



Blacklegged tick surveillance in Ontario:

A systematic review



June 2016

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How to cite this document:

Ontario Agency for Health Protection and Promotion (Public Health Ontario). Blacklegged tick surveillance in Ontario: a systematic review. Toronto, ON: Queen's Printer for Ontario; 2016.

ISBN 978-1-4606-7575-5 [PDF]

Public Health Ontario acknowledges the financial support of the Ontario Government.

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Acknowledgements

The authors wish to express their sincere appreciation for the effort and dedication demonstrated by Ontario's 36 Public Health Units (PHUs), Ministry of Health and Long-Term Care, and Public Health Agency of Canada (PHAC) throughout the development of Public Health Ontario's (PHO) Lyme disease surveillance products. We thank Joan Mays (Leeds, Grenville and Lanark District Health Unit), Nina Jain-Sheehan (Niagara Regional Health Unit) and Donna Stanley (Northwestern Health Unit) for their input and work on the report. We thank PHO's Tina Badiani, Lisa Fortuna, Kiren Gill, Steven Janovsky, Cathy Mallove, George Pasut and Brian Schwartz for reviewing the report. We would like to thank L. Robbin Lindsay (PHAC) for his continued help with developing tick surveillance guidelines and programs in Ontario. In addition, we thank PHO's Library Services for their assistance in developing our search strategy.

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Introduction

Purpose and Objectives

In order to provide the latest, evidence-based advice on surveillance, PHO performed a systematic literature review to assess methods and best practices for blacklegged tick surveillance. This review assesses the applicability of currently available methods to Ontario and provides the foundation for the updated Tick Surveillance section in PHO's <u>Technical report: Update on Lyme disease prevention and</u> <u>control: Second edition</u>. Assessing the gamut of available methods that could apply to the Ontario context is especially important, as most studies reviewed were carried out in jurisdictions with differing social, environmental and ecological conditions as well as healthcare, public health and surveillance systems. The primary objectives of this report are to:

- assess blacklegged tick surveillance methods reported in the literature and the relevance of these methods in Ontario;
- explain the purpose of different surveillance methods and how they relate to one other; and
- based on the above, assess the appropriateness of Ontario's current tick surveillance methods and determine whether other methods could be applied.

Background

Lyme disease is a bacterial spirochete infection caused by *Borrelia burgdorferi* and is transmitted to humans through the bite of an infectious blacklegged tick, *Ixodes scapularis*. Lyme disease is the most common vector-borne disease in North America, with an estimated 300,000 cases annually in the United States (US) alone.¹⁻³ Lyme disease was first recognized in 1975, when it was initially described as a cluster of juvenile rheumatoid arthritis cases in several towns in Connecticut, US.⁴ Soon after the description of Lyme disease in the early 1980s, the blacklegged tick was identified as the vector of *B*. *burgdorferi* in New York, US.^{5,6} Lyme disease is found throughout eastern North America, including southern portions of Canada, wherever blacklegged ticks are present; however, disease rates are highest in the Northeast and Upper Midwestern US states.⁷

In Canada, *I. scapularis* distribution is limited primarily to the southern portions of Manitoba, Ontario, Quebec, New Brunswick and Nova Scotia.^{8,9} In the early 1970s, the first population of blacklegged ticks in Canada was identified at Long Point Provincial Park, Ontario, along the northern shore of Lake Erie.¹⁰ Beginning in the mid-1990s and through the 2000s, additional established populations of blacklegged ticks were detected along the northern shores of Lake Erie (Point Pelee National Park, Rondeau Provincial Park, Turkey Point Provincial Park and the Wainfleet Bog Conservation Area), Lake Ontario (Prince Edward Point National Wildlife Area) and the St. Lawrence River (St. Lawrence Islands National Park), Northwest Ontario (Rainy River), Southwest Ontario (Pinery Provincial Park) and urban-suburban parks (Rouge Valley).¹¹⁻¹⁵ Since 1988, the majority of human cases of Lyme disease acquired in Ontario have originated from Southern Ontario, especially in areas of Southeastern Ontario where blacklegged tick populations are expanding.

Multiple variables are responsible for the expansion of blacklegged ticks in Ontario. A driving force behind the recent expansion in Ontario and other areas is climate change, specifically the increase in the mean annual degree days above 0°C.^{16,17} Other factors that contribute to blacklegged tick expansion include land use changes (i.e., farmland to forest; encroaching human populations; forest fragmentation) and changes in the range of the main hosts for ticks (i.e., white-footed mouse *Peromyscus leucopus*, white-tailed deer *Odocoileus virginianus*). All tick surveillance indicators suggest that the current geographic range of blacklegged tick populations is expanding in southern Ontario and will likely continue to do so, as available habitat permits.¹⁸

Blacklegged tick populations can occur sporadically over a wide geographic range in Canada, due to larvae and nymphs readily attaching themselves to migratory birds.¹⁹ Birds help transport blacklegged ticks from areas in the US and Canada to disparate locales across Canada. Bird-borne (adventitious) ticks create the possibility of infectious tick bites almost anywhere in Ontario. Human cases of Lyme disease may occur outside of known Ontario risk areas; however, the risk of exposure is considerably less than in identified risk areas. The risk of Lyme disease is usually greater in tick-established areas because of a greater probability of bites from infectious ticks compared to areas where blacklegged ticks are not established.¹⁵

Methodology

Search Strategy

PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines for conducting a systematic review were followed in the development of this review. A scientific literature search of English language articles was conducted using six electronic databases: Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations and Ovid MEDLINE(R) (Ovid interface: January 1, 1946 to April 16, 2015); Embase (Ovid Platform: January 1, 1988 to Week 15, 2015); Scopus (January 1, 1995 to April 16, 2015); BIOSIS Previews (2002 to Week 15, 2015); Environment Complete (January 1S, 1995 to April 16, 2015); Cochrane Database of Systematic Reviews (January 1, 1995 to April 16, 2015). The literature search used subject headings and keywords that included "ticks", "ixodes", "blacklegged tick", "passive", "active", "surveillance", "collect", "monitor", "host", "data collection", "biosurveillance" and "epidemiological monitoring." The primary search strategy, developed in Medline, was customized into other databases to account for database-specific vocabulary and functionality differences. All searches were current as of April 16, 2015 (full search strategy for Ovid Medline, Table 1).

