Ticks and Tick-borne Diseases 15 (2024) 102413

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Original article

The distribution of *Babesia odocoilei* in *Ixodes* species ticks in Canada: Implications for one health surveillance

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ARTICLE INFO

Keywords:
Cervid babesiosis
Babesia odocoilei
Tick-borne diseases
Canada

ABSTRACT

Ixodes scapularis and *Ixodes pacificus* are vectors of a range of pathogens of public health significance in North America. These ticks transmit pathogens to and from wild animal reservoir host species, but also bite humans and expose them to the pathogens. We describe the geographical and temporal distribution of the pathogen *Babesia odocoilei*, the causative agent of cervid babesiosis. *Ixodes* spp. ticks collected through active and passive

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https://doi.org/10.1016/j.ttbdis.2024.102413
Received 5 August 2024; Received in revised form 25 October 2024; Accepted 9 November 2024
Available online 20 November 2024

North America
Surveillance

surveillance were submitted to the National Microbiology Laboratory of the Public Health Agency of Canada for analysis of the presence of *B. odocoilei* from 2018 to 2021. Generalized linear models were constructed to evaluate the temporal change of *B. odocoilei* prevalence across Canada. *Babesia odocoilei*-positive *I. scapularis* are widespread across south-central and eastern regions of Canada, with an overall prevalence of 12.0 % in both nymphs (CI 95 % : 11.4–12.6) and adults (CI 95 % : 11.9–12.1) collected in passive surveillance and 13.2 % (CI 95 % : 12.9–13.5) and 10.0 % (CI 95 % : 9.8–10.2) in nymphs and adult, respectively, collected in active surveillance. A single *I. pacificus* tick tested positive in active surveillance out of 29 ticks collected in British Columbia, while no *B odocoilei*-positive *I. scapularis* were found in passive surveillance among the 11 adult ticks tested. Although *B. odocoilei* infection prevalence of adult *I. scapularis* was significantly higher in 2019 (14.1 %) than in 2018 (7.4 %), it remained stable from 2019 to 2021, suggesting that this pathogen may already be well established in endemic tick populations. The results provided in this article represent, to date, the most comprehensive picture of *B. odocoilei* distribution and prevalence in ticks in Canada and highlight the interest of maintaining One Health surveillance approaches to give added insight into disease transmission cycles for less well-characterized microorganisms.

1. Introduction

In many parts of the world, including Canada, tick-borne diseases (TBDs) represent a growing public and animal health challenge due to the expanding range and increased abundance of vector ticks, driven by climatic and ecological changes (Bouchard et al., 2019; Boyer et al., 2022; Kulkarni et al., 2015; Ogden et al., 2006; Ostfeld and Brunner, 2015). Adding to this challenge, the complex transmission cycles of some of the known tick-borne pathogens remain incompletely understood. The TBD with the highest incidence in humans in Canada is Lyme disease (LD) and cases are nationally notifiable (Elmieh, 2022). Cases of less common TBDs such as anaplasmosis, human babesiosis and Powassan virus disease are also thought to be increasing and became nationally notifiable in early 2024. While ongoing monitoring exists for the emergence of TBDs of humans, livestock (Chilton et al., 2018) or domestic animals (Duplaix et al., 2021) in Canada, those affecting solely wildlife species may be overlooked.

Ixodes scapularis and *Ixodes pacificus*, known as the blacklegged tick and the western blacklegged tick, respectively, are well studied in North America as they are primary vectors for *Borrelia burgdorferi* sensu stricto (s.s.), the primary agent of LD in humans, dogs and horses. These ticks are vectors for other pathogens of both humans and non-humans such as *Anaplasma phagocytophilum* and *Babesia microti;* pathogens of humans such as *Borrelia miyamotoi*, Powassan virus, as well as species that are not considered to affect humans such as *Babesia odocoilei* (Bouchard et al., 2019; Kukina et al., 2018; Zintl et al., 2003).

Historically, most ticks collected in Canada as part of surveillance for TBDs of public health importance have been submitted to the Public Health Agency of Canada's National Microbiology Laboratory (NML), through academic research groups or provincial public health laboratories for identification and/or testing for tick-borne pathogens affecting humans (Koffi et al., 2012; Guillot et al., 2020; Ogden et al., 2010). Testing for pathogens that primarily affect domesticated and/or wild animals, has not routinely occurred; however, since 2018, molecular testing of ticks for *B. odocoilei* has been conducted at the NML to distinguish this pathogen from related species such as *B. microti, Babesia duncani* and *Babesia divergens*, which are known zoonotic disease agents.

Babesia spp. are tick-borne apicomplexan parasites that mostly infect erythrocytes in their mammalian hosts (Krause, 2019). The most well-known *Babesia* spp. associated with cervids and other ungulates are *B. odocoilei* in North America and *B. divergens* in Europe (Bajer et al., 2022; Fannelli, 2021). Infections of *B. odocoilei* have been associated with disease in cervids such as elk (*Cervus elaphus canadensis*), caribou (*Rangifer tarandus caribou*), and reindeer (*Rangifer tarandus*) held in captivity (Gallatin et al., 2003; Mathieu et al., 2018), while *B. divergens* is well recognised as a pathogen of cattle (Zintl et al., 2003). Similar to many other species of *Babesia, B. odocoilei* can be transmitted transovarially from an infected female to its offspring (Zembsch et al., 2021). Infection with *Babesia* spp. may cause clinical disease in animals as a consequence of intravascular haemolysis, with resulting potentially fatal anaemia (Carter and Rolls, 2022; Fahrimal et al., 1992; Mathieu et al., 2018; Milnes et al., 2019; Waldrup et al., 1989; Zembsch et al., 2021). Subclinical infections can also occur, and infected animals often become asymptomatic carriers (Gallatin et al., 2003; Schoelkopf et al., 2005). Unlike *B. divergens*, which can cause disease in immunosuppressed, particularly splenectomised, humans, the zoonotic potential of *B. odocoilei* is yet to be confirmed (Kukina et al., 2018; Zintl et al., 2003). No cases of human babesiosis caused by *B. odocoilei* have been confirmed in Canada, although two publications suggest the possibility of human infection (Maggi et al., 2024; Scott et al., 2021a).

