TICK SURVEILLANCE PRACTICES IN THE NORTHEAST

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TICK SURVEILLANCE PRACTICES IN THE NORTHEAST

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Presented by:
Bryon Backenson, New York State Department of Health
Kirby Stafford, Connecticut Agricultural Experiment Station
Melissa Prusinski, New York State Department of Health
Maria Diuk-Wasser, Columbia University
Patti Casey, Vermont Agency of Agriculture
Charles Lubelczyk, Maine Medical Center Research Institute

Tick Surveillance: Overall Considerations

Bryon Backenson, MS
Bureau of Communicable Disease Control
New York State Department of Health

Definition: Public Health Surveillance

"The continued watchfulness over the distribution and trends of incidence (of disease) through the systematic collection, consolidation and evaluation of morbidity and mortality reports and other relevant data" and the regular dissemination of data to "all who need to know."

A. Langmuir, NEJM 1963; 268:182-92.

(in other words, "Information for Action")

Data Sources and Methods for Public Health Surveillance

- Notifiable Diseases
- Laboratory Reporting
- Vital Records (death certificates)
- Administrative data (hospital discharge, billing data)
- Syndromic surveillance
- Zoonotic (Animal), reservoir and vector surveillance
- Ongoing surveys (Behavioral Risk Factor Survey, National Immunization survey)
- Prescription drug or OTC drug purchase data

What is the Purpose of Vector Surveillance?

- Identify where vectors are breeding or abundant
- Identify the species present
  - Species is important in determining risk of transmission to humans
- Monitor vector population densities (in different life stages) by species over time
- Identify infectious agents in the vector population

Detect pathogens and prevent their spread!

Determine risk and prevent disease!

What Public Health Surveillance Is:

- Systematic
- Ongoing
- Data Collection
- Data Analysis
- Interpretation
- Dissemination
- Link to public health practice

What Surveillance Is Not:

- A "study" or "research"
  - Time limited
  - Doesn't usually focus on whole population
  - Test a hypothesis

Questions for Thought:

- Why are you considering vector surveillance?
- What do you want to do, and can you actually do it?
  - Resources?
  - Personnel?
  - Equipment?
  - Skills?
  - Data Collection?
  - Data Analysis?
  - Interpretation?
  - Dissemination?
  - Link to public health practice!
    - Control?
    - Education?

Tick Surveillance

- What, if any, species are you trying to target?
  - Techniques may vary
- How much is enough? Too much? What is representative?
  - Balance of collections vs. results

Passive vs. Active surveillance

→ Passive
- People bring/send you specimens (Doesn't always mean less work!)
  - Tick identification services
  - Tick spotter apps
  - Veterinary surveys
- What level of service are you going to provide to the submitter?

→ Active
- You go out and do collections
  - Dragging and flagging
  - Walking surveys
  - CO_2 and other traps
  - Host surveys

Passive Tick Surveillance: Pros and Cons

- Pros
  - → Gain tick distribution data via crowdsourcing
  - → More granular than active surveillance?
  - → Identify risk groups
  - → Interact with public/education opportunities
- Cons
  - → Expensive startup—microscope, storage, etc.
  - → Ongoing costs? Mailing?
  - → Time consuming, fun with packaging
  - → Data interpretation can be limited
  - → Potential data and specimen management issues
  - → Potential medical liability issues?
  - → Dealing with confidential patient data

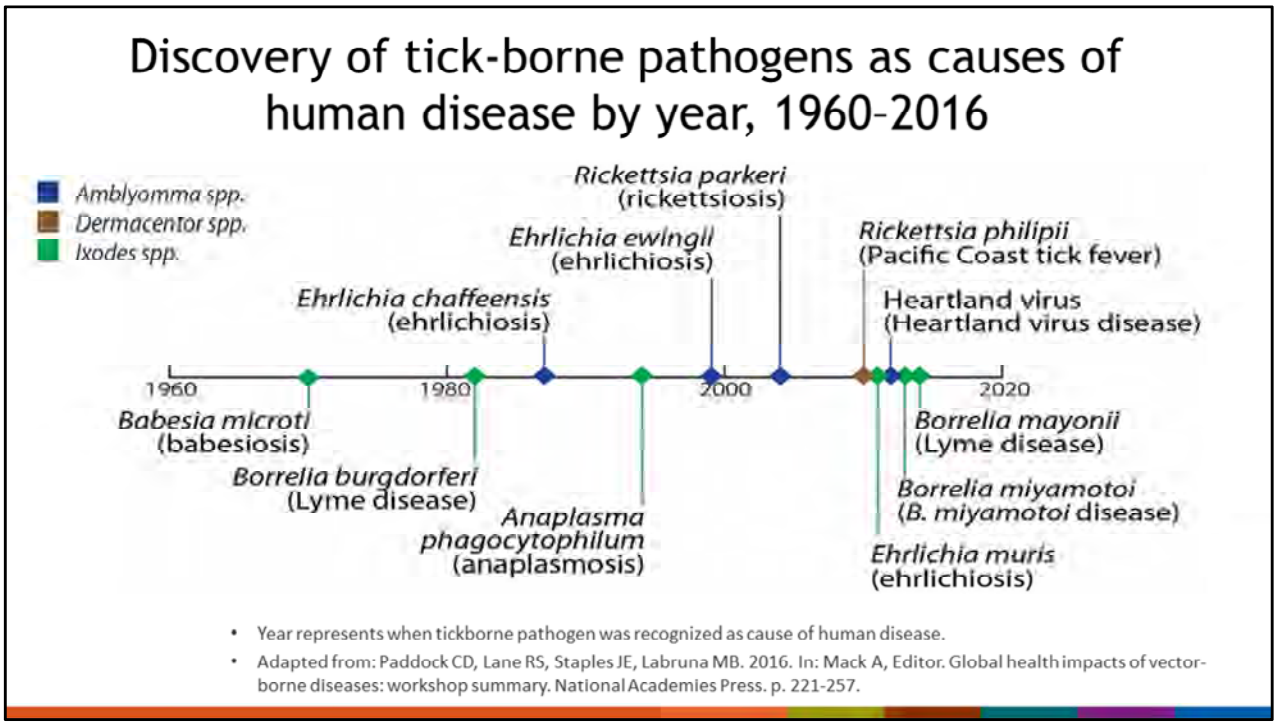
To test or not to test?

Active Tick Surveillance: Pros and Cons

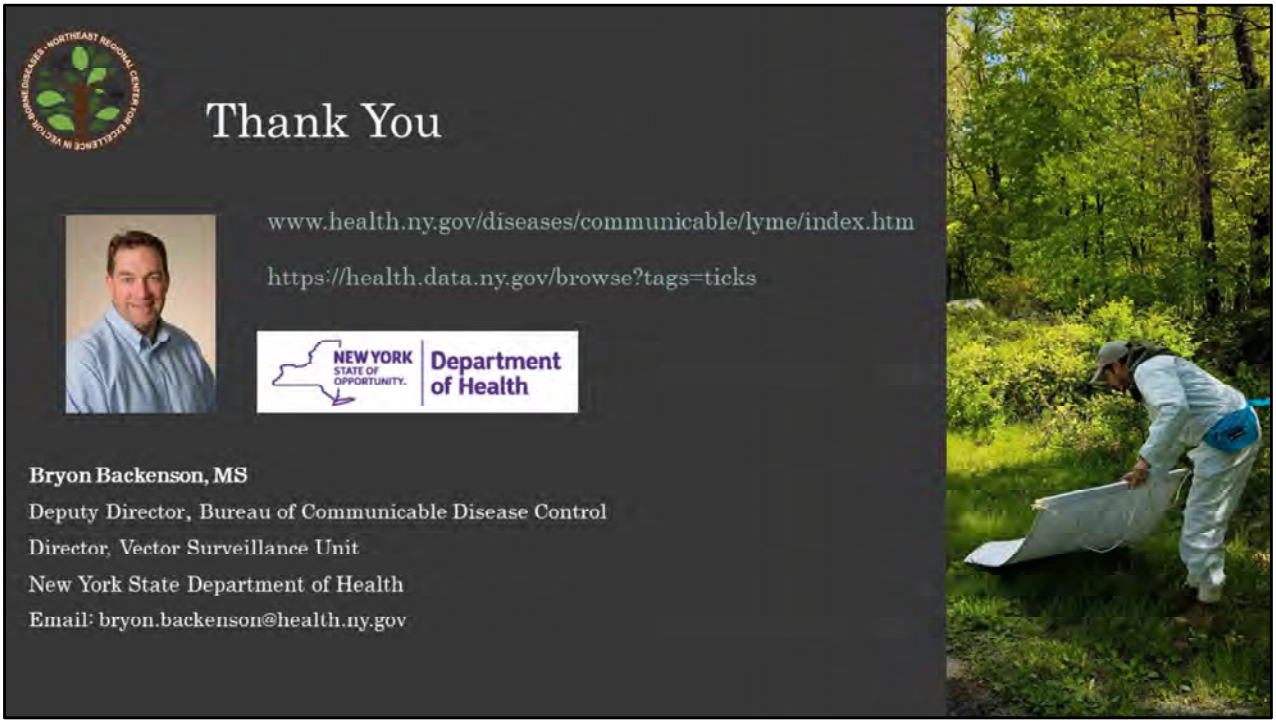
- Pros
  - → Maximum control over surveillance
  - → Can be "more scientific"
  - → Provides high quality data
  - → Interact with public/education opportunities
- Cons
  - → Time and resource intensive
  - → External factors can influence success
  - → Typically collect then test, collect then test
  - → Depending on resources, can become geographically limited—not as many data points
  - → Safety concerns

To test or not to test?

Why is all this important?



Bourbon virus diseases not yet included
Rickettsia philipii = *Rickettsia* species 364D
Ehrlichia muris = E. muris eauclairensis



Tick Surveillance
A Review of Methods by Species

Notes on Collecting

Blacklegged Ticks

Lone Star Ticks

American Dog Ticks

Asian Longhorned Ticks

Kirby C. Stafford, Ph.D.
Chief Scientist, State Entomologist
Connecticut Agricultural Experiment Station
New Haven, CT

- Once the tick is settled into a waiting posture on a stem or leaf, host's approach, may cause the tick to assume characteristic questing posture oriented towards stimulus (vibration, shadow, CO_2) with front legs raised and often waving.
- Classic method of collecting free-living, host-seeking Ixodid ticks (hard ticks) is called dragging or flagging for these ticks. Up to 90% of the tick's life cycle may be spent off-host.

Drawing of *I. persulcatus* from Pomerantzev, 1959; picture of questing *I. scapularis* nymph (ALDF), *D. variabilis* in waiting posture, and questing *H. longicornis* (CDC-James Gathany) (side images K. C. Stafford except ticks on drag from USDA).

Note: References in the notes are summarized in slides at the end of the presentation

References

Balashov 1972

Pomerantzev, 1959

Tick Surveillance

1. Sample host-seeking (questing) ticks
2. Sample host-feeding ticks

Method used will depend, in part, on the goals of sampling

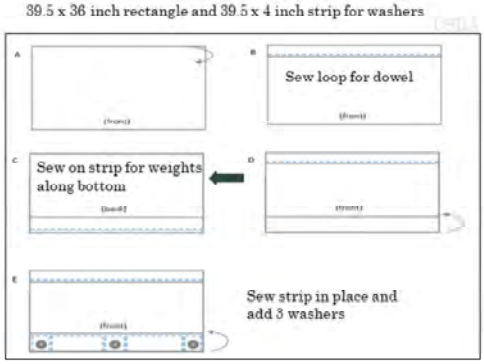
Methods

- Dragging, Flagging, Sweeping
- Walking surveys
- Carbon dioxide (CO_2) traps
- Collect ticks small to medium-sized mammals, birds, lizards
- Collect ticks from white-tailed deer
- Ticks from humans/pets submitted to you (passive surveillance)

Gladney, W. J. 1978.