Table 1. Ovid Medline search strategy for tick surveillance

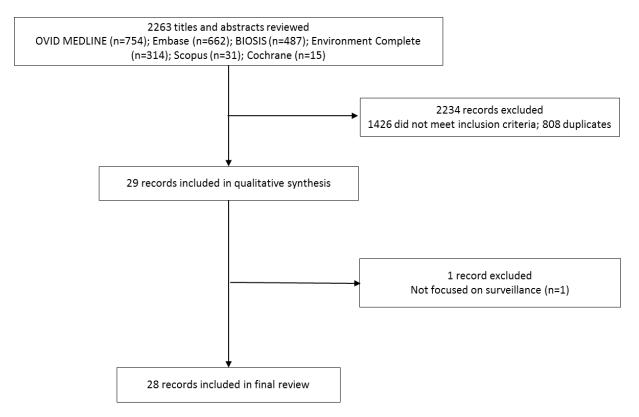
#	Searches
1	(ticks/ or ixodidae/ or tick infestations/) and ((lyme or burgdorferi or borreliosis or LD or LB).tw,kf,kw. or Lyme disease/ or Borrelia burgdorferi/)
2	ixodes/ or (i scapularis or black legged tick? or blacklegged tick? or ixod\$ tick? or ixode? or deer tick? or bear tick?).tw,kf,kw.
3	1 or 2
4	((tick or active or passive) adj1 (sampl\$ or surveillance or monitor\$ or collect\$ or trap\$)).tw,kf,kw.
5	((host or deer or mouse or mice or small mammal? or rodent? or bird? or lizard?) adj2 (capture or examin\$)).tw,kf,kw.
6	surveillance.hw. or data collection/ or epidemiological monitoring/ or biosurveillance/ or *lyme disease/ep or *lyme disease/sn or spatial analysis/ or (((monitor\$ or collect\$ or trap\$ or sampl\$ or surveillance or check\$ or screen\$ or assess\$) adj2 (risk? or method\$)) or drag\$ or sweep\$ or flag\$ or blanket or ((carbon dioxide or "CO2") adj2 trap\$) or nymphal infection prevalence).tw,kf,kw.
7	4 or 5 or 6
8	3 and 7
9	limit 8 to english language
10	limit 9 to last 20 years

Study Selection

Two reviewers independently screened titles and abstracts against eligibility criteria and differences resolved by consensus (MPN, Nina Jain-Sheehan) (Figure 1). Articles included in the review met the following inclusion criteria: 1) described Lyme disease risk areas; 2) were published on or after January 1, 1995; and 3) included field-collections of *I. scapularis* or related tick species. Excluded studies were

those that only described data, with no reference to tick surveillance. Three studies published before 1995 were included at the discretion of the authors.²⁰⁻²²

Figure 1. Literature search and study selection



Data Extraction and Quality Assessment

A data extraction table was populated with the year the study was published, first author, citation, location of study, target tick species, tick stages targeted in study, passive tick collection methods used, primary variables collected, dates of tick collection, active collection methods used and primary results of study.

To evaluate the quality of eligible primary studies and to reduce the risk of bias, critical appraisals were completed by two independent reviewers for each paper and disagreements were resolved by consensus (Curtis Russell, Mark P. Nelder; Appendix 1). Quality assessments of all studies were performed using the Public Health Ontario MetaQAT Tool.²³ All studies were assessed using the MetaQAT tool based upon four major categories: 1) assessment of relevancy (two specific questions); 2) assessment of reliability (seven questions); 3) assessment of validity (eight questions); and 4) assessment of applicability. We did not calculate an overall quality score, as per agreement in the literature.²⁴

Findings

Study Characteristics and Quality Assessment

Twenty-eight studies were included in the review (Table 2).^{20-22,25-49} Seventeen studies were reported from the US, six from Europe, two from Asia, two from Canada and one from South America. In the US, the majority of studies were from New Jersey (n= 3), New York (n = 3), California (n = 2) and Massachusetts (n = 2). Seventeen studies included *I. scapularis* in their surveillance; six targeted the blacklegged tick's sister taxa in Europe, *Ixodes ricinus*; two targeted the western blacklegged tick *Ixodes pacificus*; two targeted other *Ixodes* species and one did not include *Ixodes* species. Four studies were reported during the period from 1989 through 1999; seven from 2000 through 2009; and 18 from 2010 through 2015.

61% (17/28) of studies met 100% of quality criteria; another 25% (7/28) met 75% of quality criteria (Appendix 1). 89% (25/28) of studies included sufficient detail to allow for replication.

Year; location; reference	Target tick; stages [*]	Passive methods	Primary variables (dates of collection) [†]	Active methods	Primary results
1989; New York; Ginsberg ²¹	Multiple habitat, flag size		stage, site, date	Dragging/ flagging, walking, CO ₂ - baited traps, small mammal trapping, pitfall traps, leaf litter samples	More larvae collected from rodents; walking more effective than dragging/flagging; pitfall traps and leaf litter samples collected few ticks
1992; New York; Falco ²⁰	; New /cit N NA method tick		Site, collection method, tick stage (May–Aug)	Dragging, CO ₂ - baited traps, small mammal trapping	Dragging collected more nymphs than CO ₂ - baited traps; dragging collected more larvae than CO ₂ -baited traps
1992; New Jersey; Solberg ²²	Collection time, Multiple collection method, species NA date, tick species (<i>Is</i>); L, N, A and stage (May, July, Nov)		Dragging, walking, CO ₂ - baited traps, mark-recapture	CO ₂ -baited traps collected more ticks than dragging or walking; no difference between walking and dragging	
1997; NewMultipleJersey;speciesNASchulze25(Is); L, N, A		NA	Vegetation type, collection method, tick species and stage (Apr, May, Aug possibly others)	Dragging, walking, CO ₂ - baited traps	Effectiveness of method was species- and stage- specific; walking most effective for <i>Is</i>