The natural transmission cycle of *B. odocoilei* remains mostly unstudied. White-tailed deer (*Odocoileus virginianus*) are thought to be the main reservoir, but it remains unknown if other species contribute to the transmission cycle. In southern Québec, *B. odocoilei* DNA was detected in the liver of a shrew (Crandall et al., 2022), but it is unknown if shrews can act as reservoirs of the pathogen or if this animal was just transiently infected. To our current knowledge, blacklegged ticks and western blacklegged ticks are the main vectors of *B. odocoilei*, but the presence of cervid babesiosis (confirmed by blood smear evaluation, PCR, and/or DNA sequence analysis of clinical samples) in geographic locations where these tick species have not become established, such as in Saskatchewan (Pattullo et al., 2013), suggests there may be other tick vector species, e.g., *Dermacentor albipictus* (winter tick).

Cervid babesiosis in domesticated or captive ungulates has been reported across several US states, including New Hampshire, New York, Pennsylvania, Indiana, Texas, Minnesota, and Wisconsin (Holman et al., 1994; Gallatin et al., 2003; Petrini et al., 1995; Schoelkopf et al., 2005). The first infected animal in Canada was found in 2012 in central Saskatchewan (Pattullo et al., 2013) and six years later, cervid babesiosis was detected in several provinces, including Manitoba, Ontario, and Québec (Mathieu et al., 2018). Despite these findings, the geographical distribution of *B. odocoilei* in Canada remains poorly documented. Surveillance reports produced by federal, provincial public health and the Canadian Lyme Sentinel Network (CaLSeN) focus on pathogens that infect humans, and data on *B. odocoilei* have been reported only in a few research publications to date (Crandall et al., 2022; Robinson et al., 2022; Scott et al., 2021b). Given the growing presence of ticks, both in geographic distribution and density, it is pertinent to assess whether *B. odocoilei* is an emerging pathogen showing increasing prevalence within tick populations. In this article, we use data from *Ixodes* spp. ticks collected for public health surveillance or research activities to describe the distribution of *B. odocoilei* in *Ixodes* spp. in Canada and explore if there is evidence of its emergence in tick populations.

2. Materials and methods

We conducted a retrospective analysis of infection prevalence of *B. odocoilei* in *Ixodes* spp. collected between 2018 and 2021 submitted to the NML branch of the Public Health Agency of Canada by provincial/territorial, CaLSeN, or academic partners (Guillot et al., 2020; Wilson

et al., 2023) or to the University of Ottawa laboratory. This study analysed data on ticks collected by both passive surveillance and by drag sampling during active field surveillance (termed active surveillance in this article).

2.1. Passive surveillance

Passive tick surveillance has been conducted since the early 1990s in Canada (Ogden et al., 2006). The program included the voluntary collection of ticks by members of the public when a person or their pet had an attached tick, which was then submitted directly to the NML or via medical or veterinary clinics, regional public health authorities, or other institutions. Tick specimens were morphologically identified to species using standard keys (Durden and Keirans, 1996; Lindquist et al., 2016). Tick engorgement was evaluated using a binocular microscope and classified using standard keys (unfed, slightly, partly, and fully). *Ixodes scapularis* and *I. pacificus* tick submissions with documented location of likely origin (census subdivision: CSD) and without travel history outside their province of residence were used for this study. Here, a tick submission refers to the event of tick submission by an individual; submissions may consist of either a single tick or multiple ticks, depending on how many ticks the individual found and submitted for analysis.

2.2. Active surveillance

For our study, we used a federal database that comprises ticks collected by drag sampling by provincial public health agencies, academic, and other organizations and subsequently tested at the NML. Drag sampling involves dragging a 1 m^2 piece of white flannel horizontally on the ground to capture questing ticks (Rulison et al., 2013). Studies using this method have been ongoing in Canada since the 2000s for surveillance and research purposes. Ticks collected in the course of research initiatives are sent to the NML and then collated into the active surveillance database and can be subsequently used for surveillance purposes. Within the federal database, the location of tick collection is georeferenced as the centroid of the park or the municipality where the specimens were found. Additional data from the University of Ottawa laboratory supplemented the dataset, as some of the specimens from active surveillance conducted in Ontario are directed to this laboratory for tick identification and laboratory analyses.

2.3. Laboratory analyses

Adult, nymphal, and larval tick specimens from passive and active surveillance were analysed. Ticks originating from multiple submissions in passive surveillance i.e., more than one tick collected from a single individual, were pooled together for testing, whereas single submissions were processed individually. DNA from tick specimens collected in passive surveillance was extracted using DNeasy 96 tissue kits (Qiagen, Hilden, Germany) while RNA was extracted from ticks collected in active surveillance using Qiagen RNeasy 96 kits, in both cases according to manufacturer's protocols. Extracted DNA and RNA was tested for pathogens at the NML including *Babesia* species, *Borrelia* species, and *A. phagocytophilum* and Powassan virus as previously described (Robinson et al., 2022).

Briefly, *Babesia* testing was performed using a triplex real-time in-house PCR assay that includes i) primers for the 18S rRNA gene of pan-*Babesia* species, with confirmation of *B. microti* and *B. odocoilei* infection via species-specific probes; and ii) a *B. odocoilei*-specific primer pair and confirmatory probe (Table S1). All triplex PCR reactions were run for 50 °C for 2 min and 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1min. This assay system can detect *B. odocoilei* that co-occur in *B. microti* infected ticks. Samples that were pan-*Babesia* and *B. odocoilei* positive in the triplex were considered *B. odocoilei* positive, while samples that were pan-*Babesia* and *B. microti* positive were further confirmed using a real-time PCR targeting the chaperonin-containing t-complex eta of *B. microti* (Nakajima et al., 2009). Samples that were only pan-*Babesia* positive in the triplex were sent for Sanger sequencing for species-level identification. The University of Ottawa laboratory extracted DNA from whole tick specimens using QIAamp DNA Mini Kits and RNA using RNesay Mini Kits (Qiagen). Primers, probes, and the run protocol were identical to procedures used at the NML, but only adults and nymphs were tested.

2.4. Data analysis

2.4.1. Distribution of Babesia odocoilei-infected Ixodes spp. ticks

The geographical distribution of positive *Ixodes* spp. from passive and active surveillance was mapped using ArcGIS version 10.8.2, Esri, Redlands, California, USA. Data from 2018 to 2021 were aggregated at the CSD for ease of visualisation. Estimates of the prevalence of *B. odocoilei* in ticks in passive surveillance data are presented at the centroid of the CSD, while for ticks collected in active surveillance, the prevalence estimates are presented at the centroid of the sampling sites or CSD in which the tick was collected if precise sampling location was not available. For passive surveillance, prevalence is determined by calculating the percentage of positive submissions, as some submissions involved multiple ticks. Since only a small proportion of submissions included multiple ticks (see results), it was considered more informative to retain these data rather than exclude them. To ensure more reliable prevalence estimates, the prevalence of infection with *B. odocoilei* in ticks was displayed only from locations with at least 30 tick specimens. The boreal forest layer was overlayed to depict ecozones that are assumed to be less suitable for the establishment of *I. scapularis* and *I. pacificus*, and provide context for CSDs with significant amounts of unsuitable habitat from where ticks are more likely to have been adventitious ticks rather than ticks from established populations (Ginsberg et al., 2004; Ginsberg and Zhioua, 1996; layer data from Natural Resources Canada – Canadian Forest Service : Boreal forest (canada.ca)).