Drag Sampling

- Inexpensive, easy to use.
- Make a tick drag using flannel-like sheeting, materials vary between researchers; corduroy, polar fleece, muslin, etc.
- A number of researchers did not favor the plastic backed fleece suggested in the CDC surveillance guidelines for *I. scapularis*.
- Material selection also dependent on vegetation to be sampled. Tick sweep variant of flag.



Tick sweep variant of tick flag (USDA K7292-5)

Sampling lawn edge

Surveillance for *Ixodes scapularis* and pathogens found in this tick species in the United States Cdc-pdf [PDF – 34 pages] Print only

Drag Sample Goals/Considerations

- Objective sampling (presence/absence, density est., pathogens, risk, phenology?)
- Seasonality - life stage
- Size of transect or area plot (e.g., 100 m or 100 m^2)
- Frequency check drag (10-20 m)
- Other issues (e.g., sample site selection, density vegetation, personal protection, shipping for pathogen testing)

Teaching drag sampling at the NEVBD Boot Camp May 2019 (note Tyvek suits)

Note: Popular Science, November 2004, listed tick collecting or tick dragger as one of the worst jobs in science. Welcome to doing tick surveillance. ☺

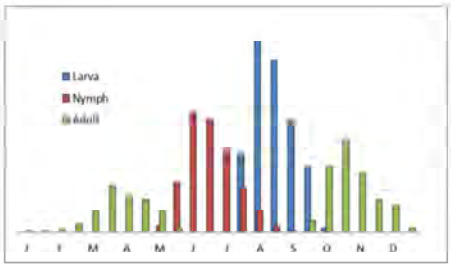
Other considerations:

- Sample site selection
- Time of day – diel activity
- Density of vegetation
    - Dense vegetation such as Japanese barberry, *Rubus* or *Smilax* spp., and honeysuckle often limits use of drag sampling, and use of flag or sweeps at the bottom or on the top of the vegetation may be the only option.
- Personal protection
    - Many researchers use Tyvek suits and tape bottom to prevent tick bites.
    - The CDC suggests use of EPA registered tick repellents DEET, picaridin, IR3535, Oil of Lemon Eucalyptus (OLE), para-menthane-diol (PMD), or 2-undecanone, or treat clothing and gear with products containing 0.5% permethrin for personal protection.
    - Personally, I use permethrin-treated clothing and, properly used, I have not found it to influence my tick collecting. I have had very, very few tick bites. I suspect that the use of DEET could potentially contaminate tick drags.

Seasonal Activity

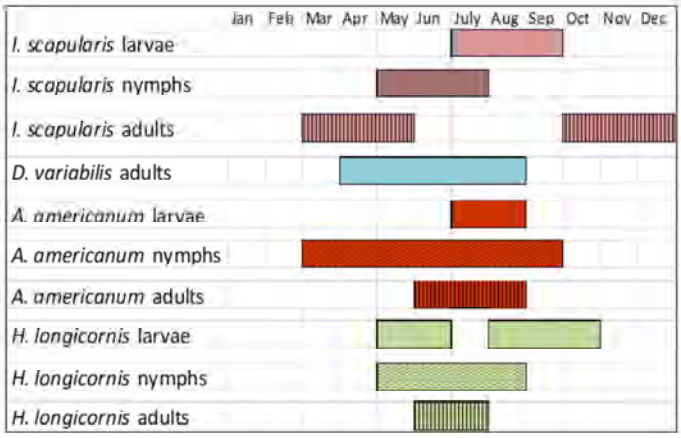
Remember tick activity is not a block.

- Activity begins, peaks and trails off through the season.
- Need 2-3 visits per site within the season.



Generalized seasonal activity of *Ixodes scapularis* ticks (adults, nymphs, and larvae), northeastern U.S. (not to scale) (Stafford).

Seasonal activity: focus may vary with tick species & stage; could be sampling most of the year.



Based on NYSDOH & CAES passive surveillance, & Tufts et al. 2019
Seasonal activity for NE and mid-Atlantic region only

Reference: Tufts et al. 2019

Tick Density, Storage, & Shipping Ticks for Pathogen Testing

Density estimate definitions:

- DON – density of nymphs per unit area, generally number/100 m^2
- DIN – density of infected nymphs per unit area, need pathogen infection data
- ERI or entomological risk index = DIN

- Shipping likely infected ***live*** ticks may require UN3373 Biological Substance Category B certification and labeling.
  - Includes diagnostic samples, but diagnostic specimens do not include live infected animals.* Gray area for ticks.
  - Good idea if shipping large numbers.
- To screen for non-viral pathogens send in very small volumes of ethanol (so exempt from labeling flammable liquids).
- To test for arboviruses, freeze at -80°C and ship on dry ice.
- Store and ship in DNA/RNA Shield™, RNA*later*™, or similar material.

*"Human or animal specimens for which there is minimal likelihood that pathogens are present are not subject to these Regulations if the specimen is transported in a packaging which will prevent any leakage and which is marked with the words "Exempt human specimen" or "Exempt animal specimen", as appropriate.

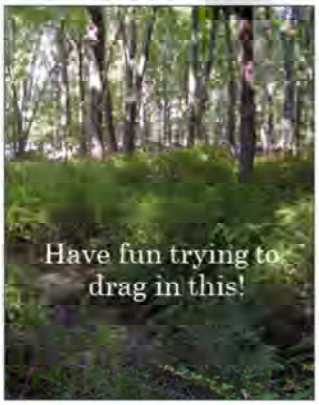
Blacklegged Tick
Ixodes scapularis

- Adult blacklegged ticks have an apparent threshold of questing activity around 4°C (39.2 °F).
- Humidity, solar exposure, soil temperature, may affect activity.
- Activity of nymphs increases slightly dawn & dusk, but it appears a fraction of ticks quest most of the time, regardless of conditions.

Drag Sample Notes *I. scapularis*

- Dragging efficiency independent of tick density, but variable among personnel.
- Number of nymphs recovered by dragging > CO_2 > number from mice, although results are site variable.
- CO_2 traps are not very effective; blacklegged ticks are ambush strategists and don't move far.
- What proportion of the population are you picking up in drag sampling?
  - Nymphal drag efficiency is 3.3-9.1%
  - Adult drag efficiency is 1.9-6.4%.
- Most ticks are in woods, but 80% of nymphal ticks on lawns are recovered within 3 m lawn edge.

Low to no shrub cover, with leaf litter, easy to drag



75-100% shrub cover

- Ticks do not fly, jump, or drop from trees!
- Walking surveys may recover more adults than dragging.
- Nymphs are almost exclusively collected by dragging.
- Walking surveys work well to collect adult ticks from the shrub layer; walking surveys are not suitable for density estimates.
- Dense vegetation such as Japanese barberry, *Rubus* or *Smilax* spp. often limits placement of sample plots or transects or use of drags and limits sampling to the top of the vegetation where adult ticks may be found.
- In the southeastern states, nymphs are rarely collected by drag sampling (don't quest above leaf litter) (adults only).

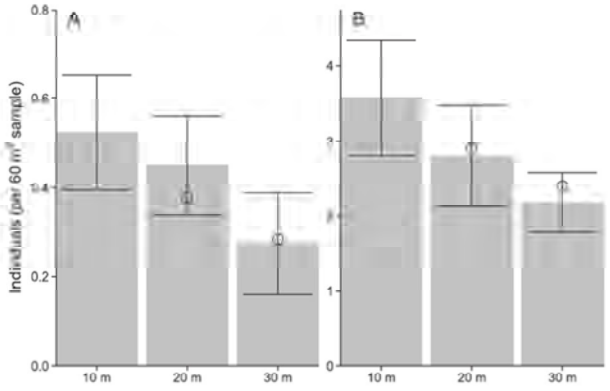
References

Daniels et al. 2000; Carrol et al. 1992; Ginsberg, H. S. and C. P. Ewing. 1989.
Stafford & Magnarelli 1993; Falco & Fish 1992; Schulze et al. 1997

How often check drags for ticks?

- The retention of ticks on drags varies significantly between vegetation densities.
- Drags should be checked at 10-m intervals in dense vegetation, whereas in sparse vegetation this distance can be extended to 20-m without significant loss of acquired *I. scapularis* or *A. americanum* ticks.
- No ticks collected does NOT mean no ticks are present.

Number of *I. scapularis* ticks per 60-m^2 sample depending on the distance between drag-cloth checks. (A) adults and (B) nymphs (Borgman-Winter and Allen, 2019)



Distance between cloth checks

Reference: Borgman-Winter and Allen, 2019

Sampling Host-Feeding *I. scapularis*

Sherman box traps are used for small mammals, mainly white-footed mice *Peromyscus leucopus*

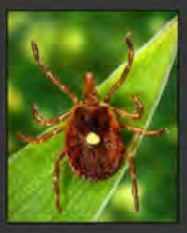
Issues sampling small mammals

- **Expensive**
  (cost traps, bait, tags, anesthesia, gloves, respiratory protection)
- **Labor intensive**
  (setting & retrieving traps, processing animals)
- **Requires skill**
  (especially for use of anesthesia and obtaining serological [blood] samples)
- **Logistics, risks**
  (wildlife permit acquisition, IACUC approval, trap transport, set up sample size grids, potential exposure to animal pathogens)

Issues sampling white-tailed deer

- Availability and access to deer hunter check stations, fall season timing for adult ticks only.
- Live sampling (tranquilize deer).

Lone Star Tick
Amblyomma americanum



- Lone star ticks will engage in both vegetative ambush and active pursuit of hosts.
- Deer, coyotes and a number of medium-sized mammals are hosts for all stages of the tick.
- Wild turkeys are important hosts. Rodents do not appear to be important hosts for immature *A. americanum*.

Sampling *A. americanum*

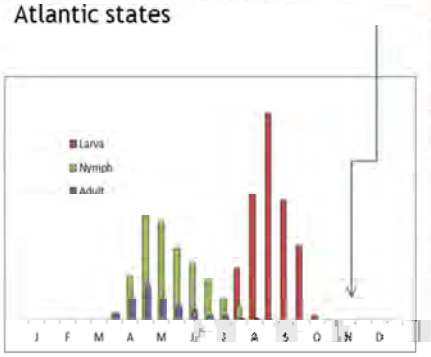
Sample Questing Ticks

- Dragging or flagging
- Walking
- CO_2 traps

- Dragging, walking surveys, and carbon dioxide-baited traps are effective for collecting all life stages of the lone star tick.
- Only dragging/flagging acceptable for density estimates.
- Dense cover such as honeysuckle or barberry limits sampling to the top or edge of the vegetation.

Sample Host-Feeding Ticks

No to few ticks present on white-tailed deer during fall hunting season in the northeast and mid-Atlantic states



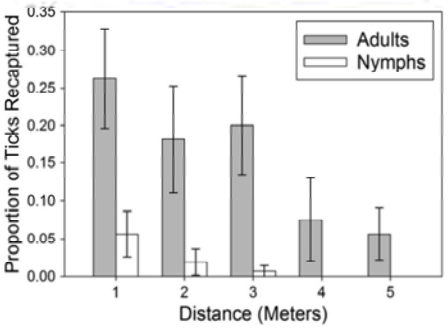
Usually research, i.e., tranquilize deer during appropriate season

Dense vegetation
Amur Honeysuckle

Reference: Shulze et al. 1997

Notes on Use of Carbon Dioxide Traps for *A. americanum*

- **Only provides a presence and relative estimate of tick abundance.**
- Requires placement numerous sites and "rebaiting" with dry ice.
- CO_2 sampling effective for nymphs and adults (10-20% recovery), sampling radius is 3.1-m.
- The proportion of *A. americanum* recovered by CO_2 was found life-stage and distance dependent, but not habitat dependent; adults captured at 5 m, no nymphs from 4-5 m, less effective for larvae.