Table 2. Summary of 28 studies reviewed for tick surveillance

Year; location; reference	Target tick; stages [*]	Passive methods	Primary variables (dates of collection) [†]	Active methods	Primary results
2000; New York; Daniels ²⁶	<i>ls</i> ; L, N, A	NA	Habitat, year, collection method, tick stage, drag efficiency, mortality, population size (Mar–Dec)	Dragging, mark- release- recapture, removal	Dragging effective in estimating population size
2000; California; Tällenklint- Eisen ²⁷	I. pacificus; N	NA	Climate, topography, temperature, RH, vegetation, treatment, sampling efficiency (Apr–Jun)	Dragging	Dragging effective at estimating population size for nymphs
2005; Louisiana; Mackay ²⁸	ls; A	NA	Temperature, location, habitat, date, collection method, sampling effort (all year)	Dragging, CO ₂ - baited traps	CO ₂ -baited traps collected more ticks than flagging
2006; Canada; Ogden ²⁹	<i>ls</i> ; L, N, A	Tick submissions	Hosts, location, date of collection, travel, tick stage and fed/unfed status (all year)	NA	Passive surveillance effective at determining tick distribution and infection rates
2007; Maine; Rand ³⁰	Multiple species (<i>Is</i>); L, N, A	Tick submissions	Host, location, date, attachment site, symptoms, tick species and stage, distance from coast (all year)	NA	Passive surveillance effective at determining tick distribution and infection rates
2009; Poland; Supergan ³¹	Multiple species (<i>I</i> . <i>ricinus</i>); N, A	NA	Risk scale, habitat type, tick species and stage (Apr–Jul)	Flagging	Flagging effective for monitoring local tick populations
2010; California; Castro ³²	l. pacificus; A	NA	Date, habitat, temp, RH, wind, time of day, collection method (Jan-Mar)	Flagging, visual inspection	Both methods effective for estimating tick abundance
2010; Missouri; Petry ³³	Multiple species (<i>ls</i>); L, N, A	NA	Season, tick species and stage, habitat, collection method (Mar–Nov)	Dragging, CO ₂ - baited traps	Technique effectiveness was habitat-, species- and stage-specific
2010; Brazil; Terassini ³⁴	Multiple species (no <i>lxodes</i>); L, N, A	NA	Collection method, tick species and stage, date (Aug– Oct)	Dragging, visual inspection	Dragging detected more immatures than visual inspection; visual more effective for detecting adults

Year; location; reference	Target tick; stages [*]	Passive methods	Primary variables (dates of collection) [†]	Active methods	Primary results
2011; United Kingdom; Dobson ³⁵	l. ricinus; L, N, A	NA	Habitat, date, soil moisture, RH, temperature, weather, collection method, tick stage and density (all year)	Dragging, leggings, heel flags	Dragging effective at sampling for abundance; leggings and heel flags improved tick collection numbers
2011; Great Britain; Jameson ³⁶	Multiple (<i>I. ricinus</i>); L, N, A	Tick submissions	Date collected, location, host, habitat, tick species and stage, tick density (all year)	Dragging	Dragging and passive tick submissions effective in monitoring tick populations
2011; New Jersey; Schulze ³⁷	Multiple species (<i>Is</i>); N	NA	Repellent treatment, tick species and stage, collection method (Jun–Jul)	Dragging, CO ₂ - baited traps	Dragging and CO ₂ - baited traps equally effective at collecting nymphs of <i>A</i> . <i>americanum</i> and <i>Is</i>
2011; Belgium; Tack ³⁸	I. ricinus; L, N, A	NA	Vegetation type, wind speed, temperature, RH, collection method (blanket size), tick stage (Apr–Sep)	Dragging	Entire blanket dragging more effective at collecting ticks than smaller strips; however, strip better at collecting larvae
2012; Romania; Gherman ³⁹	Multiple species (<i>I. ricinus</i>); L, N, A	NA	Site, tick species and stage, collection method (May–Apr)	Flagging, CO ₂ - baited traps	CO ₂ increased ability of flagging to collect more <i>I. ricinus</i>
2012; Illinois; Rydzewski ⁴⁰	<i>ls</i> ; L, N, A	NA	Date, site, number site visits, tick stage (Apr– Oct)	Dragging	Dragging effective at determining distribution and monitoring populations in urban environment
2012; South Korea; Yun ⁴¹	Multiple species (<i>Ixodes</i> spp.); L, N, A	NA	Habitat, collection method, tick species and stage, pathogen prevalence (unknown)	Dragging, flagging, BG- Sentinel traps	All methods useful for pathogen detection in ticks
2013; South Korea; Chong ⁴²	Multiple (<i>Ixodes</i> spp.); L, N, A	NA	Habitat, collection method, tick species and stage, date, tick density (Apr– Oct)	Dragging, flagging	No significant difference between dragging and flagging methods for all tick species
2013; Italy; Dantas-Torres ⁴³	Multiple species (<i>I. ricinus</i>); L, N, A	NA	Season, tick species and stage, habitat, collection method (all year)	Dragging, flagging	Method was season-, habitat- and species- specific

Year; location; reference	Target tick; stages [*]	Passive methods	Primary variables (dates of collection) [†]	Active methods	Primary results	
2013; Massachusetts, Wisconsin, Rhode Island; Rulison ⁴⁴	<i>ls;</i> N	NA	Habitat, tree density, date, collection method (May–Sep)	Dragging, flagging	Both methods effective at sampling nymphs	
2014; Connecticut, Massachusetts; Diuk-Wasser ⁴⁵	14; nnecticut, /s; N NA assachusetts;		Habitat, site, year, pathogen prevalence, human infection rates (May–Jun)	pathogen prevalence, human Dragging infection rates		
2014; Quebec; Ogden ⁴⁶	<i>ls</i> ; L, N, A	NA	Season, year, collection method, tick stage (May– Oct)	Dragging, small mammal trapping	Dragging alone effective at determining if an area is a Lyme disease rick for public	
2014; North Dakota; Russart ⁴⁷	Multiple species (<i>ls</i>); L, N, A	NA	Tick species and stage, host, pathogen prevalence, flagging effort, trapping effort, site (Jun–Jul)	Flagging, ex. humans, small mammal trapping	No comparisons of methods; all methods effective at overall survey of ticks	
2014; Ohio; Rynkiewicz ⁴⁸	Temperature, R saturation defic habitat, date, ho species NA site, collection (<i>Is</i>); L, N, A method, tick species and stag			Dragging, small mammal trapping, CO ₂ - baited traps	Suggested using all methods to estimate entire tick community	
2014; Ohio; Wang ⁴⁹	Multiple species (<i>Is</i>); L, N, A	Tick submissions	Host, location, date, collection method, pathogen prevalence, tick species and stage (all year)	Flagging, small mammal trapping, deer examination	No comparison of techniques; passive surveillance program and deer head methods discontinued due to loss of funding	

^{*}L, larva; N, nymph; A, adult ^{**}Is, Ixodes scapularis

[†]RH, relative humidity

Descriptive Analysis

To allow for comparison, a brief description of each method in the reviewed studies is provided below.