2.4.2. Emergence of Babesia odocoilei

To investigate if there is evidence of the emergence of *B. odocoilei* in Canada, two statistical models were built using laboratory results from *I. scapularis* passive surveillance specimens. Passive surveillance data were used for this purpose as this dataset contained a greater number of tick submissions across a greater number of CSDs compared to active surveillance. Active and passive surveillance datasets were not combined, as these two surveillance methods provide a different portrait of the spatial distribution of ticks. Usually, active surveillance efforts are focused in areas of scientific interest, i.e. where ticks are likely to be found, thus creating a selection bias in the analysis. Conversely, passive surveillance often reflects a bias towards high density population centres where people are most likely to live and to report ticks. There were too few *I. pacificus* specimens to perform statistical analyses. Submissions comprising of ticks of different stages were excluded as they were pooled for testing, and it was not possible to determine the infection status according to stage. Generalized linear models (GLM) with a logit link function and model diagnostics were performed with R software version 4.0.3 using the *glmmTMB, DHARMa* and *emmeans* packages (Russel, 2016; Hartig, 2022; Brooks et al., 2017).

Model 1 explored variation in the probability that *I. scapularis* tested positive for *B. odocoilei* amongst years of sampling. This model was used to assess if the odds of ticks testing positive for *B. odocoilei* in Canada has increased across time, supporting the hypothesis that *B. odocoilei* is emerging in the country. The dataset for Model 1 included all passive surveillance submissions of *I. scapularis* adults (Model 1a) or nymphs (Model 1b).

Model 2 was used to explore variations amongst years in the probability that at least one tick tested *B. odocoilei*-positive in a specific CSD, thus including only CSDs with at least one submission of an adult (Model

2a) or nymphal (Model 2b) *I. scapularis* in passive surveillance. The objective of this model was to evaluate whether the geographical range of *B. odocoilei* expanded over time, thereby assessing the hypothesis that the emergence of *B. odocoilei* by geographic range expansion is a phenomenon in Canada.

The covariates *region* of submission (Québec, Ontario, Western/Central Canada, Atlantic Canada), *tick engorgement* (unfed, slightly, partly, and fully), which could influence the likelihood that ticks test positive for microorganisms (Nelder et al., 2021), and *latitude* of the CSD were explored as covariates in Model 1. The category Western/Central Canada included the provinces of Alberta, British Columbia, Manitoba, and Saskatchewan, whilst the Atlantic Canada category included New Brunswick, Newfoundland and Labrador, Nova Scotia and Prince-Edward-Island. In Model 2, the covariates *year, latitude* of the CSD and *region* of submission were included. The number of *submissions* (log transformed) of adults (Model 2a) or nymphs (Model 2b) was included as an offset. Computed this way, the model output represents the odds of having at least one positive adult or nymph per logarithm unit of adults or nymphs submitted at the CSD level.

Model selection was conducted in a uniform manner for all models. First, univariable associations between outcome variables and covariates were tested with a Pearson χ^2 test or Fisher's exact test. Strength of association was evaluated with Cramer's V or Cohen's D. Then, covariates with a significant univariable association ($\alpha = 0.2$) were included in multivariable selection. The contribution of covariates to explaining outcome variance was tested with the Log-likelihood ratio test (LRT). A covariate was kept in the final model if it significantly explained some of the outcome variance at the level $\alpha = 0.05$. Final model residuals were tested for normality and homoscedasticity with the Kolmogorov-Smirnov and quantile regression tests, respectively (with a criterion of $\alpha = 0.05$). Model fit was tested with Pearson χ^2 test on Pearson residuals ($\alpha = 0.05$) and residual spatial dependency was tested on response residuals with Moran I test ($\alpha = 0.05$). Post-hoc Tukey-adjusted tests for multiple comparisons were conducted to assess pairwise comparisons between all possible pairs of groups, considering the categorical nature of predictor variables within the models.

3. Results

3.1. Passive surveillance

Between 2018 and 2021, 22,633 tick submissions were tested through the federal passive surveillance program. For this study, we included only 14,158 tick submissions that were *I. scapularis* (n = 14,085) or *I. pacificus* (n = 12) and that had no confirmed history of travel prior to detection of the tick. Most of these submissions were adult ticks (n = 12,988), 1034 were nymphs, and 19 were larvae. All *I. pacificus* submissions were adult ticks. Forty-nine (49) submissions included multiple life stages, from a total of 861 tick submissions (6.1 %) with multiple ticks. Overall, 12.0 % (n = 1698) of tick submissions collected through passive surveillance tested positive for *B. odocoilei* including 1561 adult (12.0 %), 124 nymphal (12.0 %), and 2 larval (10.5 %) tick submissions (Table 1). None of the 12 *I. pacificus* ticks submitted were positive for *B. odocoilei*. Other *Ixodes* spp. e.g., *Ixodes angustus* (n = 4), *Ixodes muris* (n = 54), *Ixodes dentatus* (n = 2), and *Ixodes spinipalpis* (n = 1) were negative for *B. odocoilei*. From the same subset of tick submissions (*I. scapularis* or *I. pacificus* without history of travel), 17.8 % (n = 2521) were positive for *B. burgdorferi* s.s., 1.2 % (n = 174) were positive for *A. phagocytophilum*, and 0.1 % (n = 16) were positive for *B. microti*.

