detected a population in the adjacent Niagara Regional health unit (Means and White, 1997). Since there are large bodies of water separating southern Ontario and adjacent states, it is doubtful that white-tailed deer and other terrestrial animals transport substantial numbers of lone star ticks into Ontario. Migratory birds possibly introduce *A. americanum* into certain areas of Ontario; yet, a study examining over 39,000 migratory birds in the spring in Canada did not identify any lone star ticks (Ogden et al., 2008). In Ogden et al. (2008), the proportion of migratory waterfowl examined was low ($< 0.2\%$ ducks or geese); therefore, migratory waterfowl potentially contribute to *A. americanum* entering the province (Jordan et al., 2009). Lone star ticks are likely entering Ontario through a variety of migratory bird species, supplemented by humans and companion animals returning from the United States. As increasing temperature due to climate change has contributed to the spread of blacklegged ticks into southern Canada, we expect a similar development for *A. americanum* in Ontario (Ogden et al., 2006; Clow et al., 2017). Considering increasing temperatures, presence of suitable hosts and habitat, and proximity to established populations, we expect the lone star tick to establish in Ontario with an undetermined timeline.

Travel-related *A. americanum* submissions were primarily from travellers returning from the southern United States. The southern United States supports established *A. americanum* populations and is a popular destination for Canadian vacationers; thus, we expected higher numbers of submissions from Ontarian travellers to the region. A previous report of tick submissions to public health in Ontario (1967–1977) noted three submissions from Virginia and one each from Arkansas, South Carolina and Texas (Scholten, 1977). On several occasions, travelers to the United States returned to Ontario with multiple specimens and likely entered habitat with large numbers of *A. americanum*. For example, in Missouri, tick collections over 12 days in 2013 included more than 38,000 lone star ticks, compared to 900 American dog ticks (*Dermacentor variabilis*) (Savage et al., 2016). Public and veterinary health officials should advise Canadian travellers of tick exposure risks and the appropriate use of personal protection measures (avoidance of high-risk habitats, use of tick repellents) for themselves and their companion animals (Beck et al., 2009; Ministry of Health and Long-Term Care, 2018).

While Ontario has criteria for determining blacklegged tick-risk areas, public and veterinary health in the province does not have similar criteria for *A. americanum* (Ogden et al., 2014; PHO, 2016a, 2017). Using United States-based definitions for established populations, and applying Ontario surveillance data, we propose criteria for identifying *A. americanum*-risk areas (Means and White, 1997; Springer et al., 2014, 2015; Dahlgren et al., 2016). Based on continued multiple non-travel-related submissions from individuals, adventive *A. americanum* have survived in Kingston-Frontenac and Lennox & Addington, Simcoe Muskoka District and Windsor-Essex County; however, submissions did not persist at high enough numbers to indicate permanent populations. Compared to criteria developed for the United States, an established population in Ontario requires three consecutive years of six or more submissions per year (with $\geq 1$ specimen from tick dragging and $\geq 1$ nymph per year), to ensure that a local population has completed its life cycle, which can take two years or more to complete (Troughton and Levin, 2007). The numbers used for these criteria are not underpinned by rigorous ecological research on the lone star tick, but rather defined based on convenience for the purposes of surveillance. There is an opportunity to test these criteria and thresholds to determine if they reflect established populations of the lone star tick, especially in areas with emerging populations. The United States-based definitions assume that when data indicate an established population in a county, then officials deem the county as established in perpetuity. In contrast, the Ontario criteria require multi-year assessments, an important consideration given the transient nature of the Ontario *A. americanum* populations identified (clusters of lone star tick submissions). We include standardized tick dragging in our algorithm because we cannot guarantee submissions that lack travel information truly represent Ontario-acquired ticks (PHO, 2016b). Our proposed criteria for *A. americanum*-risk areas and their utility as a surveillance tool will require annual evaluations, with improvements made as new surveillance data or approaches indicate.

Several opportunities are available to augment and improve *A. americanum* surveillance in Ontario. The passive surveillance data used to signal active surveillance could include both public- and veterinary-derived specimens. For example, we can supplement existing passive surveillance data with the submission of ticks from veterinary practices, where ticks are collected from companion animals during routine examinations. Collecting ticks during veterinary care, on condition that there is a clear exposure history, will provide an additional signal of where to perform active surveillance. While not widely used by public and veterinary health, there is an opportunity to improve passive tick surveillance through the submission of digital tick images by the public. Submission of images is a useful adjunct to passive tick surveillance, especially for *A. americanum*, a species with a patent silvery-white spot on the scutum of females and the only *Amblyomma* species routinely encountered in Ontario (Nelder et al., 2014). Health officials can supplement passive tick surveillance data by using tick images submitted from the public or their pets, including the eTick and Pet Tick Tracker web-based applications. For active surveillance, the examination of wildlife, such as hunter-killed deer, wild turkeys and/or waterfowl will help identify *A. americanum* hosts in the province (Bouchard et al., 2013; Christenson et al., 2017). Another effective active surveillance tool for *A. americanum* is the use of $CO_2$-baited traps or drag cloths, techniques that exploit the aggressive and mobile nature of lone star ticks (Kensinger and Allan, 2011; Mays et al., 2016). Data derived from public and veterinary health professionals will inform one another of potential clusters of incipient populations of lone star ticks.