Passive versus active tick surveillance

The systematic review identified that most jurisdictions (n = 26) use active methods to determine the composition of their blacklegged tick populations and *B. burgdorferi*-infected *I. scapularis*. Four studies explicitly described their passive surveillance system for ticks, including information on how ticks are

submitted and what information is collected. Only two studies employed both a passive and active component to their tick surveillance program (Ohio, US and Great Britain).^{36,49} Passive methods are generally not used in jurisdictions where blacklegged tick populations are established or where Lyme disease is endemic. Jurisdictions that use passive methods include regions where blacklegged ticks have recently invaded and are expanding their range (e.g., Canada, Maine and Ohio).^{29,30,49}

Four studies used only a passive tick surveillance method (Table 2). At least one method for active tick surveillance was used in 26 studies; at least one passive and one active method was used in two studies. Passive methods included the submission of ticks through the public (n = 4), healthcare professionals and organizations (n = 4), veterinarians (n = 3), outdoor enthusiasts or workers (n = 1), academia (n = 1), wildlife organizations (n = 1) and amateur entomologists (n = 1). The most often used active methods included tick dragging (n = 21), followed by flagging (n = 9), carbon dioxide-baited trapping (n = 9) and small mammal trapping (n = 6).

As new populations of blacklegged ticks are discovered and populations continue to expand in Ontario, passive tick surveillance still holds value as an important public health tool for determining risk areas.

Tick dragging

Tick dragging requires the dragger to attach a piece of white flannel cotton (typically 1 m²) to a 1 m long wooden dowel. Cotton is a lure that mimics a host's fur; in order for easier detection of ticks on the fabric, white cotton is used. A rope is then affixed to the dowel and used to drag for ticks across low-lying vegetation (normally <1.5 m high). For more information on how to drag for ticks, please see PHO's *Active tick dragging: Standard operating procedure*.

Tick flagging

Tick flagging is similar to dragging, but instead focuses on the collection of host-seeking ticks from dense vegetation, at a variety of heights, such as bushes, shrubs and tall grasses. As the name implies, a flag is a flannel cotton cloth (1 m²; but variable depending on the study) affixed to the end of a wooden dowel (1 m long). The flag is waved across and into vegetation or leaf litter, with ticks collected from the flag at standard intervals of distance or time.

The walking method

The walking method is similar to both flagging and dragging, however, the collector is now the primary lure.^{21,22} Transects are walked in a habitat and ticks removed from the collectors clothing (typically white cotton) at regular intervals. This method is the best method to estimate the risk of human exposure in a given area, as humans are the primary at-risk hosts.

CO₂-baited traps

 CO_2 -baited traps exploit a tick's attraction to sources of CO_2 (mimicking host respiration). CO_2 -baited traps consist of an insulated container (such as a polystyrene ice or beverage container) that contains dry ice, with holes for CO_2 discharge into the environment. The trap design is highly variable among

studies.^{21,25,28,48} Approximately 1.5 kg of dry ice (the source of the CO_2) is required for a trap left in the field for 12 hours (the minimum recommended time) at a constant temperature of 27°C.⁵⁰ To capture host-seeking ticks, two-sided tape is fastened to the outer surface of the trap, or the trap is laid on a piece of cotton. In other instances, flagging around the trap is needed to collect ticks that have not been trapped by other means. Blacklegged ticks are attracted to CO_2 ; however, they are slow-moving and multiple CO_2 -baited traps must be left in the field for 12 to 24 hours to capture ticks.

Additional surveillance methods

The studies reviewed reported on additional methods for blacklegged tick surveillance. Deer carcass examinations are another method to collect blacklegged ticks, particularly adults, as white-tailed deer are the preferred host of the adult tick. Deer examinations require the ticks to be collected from hunter-killed deer brought to hunting inspection stations.⁴⁹

In the UK, variations on the walking method employed the addition of leggings (white cotton covering both of the collector's legs) and heel flags (white cotton covering the collector's feet and trailing behind the collector by approximately 25 cm).³⁵

In South Korea, collectors used "sentinel BG" or BG-Sentinel[™] traps, a trap specifically designed to collect daytime, host-seeking *Aedes* mosquitoes.⁴¹ These traps use a proprietary lure, octenol and CO₂ to attract mosquitoes (or ticks in this case); however, the authors did not explain their methodology.

In California, visual inspection of vegetation within 1 m of a walking trail was used as an alternative method of estimating the abundance of the western blacklegged tick, *Ixodes pacificus*.³²

A single study used pitfall traps and leaf litter samples to collect blacklegged ticks.²¹ Pitfall traps are 470 ml drinking cups placed into the ground, with the lip of the cup level with the ground surface. Pitfall traps are designed to capture ground-dwelling arthropods, by way of the arthropods simply walking into the cup and becoming trapped.

Leaf litter samples are simply collections of leaf litter in suspected tick habitats, followed by processing leaf litter for arthropods. In most circumstances, leaf litter is placed in a Berlese-Tullgren funnel, below an incandescent light; arthropods move away from the heat and become trapped in a collection vial below the funnel.

Mark-recapture or mark-release-recapture was used in two of the reviewed studies to estimate absolute blacklegged tick populations in a given area.^{22,26} In mark-recapture or mark-release-recapture methods, sampled ticks are collected, marked using a dye, then released back into the habitat; an estimation of the entire population size is calculated from the proportion of marked ticks collected in a subsequent sample.

Comparison of surveillance methods

Seven studies quantitatively compared various active blacklegged tick surveillance methods.^{20-22,25,28,44,48} A method's efficacy (ability to detect blacklegged ticks or estimate relative abundance) is dependent

upon blacklegged tick abundance, the stage targeted, season and habitat heterogeneity (forest type, leaf litter depth, or microclimate). Furthermore, these dependencies are important in determining the efficiency (proportion of the absolute population collected in a sampling event) or sampling effort (person hours to collect a single blacklegged tick) of a particular method.