At least one *I. scapularis* or one *I. pacificus* was submitted from a total of 1178 CSDs (out of 5161 CSDs in Canada) and at least one *B. odocoilei*-infected *I. scapularis* was submitted for 458 (38.9 %) of these CSDs (Fig. 1). There were 26 CSDs with a *B. odocoilei* prevalence in the upper quartile (14.6–22.5 %), 54 CSDs in the interquartiles (9.6–14.5 %) and 25 in the lower quartile (>0–9.5 %) and all infected ticks were

Table 1
Number and percentage of B. odocoilei-positive (Bo+) I. scapularis (a) and I. pacificus (b) per stage and per province from the ticks collected from 2018 through 2021 from passive surveillance data.

a) Ixodes scapularis

c	2018						2019						2020						2021					
	Adult			Nymph			Adult			Nymph			Adult			Nymph			Adult			Nymph		
Province	Bo+	N	%	Bo+	N	%	Bo+	N	%	Bo+	N	%	Bo+	N	%	Bo+	N	%	Bo+	N	%	Bo+	N	%
Alberta	0	2	0.0	0	0	.	0	0	.	0	0	.	0	0	.	0	0	.	0	1	0.0	0	0	.
Manitoba	1	86	1.2	0	19	0	6	101	5.9	3	27	11.1	1	2	50.0	0	1	0.0	0	3	0.0	0	1	0.0
New Brunswick	47	621	7.6	9	71	12.7	83	590	14.1	6	87	6.9	38	404	9.4	2	29	6.9	3	39	7.7	0	5	0.0
Newfoundland	1	25	4.0	0	0	.	6	29	20.7	0	1	0.0	0	1	0.0	0	0	.	0	0	.	0	0	.
Nova Scotia	3	24	12.5	0	5	0	4	26	15.4	2	2	100.0	4	26	15.4	0	3	0.0	2	14	14.3	3	8	37.5
Ontario	137	1906	7.2	19	124	15.3	469	3311	14.2	31	258	12.0	251	1801	13.9	6	57	10.5	121	911	13.3	18	139	12.9
Prince Edward Island	0	2	0.0	0	0	.	1	8	12.5	0	0	.	3	13	23.1	0	0	.	0	0	.	0	0	.
Quebec	59	901	6.5	7	63	11.1	172	1084	15.9	9	68	13.2	99	602	16.4	1	21	4.8	50	431	11.6	8	45	17.8
Total	248	3567	6.9	35	282	12.4	741	5149	14.4	51	443	11.5	396	2849	13.9	9	111	8.1	176	1399	12.6	29	198	14.6

b) Ixodes pacificus

	2018			2019			2020			2021		
	Adult			Adult			Adult			Adult		
Province	Bo+	N	%	Bo+	N	%	Bo+	N	%	Bo+	N	%
Alberta	0	1	0.0	0	0	.	0	0	.	0	0	.
British Columbia	0	1	0.0	0	6	0.0	0	1	0.0	0	3	0.0

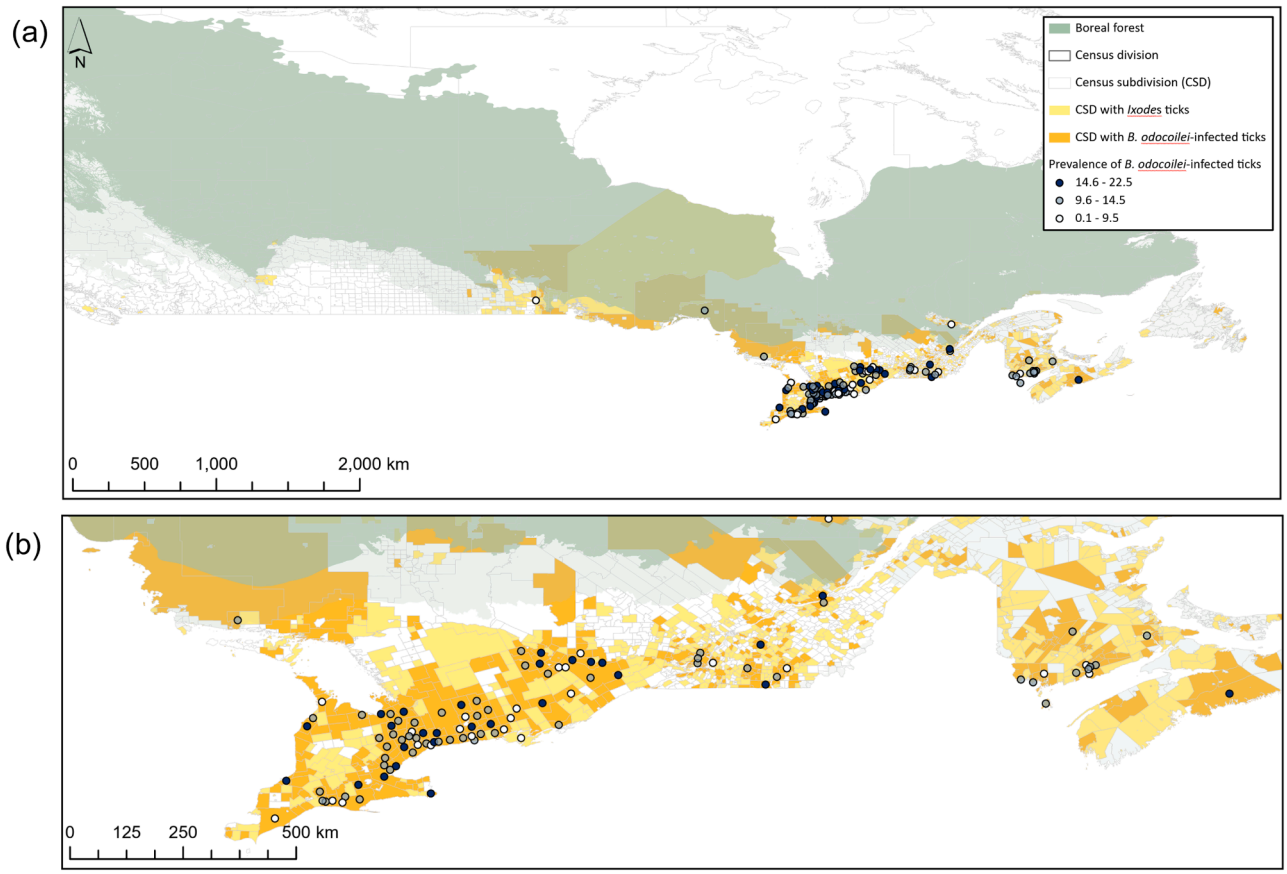

Fig. 1. Passive surveillance data, 2018–2021.
The geographic distribution of *Ixodes scapularis* and *Ixodes pacificus, Babesia odocoilei*-infected ticks, and the prevalence of *B. odocoilei*-infected ticks from federal passive surveillance data (2018–2021) in (a) southern Canada and (b) southeastern Canada.
The documented presence of at least one *I. scapularis* or *I. pacificus* submission at the CSD level is indicated by yellow, and CSDs with at least one *B. odocoilei*-infected tick are indicated by dark orange. Prevalence of *B. odocoilei* in *I. scapularis* ticks (in CSDs with at least 30 tick submissions, $n = 105$) is presented as filled circles at the centroid of the CSDs, with pathogen prevalence categorized as a percentage; lower quartile (white), interquartile (grey) or upper quartile (dark blue).