Kensinger & Allan 2011

One study found effective sampling area with 40% trap efficiency was 48.3 m^2.

Data has been used to estimate absolute numbers, but requires mark/recapture, sampling with replacement, a few assumptions, and lots of calculations!

References:
Bram, Ralph A. 1978.
Gladney, W. J. 1978.
Koch 1987
Kensinger & Allan 2011

American Dog Tick
Dermacentor variabilis

- Only the adult stage feeds on humans or pets.
- Dragging has been found to be more reliable than dry ice sampling for *D. variabilis*.
- Questing activity of adult *D. variabilis* by drag sampling best predicted by ambient temperature.

References: Harlan & Foster 1990

Sampling Notes *D. variabilis*

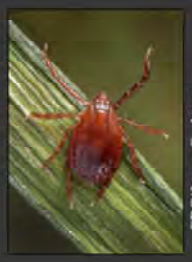
Sample Questing Ticks
- Dragging or flagging
- Walking (no data)
- CO_2 traps

Sample Host-Feeding Ticks
- Small mammals (rodents) for immature stages

- Adults tend to congregate on roadsides and similar edges.
- Dragging was found to be more reliable than dry ice sampling for adult *D. variabilis* . Fraction of the population collected by drag sample was estimated to be 8%.
- Neither dragging or CO_2 found particularly effective for collection immature stages.

While results were similar for dragging and CO_2 in prairie habitat, it was not for other habitats.

References
Carroll 1988; Semtner & Hair 1975; Sonenshine et al. 1966



Asian Longhorned Tick

Haemaphysalis longicornis

- Most Asian longhorned ticks collected to date have been from the environment, followed by white-tailed deer, dogs, raccoon, humans (although don't seem to prefer humans), cows, cats, and other livestock.
- Not found on small mammals (e.g., mice).

Sampling Asian Longhorned Ticks

***H. longicornis* has been collected from very diverse vegetation types!**
→ Woods, ecotone edge, tall grass, lawn, bare ground

Phurchhoki Sherpa, Cornell University, in determining the best collection method for *H. longicornis* found that:

- Drag or sweep method was equally effective
- 5-m check distance better than 10-m or 20-m, best for adult stage
- CO_2 traps were not effective

Sweep method

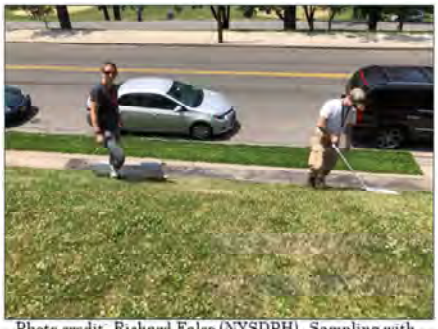


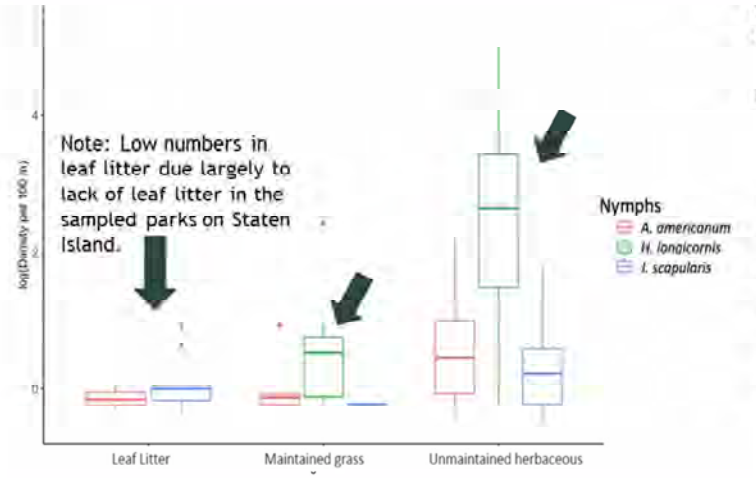
Photo credit: Richard Falco (NYSDPH). Sampling with drag and modified flag (sweep), Westchester Co., NY (Yes, they are found on lawns!)

Westchester County, NY 2018 Experience (Richard Falco)
June 3, 2018 – No *H. longicornis*
June 4, 2018 – 1 *H. longicornis* nymph
Nov. 12, 2018 – 263 nymphs, 422 adults, 115,516 larvae

Phurchhoki Sherpa, Cornell University - Determining the best collection method for *H. longicornis* *(NEVBD Trainee Seminar Series, November 13, 2019)*

Nymphal density is higher in unmaintained herbaceous habitats on Staten Island, NY

for *A. americanum*, *H. longicornis*, and *I. scapularis*

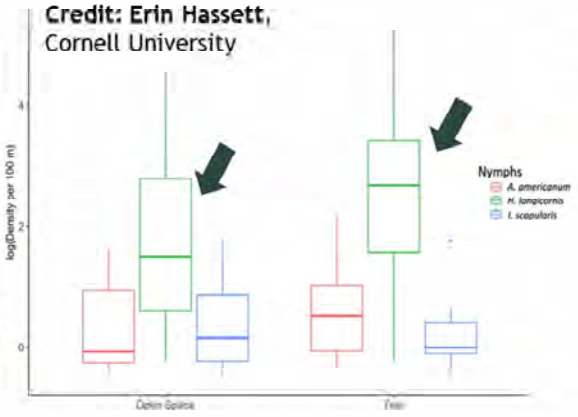


Credit: Erin Hassett, Cornell University - the Risk of Tick Exposure of Park Visitors on Staten Island, New York

Number of *H. longicornis* collected can greatly exceed that of the other tick species in either maintained grass and unmaintained herbaceous habitat.

Similar nymphal density between open spaces and trails on Staten Island, NY

for *A. americanum*, *H. longicornis*, and *I. scapularis*



- Trails were mainly leaf litter and number collected were higher than other tick species.
- Numbers of *H. longicornis* collected can greatly exceed other ticks (thousands in case of larvae).

All habitats should be sampled for *H. longicornis*!

Credit: Erin Hassett, Cornell University - Assessing Knowledge, Attitudes, Practices and the Risk of Tick Exposure of Park Visitors on Staten Island, New York *(NEVBD Trainee Seminar Series, November 13, 2019)*

Summary of tick collection methods acceptable for each tick species

	I. scapularis	*A. americanum*	*D. variabilis*	*H. longicornis*
Dragging, Flagging, Sweeping	Effective all stages	Effective all stages	Effective for adults only	Effective all stages
Walking surveys	Best for adults	Acceptable	ND*	ND*
CO_2 traps	Not effective	Acceptable	Marginal, for adults only	Not effective
Collect ticks from deer*	Acceptable (usually adults only)	Season access restrictions	Not applicable	Acceptable
Collect ticks small to medium-sized, mammals, birds, lizards	Acceptable (generally low numbers)	Not effective for small mammals	Acceptable (only effective way)	Not effective for small mammals
Ticks from humans/pets (passive surveillance)	Acceptable	Acceptable	Acceptable (adults only)	Acceptable***

*Very limiting due to timing of hunting season access
**Little or no data
***Does not seem to prefer humans, but found on dogs

Summary of tick collection methods that are acceptable or unacceptable for each surveillance objective CDC guidelines

Collection Method	Objective: Classify county status	Objective: Presence/Prevalence of pathogens in ticks	Objective: DON/DIN or DOF/DIF	Objective: Phenology
Dragging/Flagging	Acceptable	Acceptable	Acceptable	Acceptable
Walking	Acceptable	Acceptable	Not Acceptable	Acceptable
CO_2 traps	Acceptable	Acceptable for presence, but not prevalence	Not Acceptable	Not Acceptable
Ticks collected from deer	Acceptable	Acceptable for presence, but not prevalence	Not Acceptable	Not Acceptable
Ticks collected from small- or medium-sized mammals, birds, lizards	Acceptable	Acceptable for presence, but not prevalence	Not Acceptable	Acceptable
Ticks from people/pets	Acceptable, if travel history is accounted for	Acceptable for presence, but not prevalence	Not Acceptable	Not Acceptable

Surveillance for *Ixodes scapularis* and pathogens found in this tick species in the United States Cdc-pdf [PDF – 34 pages] Print only

Thank You

https://portal.ct.gov/CAES

Kirby C. Stafford III, Ph.D.
Chief Scientist (Dept. Head), State Entomologist
Department of Entomology
Center for Vector Biology and Zoonotic Diseases
CT Agricultural Experiment Station
123 Huntington Street-Box 1106, New Haven, CT 06504
Ph: (203) 974-8485; Email: Kirby.Stafford@ct.gov

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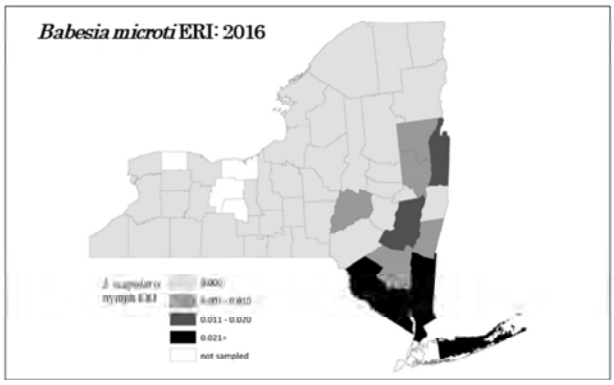
Testing Ticks for Pathogens

Melissa Prusinski
Research Scientist & Laboratory Supervisor
New York State Department of Health
Bureau of Communicable Disease Control
Vector Ecology Laboratory

Why test ticks?

- Detect pathogens
  - Determine presence/absence
  - Determine prevalence
  - Document geographic distribution
  - Track changes over time



Linden et al. 2018. *Transfusion*. 58(3): 660-668

- Estimate risk of tick-borne pathogen exposure
  - Calculate Entomological Risk Index (ERI)* – Active surveillance data
  - Determine frequency of potential exposure – Passive surveillance data

* Mather et al. 1996. *Amer J Epidemiol.* 144 (11). 1066-69

READ SLIDE.

NYSDOH experience:

Passive surveillance by statewide Tick Identification Service 1989-2011.

- Good way to track the spread of I. scapularis on a state-wide scale
- Received approximately 7,500 submissions annually
- 24-hour turn-around time on identifications (species ID, engorgement assessment, condition of mouthparts, etc.)
- Never tested passive surveillance samples for pathogens; liability, cost, and limitations associated with interpreting passive data.