- In Massachusetts, Rhode Island and Wisconsin, there was no significant difference in the number of nymphs collected by dragging and flagging; but in certain habitats; dragging collected more blacklegged ticks than flagging.⁴⁴
- On Long Island, New York, there was no difference in the number of adult blacklegged ticks collected by dragging/flagging (terms used interchangeably) and walking.²¹
- In New Jersey, walking collected more adults compared to dragging; however, dragging collected more larvae and nymphs than walking.²⁵
- In southern New York, dragging collected more nymphs compared to CO₂-baited traps; however, more larvae were collected from CO₂-baited traps than small mammal trapping.²⁰ Yet in neighbouring New Jersey, CO₂-baited traps collected more of all stages compared to dragging and walking; there was no difference in the number of ticks collected by dragging and walking.²²
- In Louisiana, CO₂-baited traps collected more blacklegged tick adults under cooler conditions (mean daily minimum air temperature <10°C), while dragging collected more ticks in warmer conditions (mean daily minimum air temperature >15°C).²⁸
- In areas where blacklegged tick populations exist in low numbers or are on the edge of their range, such as in Missouri, small mammal trapping is more effective at collecting all stages.⁴⁸
- In general, the lone star tick Amblyomma americanum is more attracted to CO₂-baited traps, as the lone star tick is a more active host-seeking species that will travel farther to find a host and moves faster toward a host; therefore, using CO₂-baited traps for blacklegged ticks is less efficient.²¹

Discussion

The systematic review identified little empirical evidence for or against the use of one active tick surveillance method over another. Other factors, besides the ability to detect or collect more blacklegged ticks, must be considered when choosing a surveillance method. Among the primary elements to consider for a surveillance program are:

- 1) cost;
- 2) ease of use;
- 3) ability to detect blacklegged ticks when population numbers are low, as well as ability to detect specific stages of blacklegged ticks; and
- 4) repeatability (Table 3).^{20-22,25,28,44,48,50}

Furthermore, an examination of a PHU's historical, blacklegged tick populations, surveillance needs and available resources are critical when deciding on an active tick surveillance method. (Please refer to *Lyme disease control and management in Ontario*).

Method	Assessment (+, yes; ±, yes/no; -, no) based on reviewed studies [*]									
	Cost	Ease of use	Low population numbers	Detecting larvae and nymphs	Detecting adults	Repeatability				
Dragging	+	+	±	±	±	+				
Walking	+	+	±	±	±	+				
CO ₂ -baited traps	-	+	±	±	±	+				
Flagging	+	+	±	±	±	-				
Small mammal trapping	-	-	+	+	-	+				
CO ₂ flagging	-	+	±	±	±	-				
Pitfall traps	±	+	-	-	-	+				
Leaf litter samples	±	+	-	-	-	+				
Deer-carcass examination	±	±	+	-	+	-				

Table 3. Comparison of active methods for the collection of blacklegged ticks, from reviewed studies

***Cost:** Is the method inexpensive? Expenses for collection materials and human resources. **Ease of use:** Is the method easy to use? Expertise needed to perform method (ability to notice and identify ticks in the field or to operate equipment)? **Low population numbers:** Is the method sensitive to detecting blacklegged ticks where populations exist in low numbers? **Ability to detect specific stages of blacklegged ticks:** Is the method effective at collecting immatures or adults? **Repeatability:** Is the method easy to replicate, within and between seasons? Includes possibilities for bias due to different people performing collection.

Cost

The cost of a particular method is an important element to consider, especially in a PHU with competing public health priorities and resources. Costs include materials to conduct the collections (e.g., drag cloths, traps, lures, vials) and more importantly, costs associated with human resources. In general, most of the materials required for tick collections are relatively inexpensive, aside from one-time costs and maintenance of various traps and equipment. Traps that employ CO₂ as a lure will accrue continual costs for replenishment and will normally require added time in the field, as well as human resources (Table 3). The same can be said for small mammal trapping, which requires not only added costs for supplies (e.g., traps, personal protective equipment, laboratory materials) but also additional human resources. Overall, dragging, flagging and walking are relatively low-cost methods that require a relatively low initial investment with low maintenance costs. The length of time it takes to collect blacklegged ticks in the field and added logistical support needed should be considered, taking into account the total person-hours required for collections, travel to and from collection sites, time for processing of ticks (sorting, identification, laboratory testing) and resources needed for data management. While certain methods are economical, they must also be feasible in the long term and take into account ease of use, their specificity in collecting various target stages, and repeatability.

Ease of Use

For the most part, the methods reviewed require little to no expertise and are readily accessible to PHU staff conducting tick surveillance. Methods that can be implemented using standardized operating procedures or one-time training are advantageous because they require minimal expertise and are easily applied in the field. In the case of visual surveillance and deer carcass examination, some expertise is required for quick recognition and identification of ticks in the field. Small mammal trapping also requires additional expertise, with collectors needing appropriate permits to collect mammals, knowledge of trap placement and rodent behaviour, blood collection equipment, anesthetization procedures, laboratory testing protocols and safety protocols (Table 3). The expertise required for small mammal trapping makes it prohibitive as a sustainable method to monitor blacklegged tick populations on a long-term basis. Walking, while simple to perform, requires collectors to readily notice ticks that have latched on to them. This is particularly important in areas with a high risk of encountering *B. burgdorferi*-infectious ticks where inexperienced collectors are possibly at a higher risk of disease. An easy-to-use method allows for a subset of PHU staff to learn the method first hand, followed by additional in-house training to newcomers by experienced staff.

Detecting blacklegged ticks when population numbers are low, as well as ability to detect specific stages of blacklegged ticks

Some methods are better than others are at detecting blacklegged ticks when they are relatively rare in the environment (such as CO_2 -baited traps, the most sensitive technique). The tick stage also affects which method is most appropriate. For example, small mammal trapping is very specific to capturing immature blacklegged ticks and is an effective technique for blacklegged tick detection when ticks are rare in the environment (Table 3). For surveillance purposes, the stage targeted by a certain method is

not necessarily vital to a program, unless immature stages and pathogen detection is important. A technique very specific to the adult stage is deer carcass examinations. Small mammal trapping and deer carcass examinations are both good at detecting blacklegged ticks when population numbers are low, and they target specific tick stages (small mammal trapping, larvae and nymphs; carcass examination, adults); however, these methods rank lower when costs, accessibility and repeatability are taken into account. Dragging, walking and flagging offer moderate abilities to detect blacklegged ticks in low population settings and for the detection of specific blacklegged tick stages.