I. scapularis from locations in south-central and southeastern Canada (Fig. 1). The 26 CSDs with *B. odocoilei* prevalence in the upper quartile were located in Ontario (19), Québec (6) and Nova Scotia (1).

Overall, the provinces with the highest percentage of *B. odocoilei*-positive blacklegged ticks collected through passive surveillance were Prince Edward Island (17.4 %, total number of *I. scapularis* tested = 23), Nova Scotia (16.7 %, total number of *I. scapularis* tested = 108), Québec (12.6 %, total number of *I. scapularis* tested = 3215), Newfoundland and Labrador (12.5 %, total number of *I. scapularis* tested = 56), and Ontario (12.4 %, total number of *I. scapularis* tested = 8507) (Table 1). Lower infection prevalences were found in Manitoba (4.6 %, total number of *I. scapularis* tested = 240) and New Brunswick (10.2 %, total number of *I. scapularis* tested = 1846). No *Ixodes* spp. were collected in Saskatchewan, only 4 and 11 tick submissions were received from Alberta and British Columbia, respectively.

3.2. Active surveillance

Between 2018 and 2021, 4862 ticks were analysed either at NML Winnipeg (where 4022 ticks were tested) or at the University of Ottawa laboratory (where 840 ticks were tested) from active field surveillance or research studies. For the total of the collected ticks, 4783 *I. scapularis* and 28 *I. pacificus,* were tested for *B. odocoilei* ($n = 4811$). Of these ticks 2938 were adults (9 were *I. pacificus*), 1866 were nymphs (19 were *I. pacificus*) and 7 were larvae (Table 2). Overall, 11.2 % ($n = 538$) were positive for *B. odocoilei*, with 293 positive adults (10.0 %) and 244 positive nymphs (13.2 %). One *I. pacificus* adult collected in the Vancouver region of British Columbia was positive for *B. odocoilei.* Other *Ixodes* spp. e.g., *Ixodes angustus* ($n = 12$), *Ixodes sculptus* ($n = 2$), *Ixodes auritulus* ($n = 2$), *Ixodes muris* ($n = 2$), and *Ixodes dentatus* ($n = 30$) were negative for *B. odocoilei*. From the subset of ticks tested at NML (3943 *I. scapularis* and 28 *I. pacificus*), 24.4 % ($n = 969$) were positive for *B. burgdorferi*, 3.7 % ($n = 146$) for *A. phagocytophilum*, 0.7 % ($n = 26$) for *B. miyamotoi*, 0.08 % ($n = 3$) for *B. microti*, and 0.05 % ($n = 2$) for Powassan virus.

For this subset of tick submissions, a total of 163 CSDs had at least one *I. scapularis* or *I. pacificus* submitted, from which 75 CSDs (46.0 %) had at least one *B. odocoilei*-infected tick (Fig. 2). The CSDs with the highest prevalence of *B. odocoilei* (i.e., upper quartile: 15.7–32.5 %) were located in southeastern Canada, in the provinces of Ontario, Québec, New Brunswick, and Nova Scotia (Fig. 2).

In active surveillance, the provinces with the highest percentage of *B. odocoilei*-positive blacklegged ticks collected were Québec (15.5 %, total number of *I. scapularis* tested = 816), New Brunswick (14.5 %, total number of *I. scapularis* tested = 1192), and Nova Scotia (12.2 %, total number of *I. scapularis* tested = 181) (Table 2). Lower infection prevalences were found in British Columbia (3.6 %, total number of *I. scapularis* tested = 28) and Manitoba (5.9 %, total number of *I. scapularis* tested = 118). No *Ixodes* spp. were collected in Alberta, Saskatchewan, or Newfoundland and Labrador, and only 2 ticks were collected on Prince Edward Island.

Table 2
Number and percentage of B. odocoilei-positive (Bo+) I. scapularis (a) and I. pacificus (b) per stage and per province from the ticks collected from 2018 through 2021 from active surveillance data.

a) Ixodes scapularis

	2018						2019						2020						2021					
	Adult			Nymph			Adult			Nymph			Adult			Nymph			Adult			Nymph		
Province	Bo+	N	%	Bo+	N	%	Bo+	N	%	Bo+	N	%	Bo+	N	%	Bo+	N	%	Bo+	N	%	Bo+	N	%
British Columbia	0	0	.	0	0	.	0	0	.	0	0	.	0	0	.	0	0	.	0	0	.	0	0	.
Manitoba	0	0	.	0	0	.	1	3	33.3	0	0	.	0	0	.	0	0	.	6	100	6.0	0	15	0.0
New Brunswick	0	0	.	0	0	.	14	87	16.1	19	107	17.8	44	317	13.9	16	127	12.6	29	219	13.2	51	335	15.2
Nova Scotia	1	12	8.3	0	0	.	8	71	11.3	13	98	13.3	0	0	.	0	0	.	0	0	.	0	0	.
Ontario	7	225	3.1	0	0	.	77	663	11.6	30	274	10.9	6	111	5.4	8	50	16.0	66	878	7.5	14	266	5.3
Prince Edward Island	0	0	.	0	0	.	0	1	0.0	0	1	0.0	0	0	.	0	0	.	0	0	.	0	0	.
Quebec	2	30	6.7	2	11	18.2	17	127	13.4	33	223	14.8	9	57	15.8	7	53	13.2	6	28	21.4	51	287	17.8
Total	10	267	3.7	2	11	18.2	117	952	12.3	95	703	13.5	59	485	12.2	31	230	13.5	107	1225	8.7	116	903	12.8

b) Ixodes pacificus

	2019						2021					
	Adult			Nymph			Adult			Nymph		
Province	Bo+	N	%	Bo+	N	%	Bo+	N	%	Bo+	N	%
British Columbia	1	8	12.5	0	2	0.0	0	1	0.0	0	17	0.0

3.3. Emergence of Babesia odocoilei

Model 1. Following model selection, the co-variates *latitude* and *engorgement* were discarded. As ticks were collected from humans and pets, rather than cervids, non-significance of the co-variate *engorgement* was not surprising considering these hosts are not recognised reservoirs of *B. odocoilei.* There was a significant association between *year* and *B. odocoilei* infection in *I. scapularis* adults (model 1a), but not in nymphs (model 1b), when adjusted for region. For adults this association was due to the increased odds of ticks being infected in 2019 (Odds ratio [OR] = 2.24, 95 % confidence interval [CI] = 1.93–2.61; $P < 0.001$), 2020 (OR = 2.12, 95 % CI = 1.80–2.51; $P < 0.001$), and 2021 (OR = 1.88, 95 % CI = 1.53–2.30, $P < 0.001$) compared with 2018 (Table 3). Ticks collected in the Western/Central Canada region had significantly lower odds of being infected by *B. odocoilei* than those collected in Atlantic Canada (OR = 0.37, 95 % CI = 0.17 - 0.72, $P < 0.01$, Table 3), Ontario (Tukey-adjusted test, $P = 0.016$) and Québec (Tukey-adjusted test, $P = 0.009$), when adjusted for year. The post-hoc Tukey adjusted tests for multiple comparisons found no significant differences in prevalence amongst the years 2019 to 2021.