Active statewide tick-borne pathogen surveillance 2008-Current

- Focused on *I. scapularis &* associated pathogens
- 300,000 ticks collected
- 700+ locations sampled
- Nearly 140,000 ticks tested
- Partner with colleges and LHDs regionally to enhance coverage
- Collaborate internally and externally to test for as many agents as possible
- Site-level results to LHDs in counties where surveillance is conducted
- Annual county-level tick surveillance data on the web at www.HealthDataNY.gov

References:

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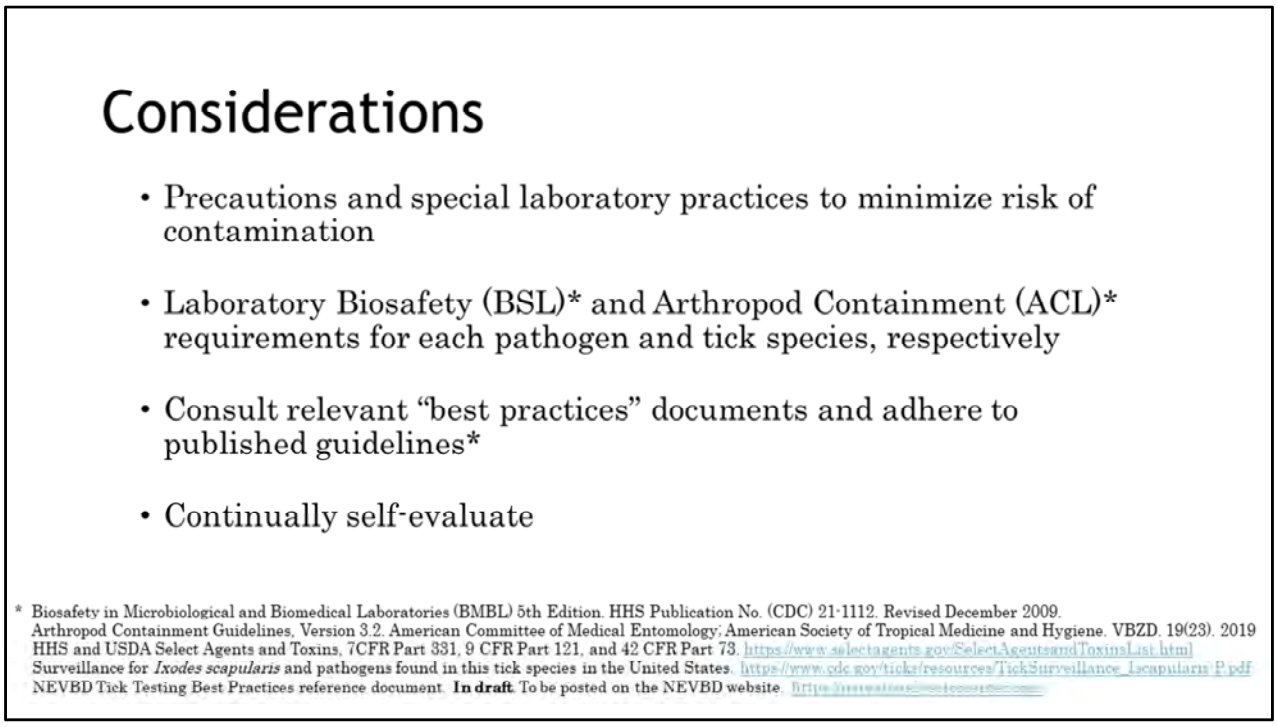


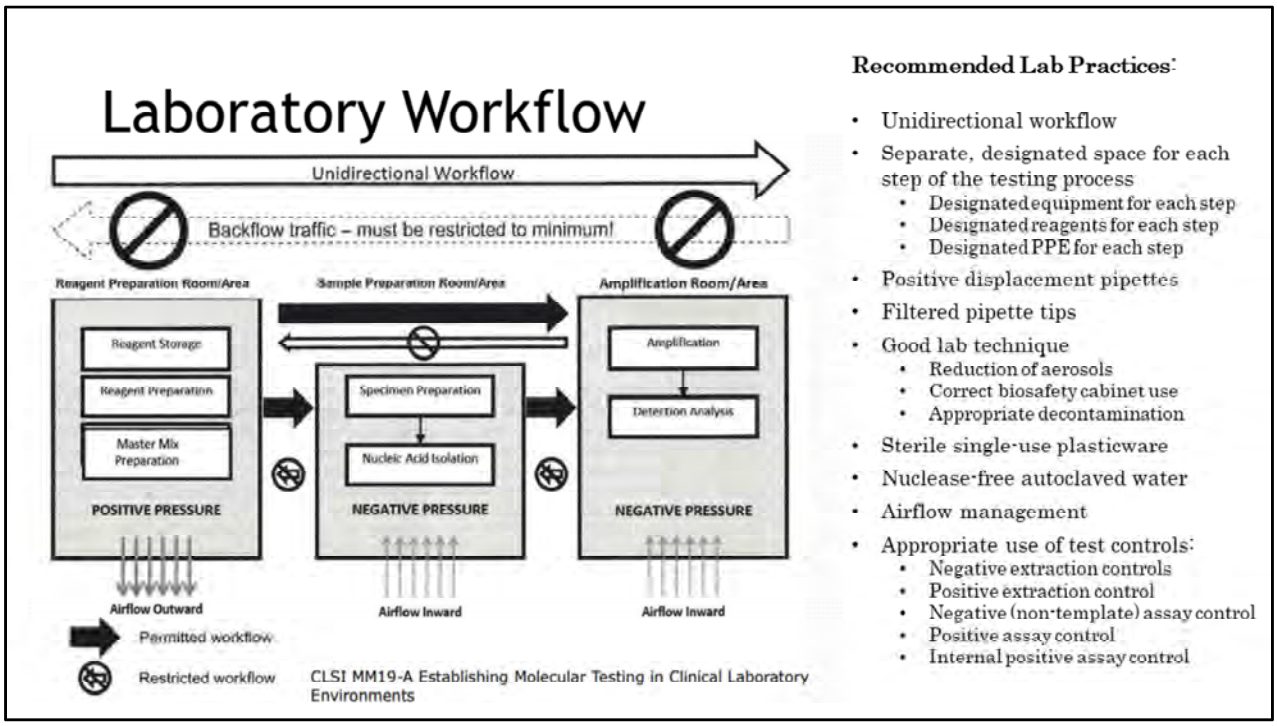
Before you consider testing ticks, determine if you have the appropriate space, trained personnel, and other resources available to do so.
READ SLIDE.

References:
Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition. HHS Publication No. (CDC) 21-1112. Revised December 2009.

Arthropod Containment Guidelines, Version 3.2. American Committee of Medical Entomology; American Society of Tropical Medicine and Hygiene. VBZD. 19(23). 2019 HHS and USDA Select Agents and Toxins, 7CFR Part 331, 9 CFR Part 121, and 42 CFR Part 73. https://www.selectagents.gov/SelectAgentsandToxinsList.html

Surveillance for *Ixodes scapularis* and pathogens found in this tick species in the United States. https://www.cdc.gov/ticks/resources/TickSurveillance_Iscapularis-P.pdf

NEVBD Tick Testing Best Practices reference document. **In draft**. To be posted on the NEVBD website. https://neregionalvectorcenter.com/



Separate designated laboratory space, reagents and equipment required for each step of the testing process (diagram).

Laboratory practices (right)

- Unidirectional flow of samples
- Avoiding aerosols
- Single-use sterilized plasticware
- Nuclease-free autoclaved water
- Airflow management
- Testing controls

References:

Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition. HHS Publication No. (CDC) 21-1112. Revised December 2009.

Arthropod Containment Guidelines, Version 3.2. American Committee of Medical Entomology; American Society of Tropical Medicine and Hygiene. VBZD. 19(23). 2019

HHS and USDA Select Agents and Toxins, 7CFR Part 331, 9 CFR Part 121, and 42 CFR Part 73. https://www.selectagents.gov/SelectAgentsandToxinsList.html

Surveillance for *Ixodes scapularis* and pathogens found in this tick species in the United States.

NEVBD Tick Testing Best Practices reference document. In draft. To be posted on the NEVBD website. https://neregionalvectorcenter.com/

Major tick vectors and most important associated human pathogens (in the Northeastern United States):

Vector Species	Pathogen	Nucleic Acid Type	BSL Designation*	Select Agent?**	Recommended Gene Targets***
Ixodes scapularis (Blacklegged tick)	*Borrelia burgdorferi*	DNA	BSL 2	No	*23S rDNA, ospA*
	Anaplasma phagocytophilum	DNA	BSL 2	No	*msp2, msp4, p44, 16S rRNA*
	Babesia microti	DNA	BSL 2	No	*16S rDNA, 18S rDNA, cox1*
	Borrelia miyamotoi	DNA	BSL 2	No	*glpQ, 23S rDNA, flaB*
	Deer tick virus/Powassan virus lineage 2	RNA	BSL 3	No	NS5 region, 3' UTR
Amblyomma americanum (Lone star tick)	*Ehrlichia chaffeensis*	DNA	BSL 2	No	*dsb, 16S rRNA*
	Ehrlichia ewingii	DNA	BSL 2	No	*dsb, 16S rRNA*
	Heartland virus	RNA	BSL 3	No	S segment
	Bourbon virus	RNA	BSL 3	No	PB1, NP1
Dermacentor variabilis (American dog tick)	*Rickettsia* species incl. *R. rickettsii*	DNA	BSL 3	Some	pan (*ompA, 17-kDa*), *23S-5S IGS, ompA*
	Francisella tularensis	DNA	BSL 3	Yes	*ISFtu2, tul4, iglC*(triplex)
Ixodes cookei (Woodchuck tick)	Powassan virus lineage 1 (prototype)	RNA	BSL 3	No	env

* Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition. HHS Publication No. (CDC) 21-1112. Revised December 2009.
** HHS and USDA Select Agents and Toxins, 7CFR Part 331, 9 CFR Part 121, and 42 CFR Part 73. https://www.selectagents.gov/SelectAgentsandToxinsList.html
*** Surveillance for *I. scapularis* and pathogens found in this tick species in the United States. https://www.cdc.gov/ticks/resources/TickSurveillance_Iscapularis-P.pdf
NEVBD Tick Testing Best Practices reference document. **In draft**. To be posted on the NEVBD website. https://neregionalvectorcenter.com/

Top medically significant ticks in the northeastern US, their major associated pathogens, and characteristics to consider.

- DNA versus RNA-based (specimen storage, prep and testing)
- Biological safety level (laboratory safety practices)
- Select agent? (Must register, requirements to work with select agents)
- Recommended gene targets (regions of the genes listed proven effective).
    - Additional tests may be necessary

List of pathogens, ticks and gene targets is not exhaustive NYSDOH experience:
NYSDOH also tests *H. leporispalustris* and *I. dentatus* for *Fransicella tularensis*, and *H. longicornis* for all pathogens listed (and then some) with NEVBD collaborators

References: Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition. HHS Publication No. (CDC) 21-1112. Revised December 2009.

Arthropod Containment Guidelines, Version 3.2. American Committee of Medical Entomology; American Society of Tropical Medicine and Hygiene. VBZD. 19(23). 2019

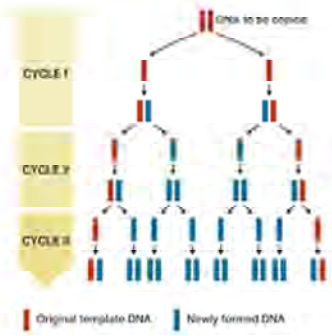
HHS and USDA Select Agents and Toxins, 7CFR Part 331, 9 CFR Part 121, and 42 CFR Part 73. https://www.selectagents.gov/SelectAgentsandToxinsList.html

Surveillance for Ixodes scapularis and pathogens found in this tick species in the United States. h ttps://www.cdc.gov/ticks/resources/TickSurveillance_Iscapularis-P.pdf

NEVBD Tick Testing Best Practices reference document. **In draft.** To be posted on the NEVBD website. https://neregionalvectorcenter.com/

Testing Methods

- Early methods were time-consuming and lacked specificity
  - Direct visualization
  - Fluorescent antibody tagging (DFA/IFA)
  - Animal passage
  - Culturing organisms
- Polymerase Chain Reaction (PCR)
  - Highly sensitive and specific technique
  - Targets and replicates unique nucleic acid sequences
  - Appropriate assay design is critical
  - Consider source of specimens when designing molecular assays and interpreting results.