Replication

Using a single tick surveillance method consistently allows for statistical comparisons of the spatiotemporal patterns of blacklegged ticks and *B. burgdorferi*. When methods change, or are modified considerably, comparative work becomes problematic and trends are difficult to discern or to interpret. Choosing a particular method must take into account the likelihood that the method may be influenced by individual or collector bias. For example, dragging over a certain distance and habitat is relatively simple to replicate and can be reproduced by different collectors at different times of the year. In some instances, individual collectors avoid certain types of vegetation such as greenbrier, making flagging more difficult to replicate, however, this bias may occur similarly in dragging and walking.²¹ Examination of white-tailed deer for blacklegged ticks, while specific for adult blacklegged ticks, is difficult to replicate, as the numbers of deer and where they are harvested is dependent upon the seasonal abundance of deer and local hunting regulations (Table 3). While standardization is essential for any method, tick dragging represents the easiest in terms of intra- and inter-season collections.

This systematic review identified and assessed the methods used for monitoring blacklegged tick populations; however, there are several limitations. The review may be subject to language bias, due to the exclusion of non-English studies; however, we are not aware of any non-English studies on blacklegged tick surveillance in North America. Since we did not perform a search of the grey literature, our results may be biased towards positive results due to publication bias. Only a few studies reviewed tested the efficacy of various methods against one another; therefore, our ability to assess which method was preferential was limited. The efficacy of a method was affected by blacklegged tick abundance in a study area, the stage targeted, season and habitat heterogeneity. Due to the heterogeneity of the settings where studies were performed, it was difficult to compare methods across studies.

Conclusions

Passive surveillance

From the studies reviewed, passive tick surveillance is not a common practice in areas where Lyme disease has been present for some time (e.g., New York); however, passive surveillance does offer important information in regions with newly established and/or expanding blacklegged tick populations, such as many areas within Ontario. Currently, there is no evidence to support changing the current use of a passive tick surveillance program in Ontario, except where indicated by a PHU's historical surveillance (Technical Report: Update on Lyme disease prevention and control, 2nd edition).

Active surveillance

PHO's systematic review, and its assessment of the available active blacklegged tick surveillance methods, has identified dragging as the best option, supplemented by small mammal trapping where specified. Tick dragging represents a relatively low-cost, easy to use, repeatable method for blacklegged tick monitoring. While walking scored relatively high with dragging in our comparison of active surveillance methods, dragging alone is considered the best measure of human disease risk.^{46,51} Small mammal trapping is not advantageous on a long-term basis; however, where there is a need for targeting samples for immature stages or pathogen detection, this is a preferred method.

References

1. Aguero-Rosenfeld ME, Wang G, Schwartz I, Wormser GP. Diagnosis of Lyme borreliosis. Clin Microbiol Rev. 2005;18(3):484-509. Available from: <u>http://cmr.asm.org/content/18/3/484.long</u>

2. Wormser GP, Dattwyler RJ, Shapiro ED, Halperin JJ, Steere AC, Klempner MS, et al. The clinical assessment, treatment, and prevention of Lyme disease, human granulocytic anaplasmosis, and babesiosis: clinical practice guidelines by the Infectious Diseases Society of America. Clin Infect Dis. 2006;43(9):1089-134. Available from: <u>https://cid.oxfordjournals.org/content/43/9/1089.full</u>

3. Kuehn BM. CDC estimates 300,000 US cases of Lyme disease annually. JAMA. 2013;310(11):1110.

4. Steere AC, Broderick TF, Malawista SE. Erythema chronicum migrans and Lyme arthritis: epidemiologic evidence for a tick vector. Am J Epidemiol. 1978;108(4):312-21.

5. Burgdorfer W, Barbour AG, Hayes SF, Benach JL, Grunwaldt E, Davis JP. Lyme disease-a tick-borne spirochetosis? Science. 1982;216(4552):1317-9.

6. Steere AC, Grodzicki RL, Kornblatt AN, Craft JE, Barbour AG, Burgdorfer W, et al. The spirochetal etiology of Lyme disease. N Engl J Med. 1983;308(13):733-40.

7. Adams DA, Fullerton J, Jajosky R, Sharp P, Onweh DH, Schley AW, et al. Summary of notifiable infectious diseases and conditions - United States, 2013. MMWR Morb Mortal Wkly Rep. 2015;62(53):1-119. Available from: http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6253a1.htm

8. Ogden NH, Trudel L, Artsob H, Barker IK, Beauchamp G, Charron DF, et al. *Ixodes scapularis* ticks collected by passive surveillance in Canada: analysis of geographic distribution and infection with Lyme borreliosis agent *Borrelia burgdorferi*. J Med Entomol. 2006;43(3):600-9.

9. Koffi JK, Leighton PA, Pelcat Y, Trudel L, Lindsay LR, Milord F, et al. Passive surveillance for *I. scapularis* ticks: enhanced analysis for early detection of emerging Lyme disease risk. J Med Entomol. 2012;49(2):400-9.

10. Watson TG, Anderson RC. *Ixodes scapularis* Say on white-tailed deer (*Odocoileus virginianus*) from Long Point, Ontario. J Wildl Dis. 1976;12(1):66-71.

11. Ogden NH, Lindsay LR, Morshed M, Sockett PN, Artsob H. The emergence of Lyme disease in Canada. CMAJ. 2009;180(12):1221-4. Available from: <u>http://www.cmaj.ca/content/180/12/1221.long</u>

12. Scott JD, Fernando K, Durden LA, Morshed MG. Lyme disease spirochete, *Borrelia burgdorferi*, endemic in epicenter at Turkey Point, Ontario. J Med Entomol. 2004;41(2):226-30.

13. Barker IK, Surgeoner GA, McEwen SA, Artsob H. *Borrelia burgdorferi*, the agent of Lyme disease, in tick vectors and wildlife reservoirs in southern Ontario. Ontario Disease Surveillance Report. 1988;9:151.