Model 2. There was a significant association between year and the odds of a CSD having at least one *B. odocoilei* positive *I. scapularis* adult (model 2a, Table 4). There was a significant increase in the number of CSDs with positive ticks between 2018 and subsequent years (Table 4), but not between 2019 and 2020 ($P = 0.939$), 2020 and 2021 ($P = 0.212$), or 2019 and 2021 ($P = 0.100$), when tested with a post-hoc Tukey adjusted test for multiple comparisons. Again, ticks collected in the Western/Central Canada region had significantly lower odds of being infected by *B. odocoilei* than those collected in Atlantic Canada (OR = 0.35, 95 % CI =0.14 - 0.76, $P < 0.05$, Table 4), Ontario (Tukey-adjusted test, $P = 0.009$) and Québec (Tukey-adjusted test, $P = 0.016$). Ontario showed significant higher odds of a CSD having at least one *B. odocoilei* positive *I. scapularis* adult than the Atlantic region (OR = 1.34, 95 % CI =1.01 - 1.79, $P < 0.05$, Table 4), when adjusting for year.

4. Discussion

In this article, we provide the first description of the distribution of *B. odocoilei*-infected *I. scapularis* and *I. pacificus* submitted in passive surveillance and active surveillance in Canada. These data illustrate that *B. odocoilei* is widespread across Canada, being largely found in ticks collected in the south-central and eastern parts of the country, with some evidence of *B. odocoilei* in questing *I. pacificus* in western Canada. As reported in previous research, knowledge of the eco-epidemiology of *B. odocoilei* in Canada is limited (Milnes et al., 2019); our report supports a more in-depth understanding of *B. odocoilei* distribution across the country and provides insight into the transmission cycle of the protozoan.

In statistical models, the prevalence of *B. odocoilei* infection in adult *I. scapularis* collected in passive surveillance increased significantly from 2018 to 2019, but remained stable in subsequent years. Similarly, the probability that a CSD yielded a positive tick collected in passive surveillance increased from 2018 to 2019 but not in subsequent years. This was not seen for nymphs, but the statistical power for the nymph model was lower than for the adult model (12,988 adults versus 1034 nymphs), which could explain the differences between the models. Increasing prevalence from 2018 to 2019 could reflect processes associated with the general expansion of the geographic range of *I. scapularis* in Canada, which include increasing efficiency of pathogen transmission cycles resulting in increasing infection prevalence (Ogden et al., 2019). However, it is equally likely that this represents interannual variations in transmission dynamics, which are seen for other *I. scapularis*-transmitted pathogens in endemic areas, which may be driven particularly by interannual variation in host densities (Foster et al. 2022). A longer surveillance period is needed to determine whether interannual fluctuations in infection prevalence occur for *B. odocoilei.* Overall though, the