Early tick testing methods (direct visualization, fluorescent antibody tagging, animal passage, culture)

- Time-consuming
- Required animals or animal products
- Lacked specificity

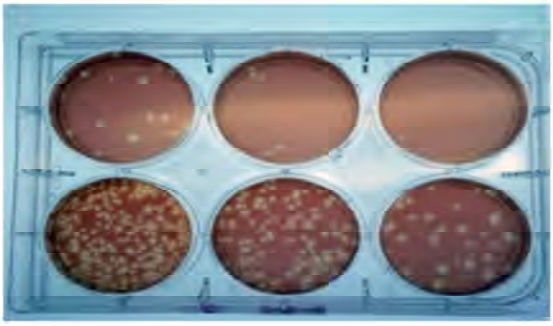
Polymerase Chain Reaction

- Sensitive and specific lab technique detecting unique target DNA sequences
- Precautions to avoid cross-contamination
- Follow all aspects of published methods
- Sequences selected must be unique to the pathogen you are looking for, otherwise false positives occur.
- Sample source important
  - Results from host tissue or engorged ticks, consider with caution
  - **If assays are not designed with specimen source in mind, false positives from host DNA, endosymbionts in ticks, or tick DNA can occur.**

Specimen Storage Options

1. Maintain alive
   - Moisture balance is key
   - 4°C to inhibit growth of mold
2. Fresh frozen at -80°C
   - No preservatives
   - Maintain cold-chain until testing
3. Ethanol preserved
   - 80-100% EtOH recommended
   - Avoid denatured ethanol
4. RNA*later*® or similar product

Need to culture live virus? Options 1 & 2 only.



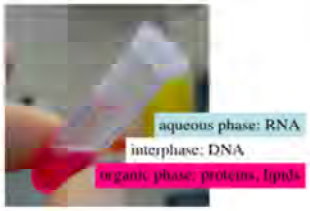
Tick samples for molecular testing:

- Best maintained alive
- Stored fresh-frozen at -80C without preservatives (maintain cold-chain until extraction)
- 80-100% ethanol
- "RNALater" or similar product

Ethanol residue must be removed before processing. **Avoid denatured ethanol, can inhibit PCR**.

Nucleic Acid Extraction Methods

Organic extraction (lysis, phenol chloroform extraction, ethanol precipitation)
- Cheap and yields high quality nucleic acids
- Uses toxic volatile chemicals (safety and waste disposal concerns)
- Many steps (more time consuming and increased chance of contamination)



Chelex extraction (Chelex resin beads)
- Faster and simpler
- Inexpensive
- Does not yield as much quality nucleic acids
- Impurities may impact long-term storage of samples

Solid phase extraction (silica column purification)
- High yield of high quality nucleic acids
- Buffers are optimized for long-term sample storage
- Several kits commercially available
- Process can be automated (at an extra cost)

Magnetic bead-based extraction (MagMAX)
- High yield of high quality nucleic acids
- Automated process
- Copurify DNA and RNA from samples
- Higher equipment and kit costs

Prusinski et al. *J. Med. Entomol.* 2014. 51(1): 226-236
Piedmonte et al. *J. Med. Entomol.* 2018. 55(6): 1496-1508
Thill et al. *J. Med. Entomol.* 2005. 42: 692-696

Before PCR, need to extract and purify the nucleic acids from samples. Common methods are listed here with pros and cons for each. **READ SLIDE.**

NYSDOH experience:
- Initially, used Chelex extraction - concerns about DNA degradation during long-term storage
- Began using spin-column kits - more expensive and time-consuming, took 3.5 hands-on hours to extract 40 samples
- Automation of this process now allows us to extract 92 specimens (and 4 negative extraction controls) in about 90 minutes (30 minutes of hands-on).
    - Negative extraction controls (no sample, just buffer) ensure that cross contamination is not occurring during extraction.
    - Controls are subjected to the same testing as samples – if amplification takes place, contamination during the extraction

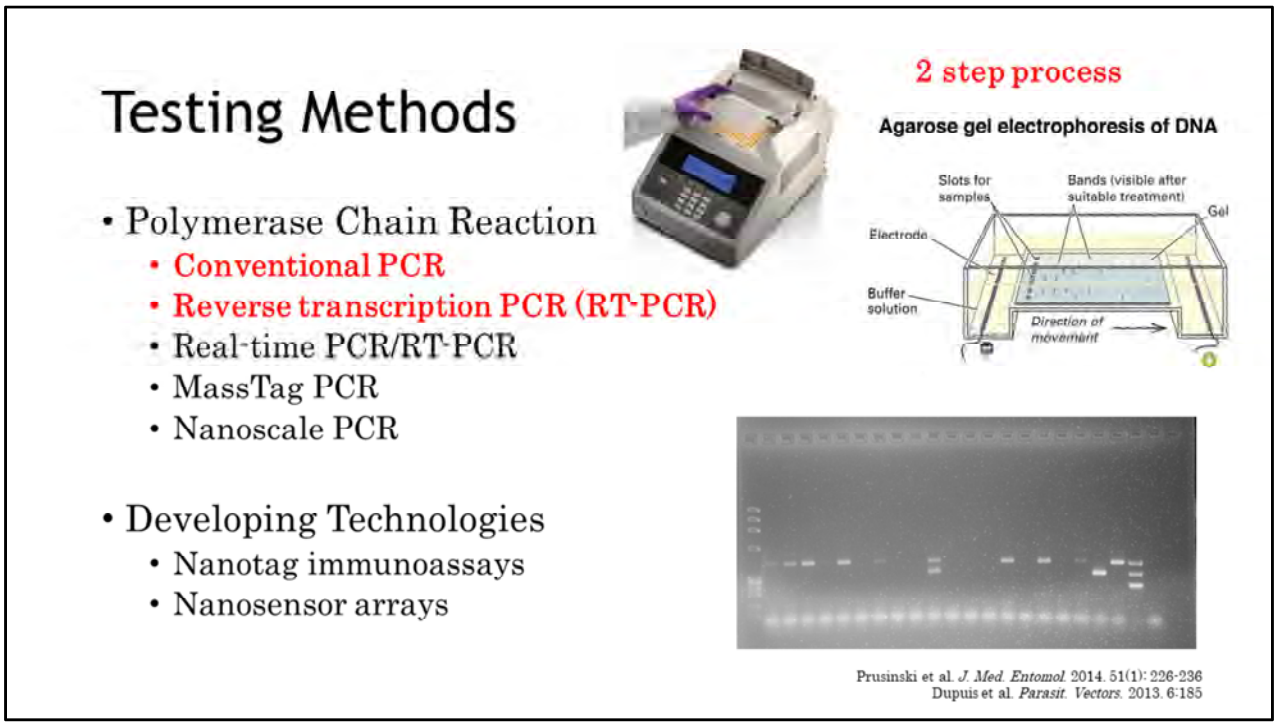
Current NYSDOH extraction costs are approximately $3 per sample using the Qiagen QIAcube HT, an automated silica column purification system, and QIAamp 96 kits.

References:

Piedmonte, N. P., S. B. Shaw, M. A. Prusinski, and M. K. Fierke. Landscape features associated with blacklegged tick (Acari: Ixodidae) density and tick-borne pathogen prevalence at multiple spatial scales in Onondaga County, New York. *J. Med. Entomol*. 2018. 55(6): 1496-1508.

Prusinski, M. A., S. J. Kogut, K. T. Hukey, J. Lee, J. E. Kokas, and P. B. Backenson. Prevalence of *Borrelia burgdorferi* (Spirochaetales: Spirochaetaceae), *Anaplasma phagocytophilum* (Rickettsiales: Anaplasmataceae), and *Babesia microti* (Piroplasmida: Babesiidae) in *Ixodes scapularis* (Acari: Ixodidae) Collected from Recreational Lands in the Hudson Valley Region, New York State. *J. Med Entomol.* 2014. 51(1): 226-236.

Thill, C. D., P. B. Backenson, M. A. Prusinski, S. J. Kogut, J. Lee, and J. L. Coleman. 2005. Detection of *Babesia microti* DNA in *Ixodes scapularis* (Acari: Ixodidae) by use of Chelex 100 resin and polymerase chain reaction. *J. Med. Entomol.* 42: 692-696.



Many variations of PCR - benefits and limitations of each.

Conventional PCR:

- Least expensive and most basic
- $1 or less per reaction in reagents
- Conventional thermal cyclers cost $2,500-$20,000 (depending on the model)
- Results must be visualized using a second technique (gel electrophoresis)
- Additional equipment and reagents
- More time-consuming
- Not ideal for high-throughput testing scenarios

Considerations:

- Negative control (nuclease free water) to test for contaminated PCR reagents
- Positive control (spiked with pathogen DNA) to make sure that the test works
- Consider including an internal control targeting a tick gene to check for PCR inhibition and positive extraction control

RT PCR (reverse transcription of RNA to DNA) - used to detect RNA-based viruses

- Specialized reagents
- Handling requirements, especially if you want to obtain live virus in culture
- Has the same limitations as conventional PCR.

Multiplex allows for simultaneous detection of multiple pathogens in individual samples in a single reaction

- Target DNA products must vary in molecular weight

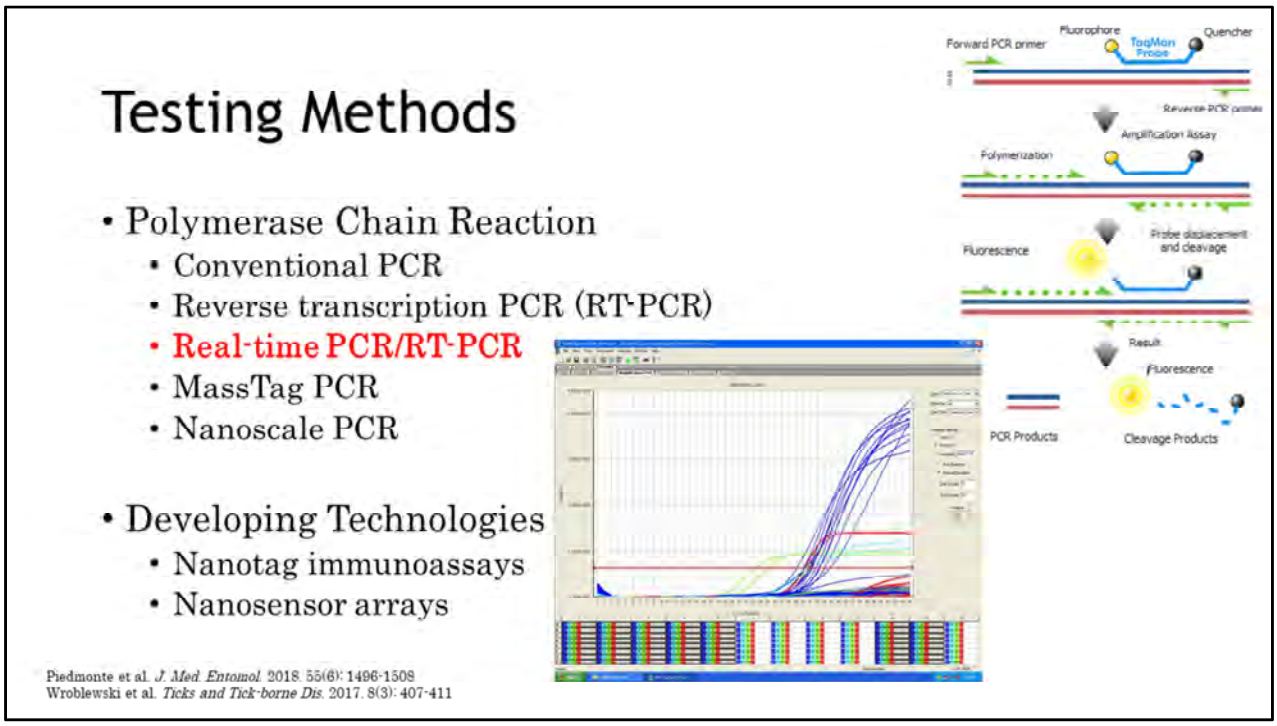
NYSDOH experience:

- When we were running a conventional PCR triplex assay (example pictured bottom right), took more than 5 hours to complete testing of 40 samples
- Total PCR testing cost was $3.77 per sample

References:

Prusinski, M. A., S. J. Kogut, K. T. Hukey, J. Lee, J. E. Kokas, and P. B. Backenson. Prevalence of *Borrelia burgdorferi* (Spirochaetales: Spirochaetaceae), *Anaplasma phagocytophilum* (Rickettsiales: Anaplasmataceae), and *Babesia microti*
(Piroplasmida: Babesiidae) in *Ixodes scapularis* (Acari: Ixodidae) Collected from Recreational Lands in the Hudson Valley Region, New York State. *J. Med Entomol.* 2014. 51(1): 226-236.