14. Barker IK, Lindsay LR. Lyme borreliosis in Ontario: determining the risks. CMAJ. 2000;162(11):1573-4. Available from: <u>http://www.cmaj.ca/content/162/11/1573.long</u>

15. Ontario Agency for Health Protection and Promotion (Public Health Ontario). Map of Lyme disease risk areas [Internet]. Toronto, ON: Queen's Printer for Ontario; 2015 [cited 2016 Feb 18]. Available from: http://www.publichealthontario.ca/en/eRepository/Lyme_Disease_Risk_Areas_Map_2015.pdf

16. Ogden NH, St-Onge L, Barker IK, Brazeau S, Bigras-Poulin M, Charron DF, et al. Risk maps for range expansion of the Lyme disease vector, *Ixodes scapularis*, in Canada now and with climate change. Int J Health Geogr. 2008;7:24. Available from: <u>http://ij-healthgeographics.biomedcentral.com/articles/10.1186/1476-072X-7-24</u>

17. Ogden NH, Maarouf A, Barker IK, Bigras-Poulin M, Lindsay LR, Morshed MG, et al. Climate change and the potential for range expansion of the Lyme disease vector *Ixodes scapularis* in Canada. Int J Parasitol. 2006;36(1):63-70.

18. Nelder MP, Russell C, Lindsay LR, Dhar B, Patel SN, Johnson S, et al. Population-based passive tick surveillance and detection of expanding foci of blacklegged ticks *Ixodes scapularis* and the Lyme disease agent *Borrelia burgdorferi* in Ontario, Canada. PLoS One. 2014;9(8):e105358. Available from: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0105358

19. Scott JD, Fernando K, Banerjee SN, Durden LA, Byrne SK, Banerjee M, et al. Birds disperse ixodid (Acari: Ixodidae) and *Borrelia burgdorferi*-infected ticks in Canada. J Med Entomol. 2001;38(4):493-500.

20. Falco RC, Fish D. A comparison of methods for sampling the deer tick, *Ixodes dammini*, in a Lyme disease endemic area. Exp Appl Acarol. 1992;14(2):165-73.

21. Ginsberg HS, Ewing CP. Comparison of flagging, walking, trapping, and collecting from hosts as sampling methods for northern deer ticks, *Ixodes dammini*, and lone-star ticks, *Amblyomma americanum* (Acari: Ixodidae). Exp Appl Acarol. 1989;7(4):313-22.

22. Solberg VB, Neidhardt K, Sardelis MR, Hildebrandt C, Hoffmann FJ, Boobar LR. Quantitative evaluation of sampling methods for *Ixodes dammini* and *Amblyomma americanum* (Acari: Ixodidae). J Med Entomol. 1992;29(3):451-6.

23. Ontario Agency for Health Protection and Promotion (Public Health Ontario), Rosella LC, Pach B, Morgan S, Bowman C. Meta-tool for quality appraisal of public health evidence: PHO MetaQAT. Toronto, ON: Queen's Printer for Ontario; 2015.

24. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JP, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. Ann Intern Med. 2009;151(4):W65-94.

25. Schulze TL, Jordan RA, Hung RW. Biases associated with several sampling methods used to estimate abundance of *Ixodes scapularis* and *Amblyomma americanum* (Acari: Ixodidae). J Med Entomol. 1997;34(6):615-23.

26. Daniels TJ, Falco RC, Fish D. Estimating population size and drag sampling efficiency for the blacklegged tick (Acari: Ixodidae). J Med Entomol. 2000;37(3):357-63.

27. Talleklint-Eisen L, Lane RS. Efficiency of drag sampling for estimating population sizes of *Ixodes pacificus* (Acari: Ixodidae) nymphs in leaf litter. J Med Entomol. 2000;37(3):484-7.

28. Mackay A, Foil L. Seasonal and geographical distribution of adult *Ixodes scapularis* Say (Acari: Ixodidae) in Louisiana. J Vector Ecol. 2005;30(2):168-70.

29. Ogden NH, Trudel L, Artsob H, Barker IK, Beauchamp G, Charron DF, et al. *Ixodes scapularis* ticks collected by passive surveillance in Canada: analysis of geographic distribution and infection with Lyme borreliosis agent *Borrelia burgdorferi*. J Med Entomol. 2006;43(3):600-9.

30. Rand PW, Lacombe EH, Dearborn R, Cahill B, Elias S, Lubelczyk CB, et al. Passive surveillance in Maine, an area emergent for tick-borne diseases. J Med Entomol. 2007;44(6):1118-29.

31. Supergan M, Karbowiak G. The estimation scale of endangerment with tick attacks on recreational towns areas. Przegl Epidemiol. 2009;63(1):67-71.

32. Castro MB, Clover JR. A comparison of visual and flagging methods for estimating adult *lxodes pacificus* (Acari: Ixodidae) tick abundance. J Vector Ecol. 2010;35(2):435-8.

33. Petry WK, Fore SA, Fielden LJ, Kim HJ. A quantitative comparison of two sample methods for collecting *Amblyomma americanum* and *Dermacentor variabilis* (Acari: Ixodidae) in Missouri. Exp Appl Acarol. 2010;52(4):427-38.

34. Terassini FA, Barbieri FS, Albuquerque S, Szabo MP, Camargo LM, Labruna MB. Comparison of two methods for collecting free-living ticks in the Amazonian forest. Ticks Tick Borne Dis. 2010;1(4):194-6.

35. Dobson AD, Taylor JL, Randolph SE. Tick (*Ixodes ricinus*) abundance and seasonality at recreational sites in the UK: hazards in relation to fine-scale habitat types revealed by complementary sampling methods. Ticks Tick Borne Dis. 2011;2(2):67-74.

36. Jameson LJ, Medlock JM. Tick surveillance in Great Britain. Vector Borne Zoonotic Dis. 2011;11(4):403-12.

37. Schulze TL, Jordan RA, Dolan MC. Experimental use of two standard tick collection methods to evaluate the relative effectiveness of several plant-derived and synthetic repellents against *Ixodes scapularis* and *Amblyomma americanum* (Acari: Ixodidae). J Econ Entomol. 2011;104(6):2062-7.

38. Tack W, Madder M, De Frenne P, Vanhellemont M, Gruwez R, Verheyen K. The effects of sampling method and vegetation type on the estimated abundance of *Ixodes ricinus* ticks in forests. Exp Appl Acarol. 2011;54(3):285-92.