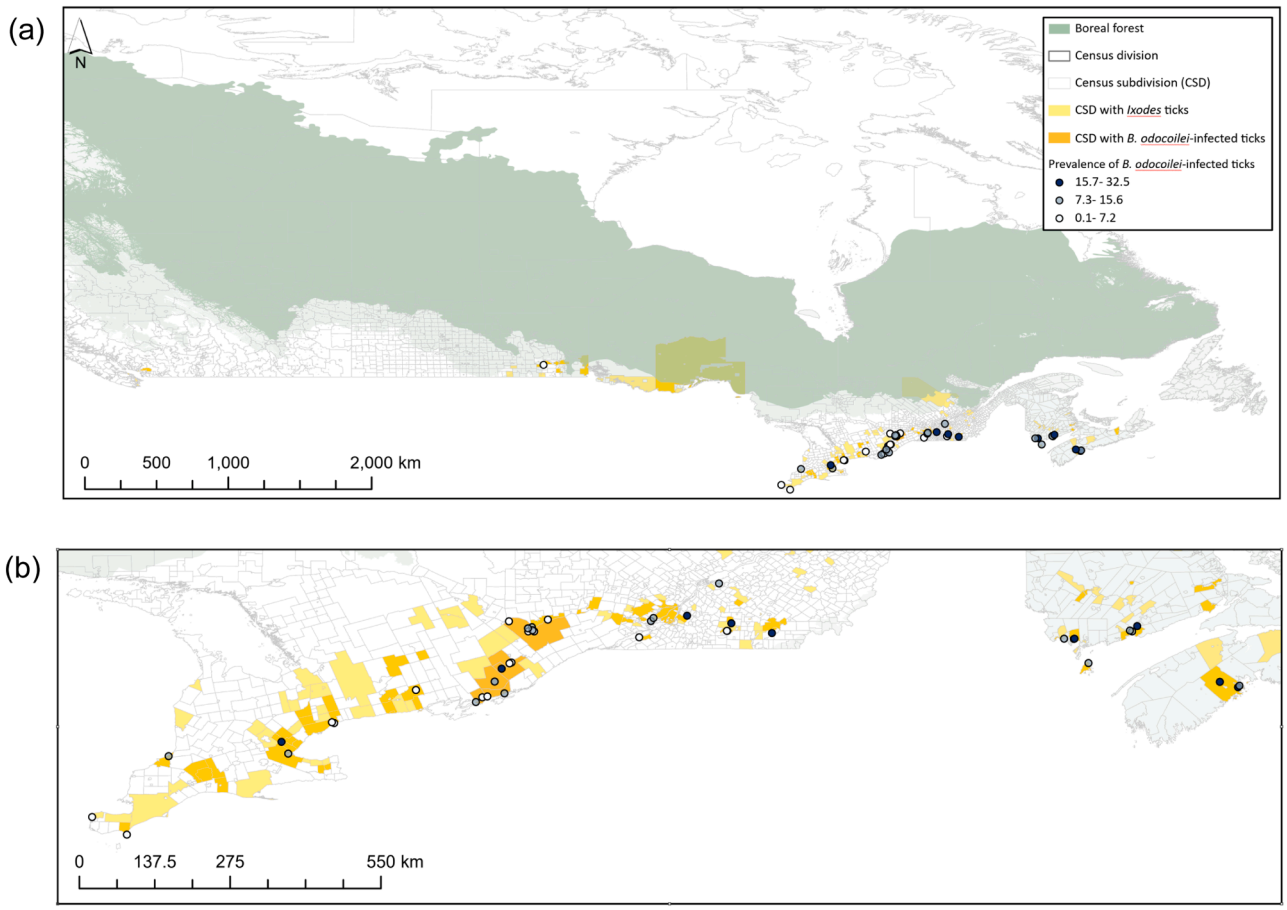

Fig. 2. Active surveillance data, 2018–2021.
The geographic distribution of *Ixodes scapularis* and *Ixodes pacificus, Babesia odocoilei*-infected ticks, and the prevalence of *B. odocoilei*-infected ticks from active field surveillance data tested at the NML (2018–2021) in (a) southern Canada and (b) southeastern Canada.
The documented presence of at least one *I. scapularis* or *I. pacificus* at the CSD level is indicated by yellow, and CSD with at least one *B. odocoilei*-infected tick are indicated by dark orange. Prevalence of *B. odocoilei* in *I. scapularis* (in sampling sites with at least 30 ticks collected, $n = 35$) is presented as filled circles at the sampling site's centroid and categorized as lower quartile (white), interquartile (grey) or upper quartile (dark blue). One adult male *I. pacificus* collected through the active surveillance system was found positive for *B. odocoilei* in British Columbia in a CSD with 28 ticks collected.

Table 3
Outputs of the binomial multivariate GLM exploring the relationship between the odds of *Ixodes scapularis* adults being infected by *Babesia odocoilei* by year and geographic region (Model 1a).

Variables		OR [95 % confidence interval]	*P*-value
Year (ref. 2018)	2019	2.24 [1.93 – 2.61]	< 0.001
	2020	2.12 [1.80 – 2.51]	< 0.001
	2021	1.88 [1.53 – 2.30]	< 0.001
Region (ref. Atlantic)	Québec	1.17 [0.97 – 1.41]	0.094
	Ontario	1.10 [0.93 – 1.30]	0.276
	Western/ Central	0.37 [0.17 – 0.72]	0.008

Table 4
Output of the binomial GLM exploring the relationship between the odds a CSD was associated with a *Babesia odocoilei*-positive *Ixodes scapularis* adult by year and geographic region (Model 2a).

Variables		OR [95 % confidence interval]	*P*-value
Year (ref. 2018)	2019	2.47 [1.90 – 3.21]	< 0.001
	2020	2.26 [1.70 – 3.00]	< 0.001
	2021	1.76 [1.29 – 2.40]	< 0.001
Region (ref. Atlantic)	Québec	1.24 [0.94 - 1.66]	0.136
	Ontario	1.34 [1.01 - 1.79]	0.022
	Western/ Central	0.37 [0.14 - 0.82]	0.045

relatively high prevalence of infection suggests that *B. odocoilei* transmission cycles may have been established for some years.

We could expect *B. odocoilei* transmission cycles become established rapidly after establishment of *I. scapularis* populations, in part, due to the likelihood that deer are the main reservoir. In north-eastern North America most *I. scapularis* ticks dispersed by migratory birds in spring are nymphs which are active in spring, and engorged nymphs moult into adults that preferentially feed on white-tailed deer (Piesman and Spielman, 1979; Wilson and Spielman, 1985; Wilson et al., 1990). Deer are resistant to infection with *B. burgdorferi* s.s. (Loken et al. 1985; Telford et al., 1988; Lacombe et al., 1993; Pearson et al., 2023), and *B. burgdorferi* s.s. is not transmitted transovarially by *I. scapularis* (Patrican, 1997). This means that imported infected ticks infrequently feed on reservoir hosts, resulting in a long multi-year gap from the initial

establishment of *I. scapularis* populations to the establishment of *B. burgdorferi* s.s. transmission cycles (Ogden et al.,. 2013). In contrast, for *B. odocoilei,* deer are likely the main reservoir hosts (Fanelli, 2021; Krause, 2019; Almazan et al., 2022). Therefore, deer would likely become infected when imported infected adult ticks feeds on them, and transmit *B. odocoilei* to other feeding ticks, including immature ticks that would likely maintain infection transstadially (Zembsch et al., 2021). In addition, the disease transmission cycle is further maintained by infected adult female ticks transmitting infection to their progeny (Zembsch et al., 2021). Further studies are needed to assess infection prevalence in archived ticks, prior to 2018, to explore rates of expansion of *B. odocoilei* transmission cycles in Canada.

Data collected through acarological surveillance can be subject to uncertainty, particularly regarding the origin of collected ticks or where they became infected; this uncertainty is considerably higher in passive surveillance (Koffi et al., 2012). Northward migrating passerines in spring can transport ticks over large distances, and these adventitious ticks may survive one moult in locations where the environment is not suitable for the establishment of reproducing, self-sustaining tick populations (Koffi et al., 2012). This means that some *B. odocoilei*-infected ticks introduced from the USA may be found in locations where endemic transmission cycles are not present (Schoelkopf et al., 2005). However, our active field and passive tick surveillance data were broadly consistent, suggesting that while passive surveillance data may overestimate the geographic range of *I. scapularis* populations and *B. odocoilei* transmission cycles, endemic transmission of *B. odocoilei* is widespread in *I. scapularis* populations in Canada.

Infection prevalence of *B. odocoilei* was low in ticks collected in south-central Canada, notably Manitoba, compared to that in ticks collected in the east. The reason behind this finding is unclear, considering that, to current knowledge, the main reservoir hosts are deer, and adult *I. scapularis* across all regions feed on deer. The finding of an infected *I. pacificus* in British Columbia suggests *B. odocoilei* is endemic across Canada where deer-feeding *Ixodes* spp. occur; further studies are needed to explore the role of *I. pacificus* in *B. odocoilei* transmission. Surveillance could also include testing other *Ixodes* spp. and the winter tick, *D. albipictus*, to determine how and if transmission of *B. odocoilei* occurs in northern territories and the prairie provinces. Overall, more intensive active surveillance is required to explore the geographic occurrence of *B. odocoilei* and understand its transmission cycle.