The consistent submission of *A. americanum* specimens allows for several avenues of investigation. For instance, we can assay submitted ticks for the presence of both animal and human pathogens (e.g., *Ehrlichia* spp., *Rickettsia* spp., HRTV). Physicians and veterinarians alike should monitor and assess patients for illnesses associated with *A. americanum* pathogens, especially those with unexplained febrile illnesses and histories of lone star tick exposures. Web-based animal tick surveillance (i.e., *Pet Tick TrackerPet Tick Tracker*), coupled with public health-based passive and active tick surveillance, is well positioned as an important One Health tool for the protection of animal and human health.

The scattered distribution of the *A. americanum*, the limited multiple specimens per submitter and the relatively low numbers encountered further suggest that the lone star tick is not yet a permanent resident in Ontario. Tick dragging throughout the province since 2008 has not detected *A. americanum* populations, but single specimens have been encountered at Rondeau Provincial Park (Chatham-Kent), Thousand Islands National Park (Leeds-Grenville and Lanark District) and the City of Hamilton (CBR unpublished data) (Nelder et al., 2014). In addition, Clow et al. (2016,2017,2018) conducted widespread drag sampling from May through October (2014–2016) throughout southern Ontario (> 100 sites, > 300-person hours of dragging) and did not find lone star ticks. In neighbouring Michigan (1985–1996), *A. americanum* was the fifth most submitted tick, yet was not routinely collected in the state's active tick surveillance (Walker et al., 1998). Public health officials in Ontario perform drag sampling for *I. scapularis* in the spring (May) and fall (October) when blacklegged tick adults are most abundant; therefore, these collections possibly miss questing adult *A. americanum*. However, if a lone star tick population is present, trained tick draggers should be able to detect specimens during these months in appropriate habitats, even if tick densities are low. To date, the scarcity of *A. americanum* during tick dragging is consistent with the premise that adventive ticks occur in Ontario but populations have not yet taken up permanent residence.

We present data based on information submitted through a passive surveillance system; therefore, our results have several limitations and should be interpreted with caution. The absence of travel information does not imply a submitter did not travel outside of Ontario, rather the information was not included on the submission form or the submitter failed to report travel. Conversely, the presence of travel history does not necessarily mean the submitter acquired the tick during travel. If the majority of our non-travel-related submissions were truly travel-related, then we would not expect the correlation we found between month of submission for travel- and non-travel-related submissions (would expect no correlation, with higher travel-related submissions during peak travel periods). In addition, we would expect a correlation between travel-related and non-travel-related submissions by PHU (with higher travel-related submissions from PHUs with higher non-travel submissions); however, the two submission types were not correlated. Our passive tick surveillance system likely underestimates the true risk of *A. americanum* exposure in the province because not all people will either notice a tick on them or submit a tick once detected. Non-submission of ticks is possibly due to variable tick awareness and healthcare-seeking behaviours of the public.

Based on existing data, we cannot confirm there are established populations of *A. americanum* in Ontario; however, this assertion comes with several caveats. First, established populations are hypothetically surviving at low numbers, but we cannot detect populations with our existing passive tick surveillance program. Second, public health officials and researchers perform routine tick dragging activities targeting blacklegged ticks at times of the year when lone star ticks are not active (or active at low numbers). Third, tick dragging for blacklegged ticks is not occurring in areas where *A. americanum* is present and we are missing these populations. Fourth, the cessation of tick submissions to PHU offices in 2014 (i.e., EOH, KFL, LGL) means additional submission clusters were possibly missed. Fifth, determining if established *A. americanum* populations exist in Ontario requires targeted tick dragging, guided in part by passive submissions.

## 5. Conclusions

*Amblyomma americanum* distribution is scattered in Ontario and submissions are likely the consequence of ongoing detection of adventive specimens. Further tick dragging is required to confirm the presence of established lone star tick populations in the province. Given the recent establishment and relatively rapid expansion of *I. scapularis* populations in Ontario, we expect climate change to facilitate the range expansion of *A. americanum* into the province. Our proposed algorithm for defining risk areas will aide public and veterinary health officials with assessing the risks posed by lone star ticks.

## Conflict of interest

The authors do not have any conflicts of interest to report.

## Author contributions

MPN, KMC - conceptualization, methodology, formal analysis, interpretation, visualization, writing original draft, review and editing; CBR, SJ - methodology, formal analysis, interpretation, visualization, writing original draft (parts), review and editing; CMJ, JSW, FR, SNP - data curation, review and editing

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ttbdis.2018.10.001.

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