39. Gherman CM, Mihalca AD, Dumitrache MO, Gyorke A, Oroian I, Sandor M, et al. CO_2 flagging - an improved method for the collection of questing ticks. Parasit Vectors. 2012;5:125. Available from: <u>http://parasitesandvectors.biomedcentral.com/articles/10.1186/1756-3305-5-125</u>

40. Rydzewski J, Mateus-Pinilla N, Warner RE, Nelson JA, Velat TC. *Ixodes scapularis* (Acari: Ixodidae) distribution surveys in the Chicago metropolitan region. J Med Entomol. 2012;49(4):955-9.

41. Yun SM, Song BG, Choi W, Park WI, Kim SY, Roh JY, et al. Prevalence of tick-borne encephalitis virus in ixodid ticks collected from the Republic of Korea during 2011-2012. Osong Public Health Res Perspect. 2012;3(4):213-21. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3747658/

42. Chong ST, Kim HC, Lee IY, Kollars TM Jr, Sancho AR, Sames WJ, et al. Comparison of dragging and sweeping methods for collecting ticks and determining their seasonal distributions for various habitats, Gyeonggi Province, Republic of Korea. J Med Entomol. 2013;50(3):611-8.

43. Dantas-Torres F, Lia RP, Capelli G, Otranto D. Efficiency of flagging and dragging for tick collection. Exp Appl Acarol. 2013;61(1):119-27.

44. Rulison EL, Kuczaj I, Pang G, Hickling GJ, Tsao JI, Ginsberg HS. Flagging versus dragging as sampling methods for nymphal *Ixodes scapularis* (Acari: Ixodidae). J Vector Ecol. 2013;38(1):163-7.

45. Diuk-Wasser MA, Liu Y, Steeves TK, Folsom-O'Keefe C, Dardick KR, Lepore T, et al. Monitoring human babesiosis emergence through vector surveillance New England, USA. Emerg Infect Dis. 2014;20(2):225-31. Available from: <u>http://wwwnc.cdc.gov/eid/article/20/2/pdfs/13-0644.pdf</u>

46. Ogden NH, Koffi JK, Lindsay LR. Assessment of a screening test to identify Lyme disease risk. Can Commun Dis Rep. 2014;40(5):83-7. Available from: <u>http://www.phac-aspc.gc.ca/publicat/ccdr-rmtc/14vol40/dr-rm40-05/dr-rm40-05-2-eng.php</u>

47. Russart NM, Dougherty MW, Vaughan JA. Survey of ticks (Acari: Ixodidae) and tick-borne pathogens in North Dakota. J Med Entomol. 2014;51(5):1087-90.

48. Rynkiewicz EC, Clay K. Tick community composition in Midwestern US habitats in relation to sampling method and environmental conditions. Exp Appl Acarol. 2014;64(1):109-19.

49. Wang P, Glowacki MN, Hoet AE, Needham GR, Smith KA, Gary RE, et al. Emergence of *Ixodes scapularis* and *Borrelia burgdorferi*, the Lyme disease vector and agent, in Ohio. Front Cell Infect Microbiol. 2014;4:70. Available from: <u>http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4044495/</u>

50. Armed Forces Pest Management Board. Tick-borne diseases: vector surveillance and control. Technical Guide No. 26 [Internet]. Silver Spring, MD: Armed Forces Pest Management Board, Information Services Division; 2012 [cited 2016 Feb 18].

51. Diuk-Wasser MA, Hoen AG, Cislo P, Brinkerhoff R, Hamer SA, Rowland M, et al. Human risk of infection with *Borrelia burgdorferi*, the Lyme disease agent, in eastern United States. Am J Trop Med Hyg. 2012;86(2):320-7. Available from: <u>http://www.ajtmh.org/content/86/2/320.full</u>

Quality assessment of tick surveillance studies reviewed

Year*	First author	Assessment of relevancy	Assessment of reliability		Assessment of validity				Assessment of applicability
		[1] Was the justification for the study clearly stated? [2] Do the study results apply to the issue under consideration?	 [1] Is the rationale for study clearly stated, and does the study focus on a clearly defined issue? [2] Can the study be reproduced with the information provided? 	[3] Are tick collection methods defined? [4] Are host species reported? [5] Are tick identification methods described? [6] Is the tick stage identified? [7] Are collection locales clearly identified?	[1] Is the research question congruent with the study design?	[2] Are the results consistent within the study; No sources of bias? [3] Can chance findings be ruled out?	[4] Are the results conclusive? [5] Are the authors' conclusions clearly derived from the results? [6] Are potential discrepancies discussed? [7] Are limitations of work described?	[8] Are there any major methodological flaws that limit the validity of findings?	[1] Can the study results be interpreted and analyzed within the context of public health?
1989	Ginsberg	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
1992	Falco	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
1992	Solberg	Yes	Yes	Yes	Yes	No	Yes	No	Yes
1997	Schulze	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
2000	Daniels	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
2000	Tallenklint- Eisen	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
2005	Mackay	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes

2006	Ogden	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
2007	Rand	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
2009	Supergan	Yes	No	No	Yes	Yes	No	Yes	Yes
2010	Castro	No	No	No	Yes	Yes	Yes	Yes	Yes
2010	Petry	No	Yes	Yes	Yes	Yes	Yes	No	Yes
2010	Terassini	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
2011	Dobson	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
2011	Jameson	Yes	No	No	No	Yes	Yes	Yes	Yes
2011	Schulze	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
2011	Tack	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
2012	Gherman	No	Yes	Yes	Yes	Yes	Yes	No	Yes
2012	Rydzewski	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
2012	Yun	Yes	Yes	No	Yes	Yes	Yes	No	Yes
2013	Chong	Yes	Yes	No	Yes	Yes	Yes	No	Yes
2013	Dantas- Torres	Yes/No	Yes	Yes	Yes	Yes	Yes	No	Yes
2013	Rulison	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
2014	Diuk- Wasser	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
2014	Ogden	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
2014	Russart	No	Yes	Yes	Yes	Yes	Yes	No	Yes
2014	Rynkiewicz	Yes	Yes	No	Yes	No	Yes	Yes	Yes
2014	Wang	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes

*Year study published

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