Many different species of *Babesia* have been described, with distinct geographical ranges, tick vectors and host preferences (Bajer et al., 2022). Of over 100 known species of *Babesia*, only a few are known to cause infections in humans, typically in immunocompromised individuals, despite many species being well-established pathogens to companion animals or livestock, e.g. *Babesia canis, Babesia gibsoni,* and *Babesia vogeli* (Young et al., 2019). For instance, in northern Europe, *B. divergens*, transmitted by *I. ricinus*, causes babesiosis in cattle and deer, but has caused disease in immunocompromised (particularly splenectomised) humans (González et al., 2015; Uhnoo et al., 1992; Zintl et al., 2003). Although *B. odocoilei* DNA has been reported in humans with non-specific clinical symptoms that may be compatible with babesiosis (Maggi et al., 2024; Scott et al., 2021a), a causal relationship between the presence of *B. odocoilei* DNA, infection and clinical human babesiosis remains to be confirmed. Molecular testing of samples of suspected or probable human babesiosis cases in Canada completed by the NML (with a standardized and validated PCR protocol) includes screening for multiple species of *Babesia,* including *B. odocoilei*; no human samples to date have tested positive for *B. odocoilei*. Moreover, in 2018, over 50,000 blood donations were analysed using a transcription-mediated implication method which permits the detection of *B. odocoilei* (personal communication; Grifols Diagnostic Solutions Inc., Emeryville, California, USA). No specimens were found positive for *B. odocoilei,* despite some blood samples originating from areas endemic for *I. scapularis* (Tonnetti et al., 2019). Further study on the eco-epidemiology of, and surveillance for, *B. odocoilei* are warranted as it is an animal pathogen potentially having impacts on wildlife and domesticated animals, particularly deer. Further vigilance is needed for evidence of infections in humans, particularly those who may be immunocompromised. However, such study requires the use of validated, standardised and specific laboratory tests, and as well as elucidation of the clinical manifestations, to avoid misdiagnosis and the unnecessary use of therapies that may have some harmful effects (Fallon et al., 2014).

Limitations of the study include the relatively short duration of *B. odocoilei* testing in ticks within the Canadian surveillance program, with only a four year portrait within which to characterize the emergence of this parasite. Future surveillance efforts should continue monitoring trends in this pathogen to validate our surveillance findings. Due to the high submission volume in highly endemic regions for *Ixodes* spp., there are restrictions on passive tick submissions from these areas, leading to limited data from these regions. The study brought together active surveillance data from a number of studies some of which were paused in 2020 due to the pandemic. Theoretically this could introduce bias if those studies were taking place in locations with particularly high or low infection prevalence. Similarly, we cannot be certain that public health measures for the pandemic did not affect spatial patterns of collection of ticks in passive surveillance. However, it seems unlikely that this had a significant impact on the results and conclusions as infection prevalence was not significantly different amongst the years 2019, 2020 and 2021 for both passive and active surveillance, and geographic differences in prevalence were also consistent between active and passive surveillance. Lastly, variations in active surveillance protocols across provinces may introduce sampling bias. However, given the extensive scale of the data, encompassing both active and passive surveillance, our findings still offer valuable insights into the distribution of *B. odocoilei*. These limitations underscore the importance of maintaining robust and representative surveillance systems to inform public health efforts regarding the evolution of less familiar pathogens.

5. Conclusion

This study comprises the first comprehensive baseline portrait of *B. odocoilei* presence in *Ixodes* spp. in southern Canada. It complements previous work which documents cases of cervid babesiosis in Canada (Pattullo et al., 2013) and highlights the need to further characterize the distribution and vector ecology of this non-human pathogen, identify potential hotspots, and conduct eco-epidemiological studies to further elucidate the factors contributing to the Canadian context. We recommend that a One Health approach, incorporating sampling from humans, animals and vectors be utilized to determine potential impacts of *B. odocoilei* on wildlife and captive animal populations and more fully understand the disease transmission cycle.

Author statement

CG, CB and JP conducted the analyses and wrote the paper. Other authors collaborated in providing data from passive and/or active surveillance. All other authors revised the present manuscript and agreed with its final content.

CRediT authorship contribution statement

Camille Guillot: Writing – original draft, Validation, Project administration, Methodology, Formal analysis, Conceptualization. **Jérôme Pelletier:** Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Cécile Aenishaenslin:** Writing – review & editing, Validation. **Heather Coatsworth:** Writing – review & editing, Validation, Data curation. **Antonia Dibernardo:** Writing – review & editing, Validation, Data curation. **Jules K. Koffi:** Writing – review & editing, Validation. **Manisha A. Kulkarni:** Writing – review & editing, Validation. **Jean-Philippe Rocheleau:** Writing – review & editing. **Christy Wilson:** Writing – review & editing, Validation, Data

curation. **Curtis Russell:** Writing – review & editing, Visualization. **Mark P. Nelder:** Writing – review & editing, Validation. **Jacqueline Badcock:** Writing – review & editing. **Justin Carr:** Writing – review & editing. **Sylvia Checkley:** Writing – review & editing. **Katie M. Clow:** Writing – review & editing. **Stephanie Cooper:** Writing – review & editing. **Susan Cork:** Writing – review & editing. **Ariane Dumas:** Writing – review & editing. **Shaun Dergousoff:** Writing – review & editing. **Nicoletta Faraone:** Writing – review & editing. **Erin Fraser:** Writing – review & editing. **Scott Graham-Derham:** Writing – review & editing. **Alejandra Irace-Cima:** Writing – review & editing. **Stefan Iwasawa:** Writing – review & editing. **Emily Jenkins:** Writing – review & editing. **Patrick A. Leighton:** Writing – review & editing. **Roman McKay:** Writing – review & editing. **Muhammad Morshed:** Writing – review & editing. **Roxane Pelletier:** Writing – review & editing. **Marion Ripoche:** Writing – review & editing. **Kateryn Rochon:** Writing – review & editing. **Karine Thivierge:** Writing – review & editing. **Maarten J. Voordouw:** Writing – review & editing. **Nicholas H. Ogden:** Writing – review & editing, Validation. **Catherine Bouchard:** Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

None to declare.

Acknowledgement

We would like to thank all people involved in the different research projects or surveillance programs that provided the data necessary to complete the analyses presented in this article. This work would not be possible without their constant efforts and hard work. A special thank you to all private owners, municipalities and parks that provided critical support in making studies on their land possible for field surveillance.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ttbdis.2024.102413.

Data availability

Data used in this article are confidential.

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