Dupuis II, A. P., R. J. Peters, M. A. Prusinski, R. Falco, R. S. Ostfeld, and L. D. Kramer. Isolation of deer tick virus (Powassan virus, lineage II) from *Ixodes scapularis* and detection of antibody in vertebrate hosts sampled in the Hudson Valley, New YorkState. *Parasit. Vectors.* 2013. 6:185.



Real-time PCR and real-time RT-PCR:

- Fluorescent dyes to view and quantify DNA replication of the target sequence in real time (concept/ process explained).
- Eliminates the need to run gels to visualize DNA product
- Good for higher throughput situations
- Can be multiplexed (different colored dyes for different pathogens)
    - No overlap in spectral output so optics can differentiate pathogens
- Scaling up the number of targets limited by loss of sensitivity and machine capabilities (number of dyes it is able to detect)

NYSDOH experience:
Real-time PCR quadplex assay to screen 92 samples in a little over 90 minutes. (results graph)
Criteria for positives explained
Do not rely on machine default threshold values, they are often way too low.
The cost of our real-time PCR quadplex assay is $2.45 per sample
Real-time PCR thermal cyclers cost about $50,000

References:
Piedmonte, N. P., S. B. Shaw, M. A. Prusinski, and M. K. Fierke. Landscape features associated with blacklegged tick (Acari: Ixodidae) density and tick-borne pathogen prevalence at multiple spatial scales in Onondaga County, New York. J. Med. Entomol. 2018. 55(6): 1496-1508.

Wroblewski, D., L. Gebhardt, M. A. Prusinski, T. A. Halse, L. J. Meehan, and K. A. Musser. Detection of Borrelia miyamotoi and other tick-borne pathogens in human clinical specimens and Ixodes scapularis ticks in New York State, 2012-2015. Ticks and Tick-borne Dis. 2017. 8(3): 407-411.

Testing Methods

- Polymerase Chain Reaction
  - Conventional PCR
  - Reverse transcription PCR (RT-PCR)
  - Real-time PCR/RT-PCR
  - MassTag PCR
  - Nanoscale PCR
- Developing Technologies
  - Nanotag immunoassays
  - Nanosensor arrays

Briese et al. *Emerg. Infect. Dis.* 2015. Feb 11(2): 310-313.
Tokarz et al. *Ticks Tick-borne Dis.* 2019. 10, 894-900.

MassTag PCR explained:

- Uses primers tagged with molecules of known masses (MassCodes)
- Each assay can have up to 20 different targets (multiplex) each tagged with different MassCodes
- Mass spectrometer reads results
- Presence of a specific MassCode indicates presence of corresponding pathogen

Columbia School of Public Health experience:

- Costs $15 per reaction
- Mass spectrometry instruments are approximately $75,000
- Thermal cycler cost additional
- Tagged primers are available from commercial vendors
- Primer sequences and protocols are freely available
- Higher throughput testing for a larger number of pathogens in a single reaction
- Higher startup, per reaction, and associated consumables costs

References:

Briese T, Kapoor A, Mishra N, Jain K, Kumar A, Jabado OJ, Lipkin WI. 2015. Virome capture sequencing enables sensitive viral diagnosis and comprehensive virome analysis. mBio 6(5):e01491-15. doi:10.1128/mBio.01491-15.

Tokarz, R., T. Tagliafierro, S. Sameroff, D.M. Cucura, A. Oleynik, X. Che, K. Jain, W.I.Lipkin. Microbiome analysis of Ixodes scapularis ticks from New York and Connecticut. Ticks and Tickborne Dis. 2019. 10:894-900.

Testing Methods

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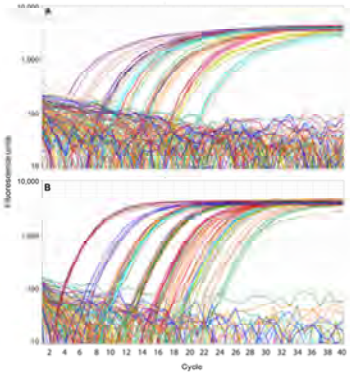


Goodman et al. *J. Vis. Exp.* 2016. (117). e54781. doi:10.3791/54781 (2016).

Nanoscale PCR explained:

- Ideal for high-throughput testing for multiple agents
- Comparable detection limits to real-time PCR

Cornell University's Animal Health Diagnostic Center experience:

- "Tick chip" assay detects up to 26 disease agents from 48 samples per run
- Plates can be custom printed with any combination of real-time PCR assays (TaqMan cycling conditions)
- Plates can run up to 54 assays on 48 samples at a time
- Up to 4 plates can be run together on the QuantStudio 12K OpenArray platform
- Pricing starts at ~$400-500 per plate (varies based on the production scale and customization.)
- Roughly $8 – $10 per sample testing cost
- Approximately $120,000 in instrument costs
- Allows for higher throughput with real-time results
- Has higher startup and associated consumables costs.

References:

Goodman, L.B., Anderson, R.R., Slater, M., Ortenberg, E., Renshaw, R.W., Chilson, B.D., Laverack, M.A., Beeby, J.S., Dubovi, E.J., Glaser, A.L. High-throughput Detection of Respiratory Pathogens in Animal Specimens by Nanoscale PCR. J. Vis. Exp. (117), e54781, doi:10.3791/54781 (2016).

Tick testing capabilities/techniques are continually being developed and refined. Some developing technologies on the horizon include nanotag immunoassays and nanosensor arrays.

Testing Service Available

- CDC tick-borne pathogen surveillance:
  - *B. burgdorferi s.s., B. mayonii, B. miyamotoi, A. phagocytophilum and B. microti*
  - CDC retains DNA extract (or send an aliquot of extracted DNA for testing)
  - Will not test ticks submitted by the general public
  - Contact CDC at: ticksurveillance@cdc.gov prior to submitting ticks or DNA for testing
- The NYS Veterinary Diagnostic Lab/Cornell Animal Health Diagnostic Center:
  - Fee - for - service basis
  - 18 assay x 3 replicate format
  - *H. longicornis* testing currently subsidized by NEVBD
  - Contact ticks@cornell.edu for more information
- Thangamani Laboratory at SUNY Upstate Medical University:
  - Citizen science based (NY State residents only)
  - Free tick testing
  - *B. burgdorferi, B. miyamotoi, Babesia sp., Ehrlichia sp.,* POWV Lineage 1, DTV/POWV Lineage 2, Heartland virus, and Bourbon virus.
  - For more information see: https://thangamani-lab.com/free-tick-testing

J. Med. Entomol

In conclusion:
If tick testing is beyond the capabilities and resources available at your institution and Don't have a governmental or academic research laboratory to partner with

Other testing services are available:
CDC

- Will test ticks submitted by public health partners in support of their surveillance efforts
- No cost, but some limitations –contact CDC first (ticksurveillance@cdc.gov)

NYS Veterinary Diagnostic Lab at Cornell

- Offers tick testing on a fee-for-service basis
- H. longicornis testing currently subsidized by the NEVBD
- Contact the Cornell Vet lab for details (ticks@cornell.edu)

Thangamani Lab at SUNY Upstate Medical University

- Citizen science based tick testing service
- Free of charge for NYS residents.
- Consult website for details (https://thangamani-lab.com/free-tick-testing)

Thank You

www.health.ny.gov/diseases/communicable/lyme/index.htm

https://health.data.ny.gov/browse?tags=ticks

Melissa Prusinski
Research Scientist and Laboratory Supervisor
New York State Department of Health
Bureau of Communicable Disease Control
Vector Ecology Laboratory
Empire State Plaza C-456 – C-467B, Albany, NY 12237
Ph: (518) 473-4439; Email: melissa.prusinski@health.ny.gov

Data Analysis and Interpretation

How do we use tick surveillance data to advance public health?

Maria Diuk-Wasser, PhD
Associate Professor
Department of Ecology, Evolution & Environmental Biology
Columbia University

Tick surveillance – CDC Guidelines

- Tick surveillance is intended to monitor changes in the distribution and abundance of ticks and the presence and prevalence of tick-borne pathogens in order to provide actionable, evidence-based information to clinicians, the public and public health policy makers.
- Key questions address when and where humans are at risk for exposure to ticks and tick-borne pathogens.

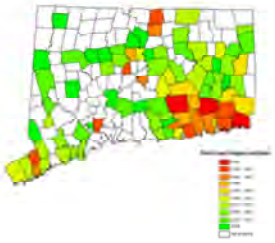
CDC guidelines - https://www.cdc.gov/ticks/resources/TickSurveillance_Iscapularis-P.pdf

Tick surveillance – what scale?

- Regional: Minimum requirements for standardized surveillance across the US (CDC guidelines)
- Local: Identify risk areas within your district

Example:
Estimated and established counties for *Ixodes scapularis*

https://www.cdc.gov/ticks/surveillance/index.html



Example:
Year when towns in Connecticut become endemic for babesiosis

Data on tick presence/absence useful to predict human cases at continental scale, with exceptions

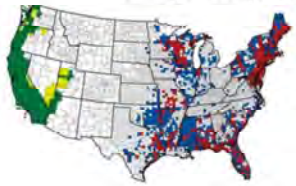
Reported cases of Lyme disease

2001

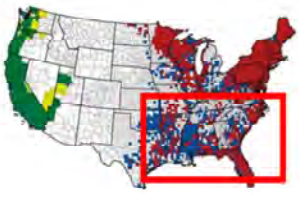
2011

CDC

Counties with *Ixodes* spp established (red/green) or reported (blue/yellow)



1996

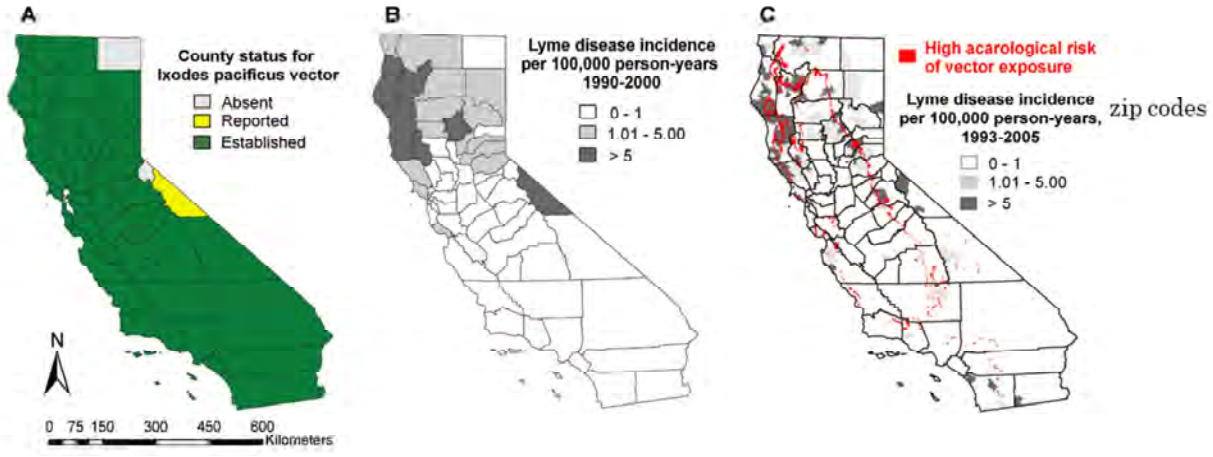


2015

Eisen et al., 2016, JME

Eisen et al. 2016. County-Scale Distribution of *Ixodes scapularis* and *Ixodes pacificus* (Acari: Ixodidae) in the Continental United States. Journal of Medical Entomology 53(2):349-386

County-level tick establishment data is too coarse to predict human cases within states



Eisen and Eisen. 2006. Critical Evaluation of the Linkage Between Tick-Based Risk Measures and the Occurrence of Lyme Disease Cases. Journal of Medical Entomology 53(5):1050-1062.

Figure legend: Assessment of Lyme disease risk in California by:
(A) counties with established populations (reports of at least six ticks or two life stages) or reported presence of the primary vector to humans, *I. pacificus* (Dennis et al. 1998);
(B) county-based Lyme disease incidence per 100,000 person-years from 1990 to 2000 (Fritz and Vugia 2001); or
(C) zip code-based Lyme disease incidence per 100,000 person-years from 1993 to 2005 in relation to the distribution of areas with high projected acarological risk of exposure to I. pacificus nymphs (Eisen et al. 2006c).

Regional mapping (CDC guidelines)

CDC goals (only for *Ixodes scapularis*)

1. Classify county status for *I. scapularis*: established, reported, or no data available.
2. Generate estimates for local prevalence of specific pathogens in relevant *I. scapularis* life stages.
3. Generate estimates of local density of host-seeking nymphs or adults, which then can be aggregated and displayed at county scale.
4. Generate estimates of local density of infected host-seeking nymphs
5. Document host-seeking phenology of all *I. scapularis* life stages in strategic locations across the tick's range and display this information at state or regional spatial scales.

Objective 1: Classify county status

- **Established**: ≥ 6 *Ixodes scapularis* of a single life stage or > 1 life stage collected per county within a 12-month period.
- **Reported**: < 6 *Ixodes scapularis* of a single life stage collected per county within a 12-month period.
- No records

Objective 2: Infection prevalence of pathogens in ticks (Infection prevalence)

- Minimum of 25 ticks (the more ticks, the more accurate)
- Pathogen prevalence and 95% confidence intervals* can be estimated per relevant tick life stage and per collection site in Excel using the software Pooled Infection Rate Add-In: https://www.cdc.gov/westnile/resourcepages/mosqSurvSoft.html
- For example, when 10 of 50 tested ticks are positive, infection prevalence is estimated as 20% (95%CI: 11-33%). Likewise, if no ticks are infected in samples of 25 ticks or 50 ticks, infection prevalence could be as high as 13% or low as 7%, respectively.

*Confidence intervals can be interpreted as "there is a 95% probability that the true infection prevalence is between [insert lower confidence limit] and [insert upper confidence limit]."

Objective 3: Map the county level density of host-seeking *I. scapularis* nymphs (DON)

- At least 1 site sampled per county
- At least 750 m^2 drag sampled per site for density estimate; drags inspected at least every 10-20 m
- Sampling timed to coincide with the peak in nymphal host-seeking activity; at least 2-3 visits to the site.
- In ecologically diverse counties, sampling at multiple sites representing the range in suitable habitat for the tick is recommended; represent average and range.
- Only distance-based assessments of DON and DIN in ArboNET.

Objective 4: Map the county level density of host-seeking infected *I. scapularis* nymphs (DIN = ERI)

- Same sampling requirements as DON.
- Test at least 25 ticks per unit of interest (transect, site, etc.)

DON * Infection prevalence = DIN

Example:

50 ticks/site * 10/50 (20%) are positive = 10 infected ticks/site

Objective 5: Describe when *I. scapularis* ticks are actively host-seeking (phenology)

- Displayed as state records of tick activity by life stage.
- Represented as categorical response
  - Records of the tick being active for a particular month of the year or not

  OR
  - No records if phenology studies were not reported from a particular state or its neighbor
- Based on weekly, bi-weekly, or monthly non-removal sampling over a 12-month period, excluding winter months too cold for tick activity in colder parts of the tick's range.

Local surveillance

Goal: focused surveillance towards actionable local goals

Potential goals and approaches

Using **passive tick surveillance** data:

- Assess association between tick surveillance and human cases

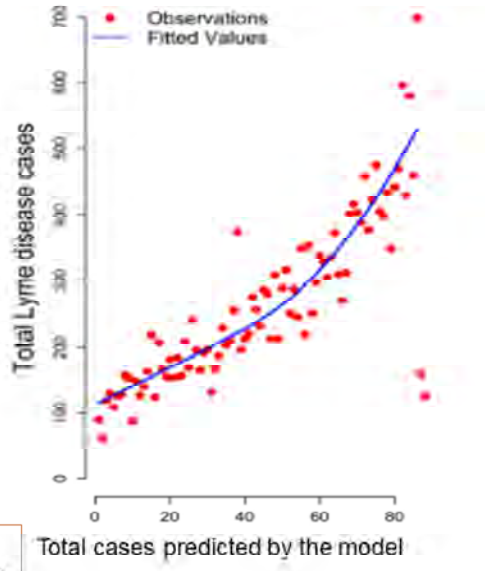
Using **active tick surveillance** data:

- Identify disease risk hot spots
- Create risk maps using environmental variables
- Monitor spread

Using passive tick surveillance: Assess the association between tick surveillance and human cases

Example: Connecticut, USA, town level

Finding of this study: The rate of submitted infected *I. scapularis* nymphs (infected nymphs / human population) are highly predictive of Lyme disease incidence for each town or county

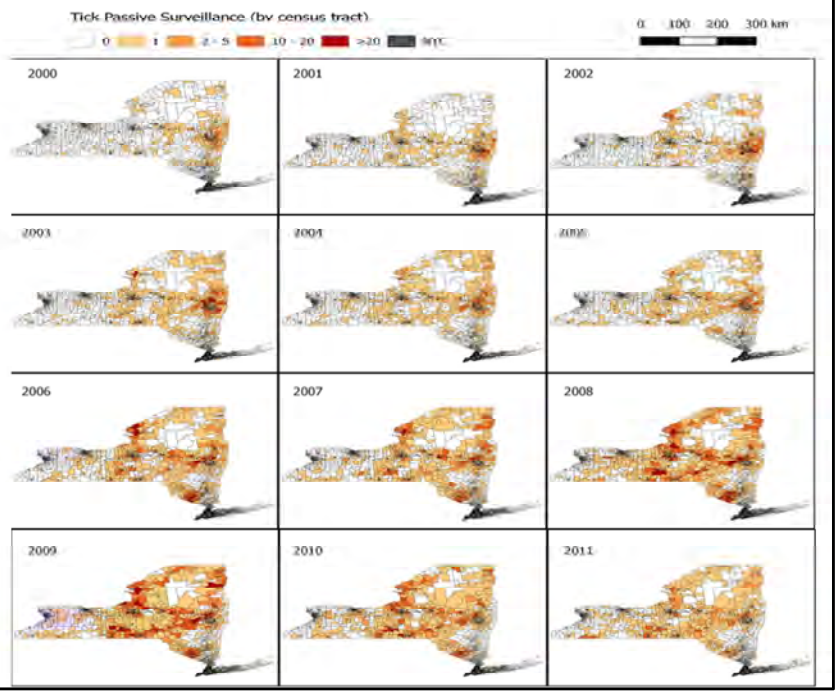


Requires: strong statistical skills (regression-based modeling)
But maps of cases and ticks submitted can be compared visually

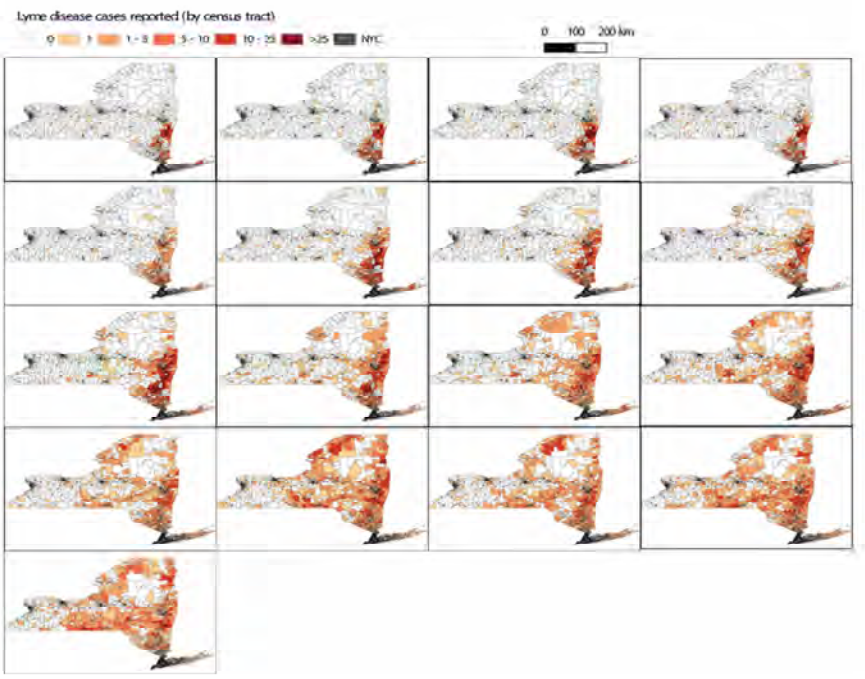
Little et al. 2019

Little et al. 2019. Predicting spatiotemporal patterns of Lyme disease incidence from passively collected surveillance data for *Borrelia burgdorferi* sensu lato-infected *Ixodes scapularis* ticks. Tick and tick-borne diseases 10:970-980.

Passive tick reporting in NY State



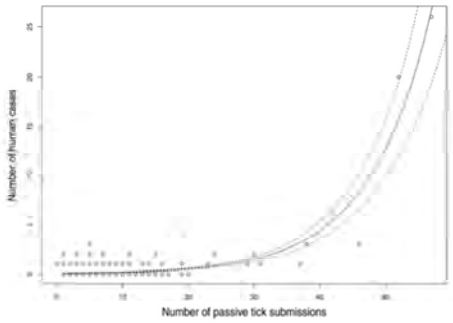
LD cases in NY State



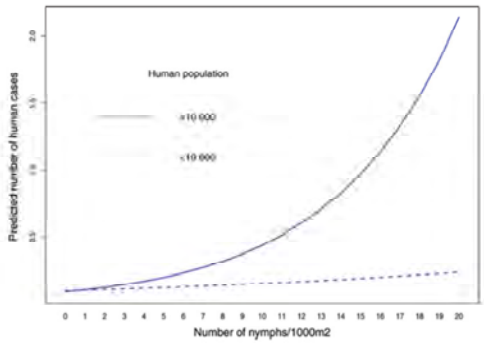
Active tick surveillance data may also be used to predict human cases

Example: Canada

Passive



Active



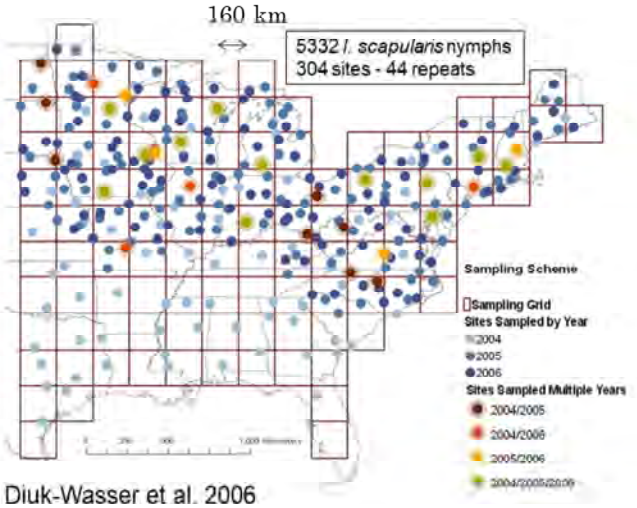
Finding: Passive surveillance can effectively identify human disease risk (cases); active surveillance effective only in endemic areas

Ripoche et al. 2018

Ripoche et al. 2018. Passive tick surveillance provides an accurate early signal of emerging Lyme disease risk and human cases in Southern Canada. Journal of Medical Entomology 55(4):1016-1026

Active tick surveillance

Example: Density of infected *Ixodes scapularis* nymphs in Eastern US (can be applied to any spatial scale)

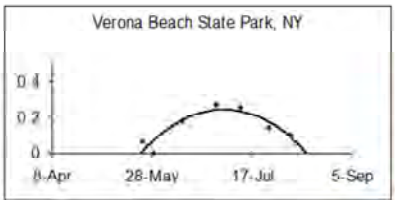


Diuk-Wasser et al. 2006

- Overlay a grid over the area to sample (can also use town/county outlines as units)
- Identify all public access parks in each grid (public databases)
- Rank parks by total forested area
- Randomly select from the top 20%
- A subset of sites sampled multiple years to maximize both spatial representation and inter-annual variability

Diuk-Wasser, MA, A Gatewood, R Cortiñas, S Yaremych-Hamer, J Tsao, U Kitron, G Hickling, J Brownstein, E Walker, J Piesman, and D Fish. 2006. Spatiotemporal patterns of nymphal host-seeking *I. scapularis* (Acari: Ixodidae) in the United States. *Journal of Medical Entomology, 43(2): 166-176.*

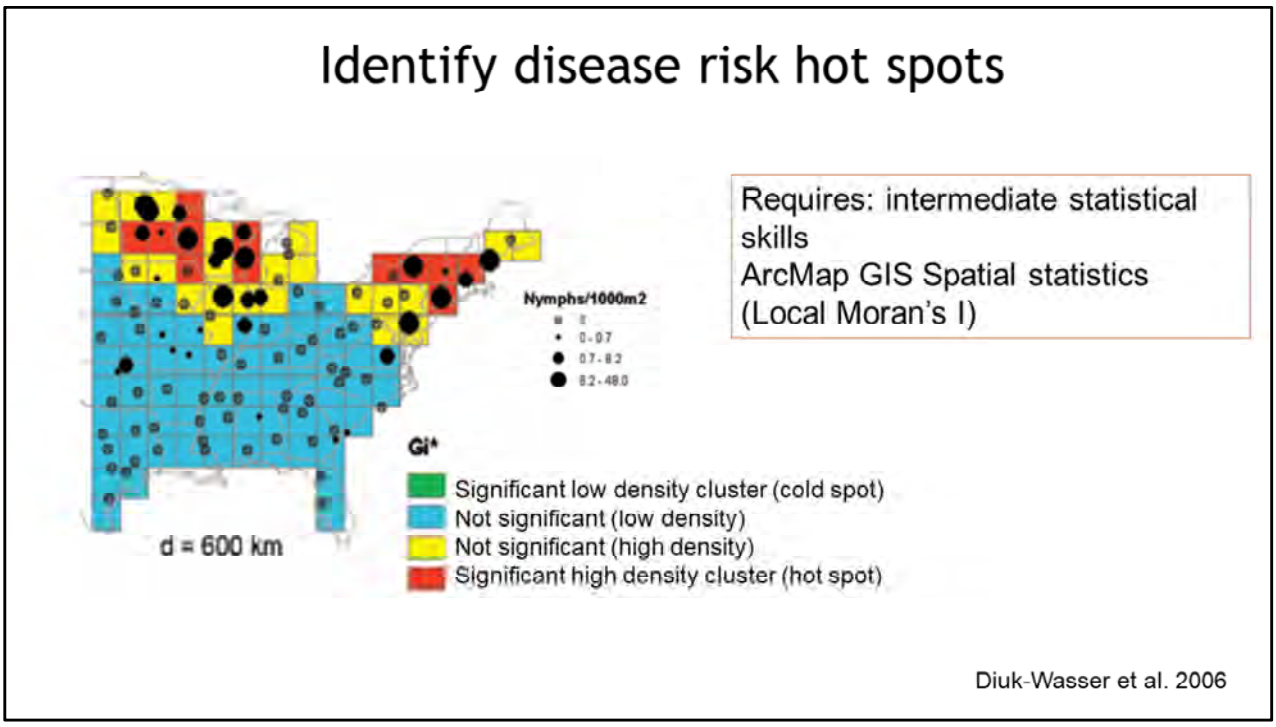
In each park...



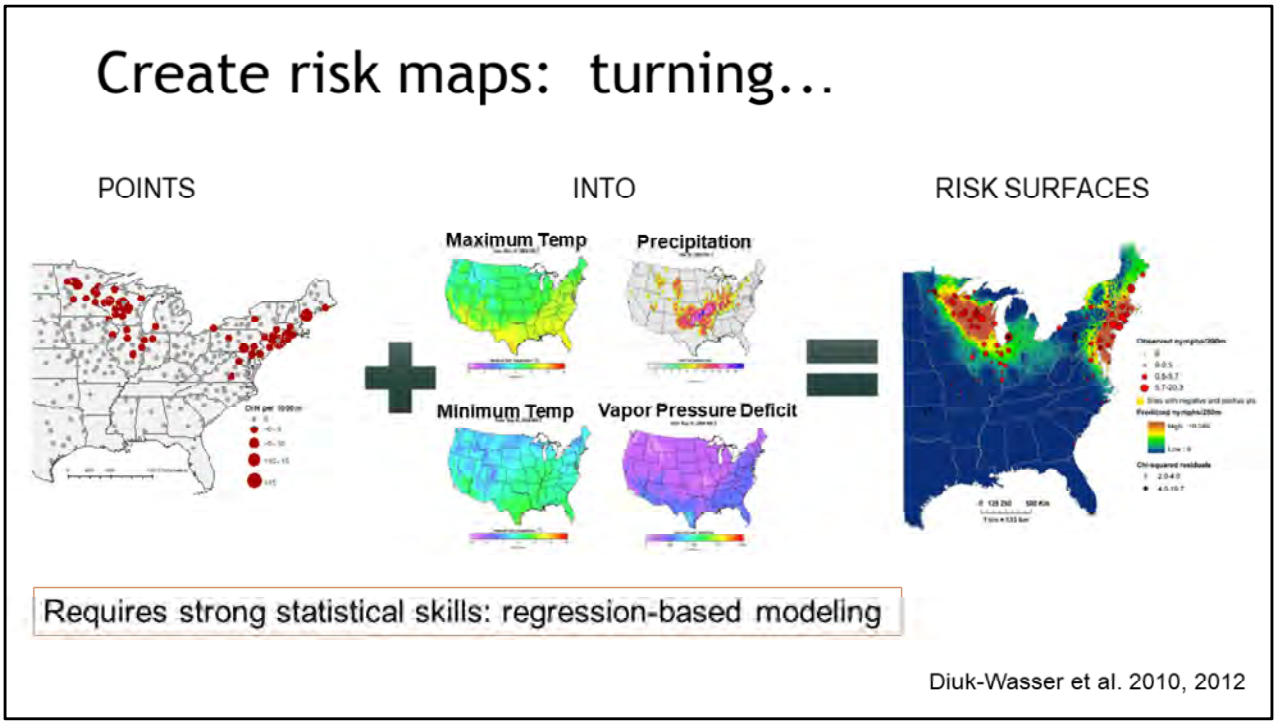
10 transects per site, 100 m² per transect (1,000 m²) is ideal
(CDC recommends minimum 750 m²)

4-6 visits/site during peak nymphal host-seeking season (CDC recommends min 3 visits)

VERY IMPORTANT:
Record individual 100m transects, even if they are next to each other (so you have REPLICATION)
OK if transect shorter than what you originally set, as long as you record the distance (this can be adjusted during analysis)



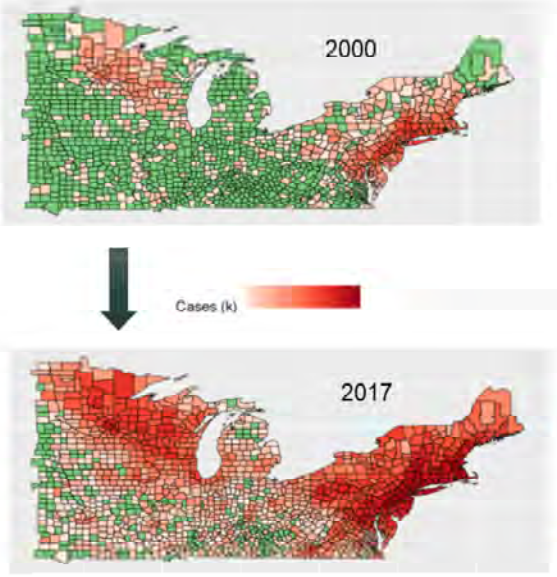
Diuk-Wasser, MA, A Gatewood, R Cortiñas, S Yaremych-Hamer, J Tsao, U Kitron, G Hickling, J Brownstein, E Walker, J Piesman, and D Fish. 2006. Spatiotemporal patterns of nymphal host-seeking *I. scapularis* (Acari: Ixodidae) in the United States. *Journal of Medical Entomology*, *43*(*2*): *166-176*.



Diuk-Wasser, MA, Vourc'h, G, Cislo, P, Hoen, AG, Melton, F, Rowland, M, Cortinas, R, Hickling, GJ, Tsao, JI, Barbour, AG, Kitron, U, Piesman, J, and D Fish. 2010. Field and climate based model for predicting the density of host-seeking nymphal *Ixodes scapularis*, an important vector of tick-borne disease agents in the eastern United States. *Global Ecol Biogeogr 19*:*504-514*.

Diuk-Wasser, MA, A Gatewood Hoen, P Cislo, R Brinkerhoff, SA Hamer, M Rowland, R Cortinas, G Vourc'h, F Melton, GJ Hickling, JI Tsao, J Bunikis, AG Barbour, U Kitron, J Piesman, and D Fish. 2012. Human risk of infection with *Borrelia burgdorferi*, the Lyme disease agent, in eastern United States. *American Journal of Tropical Medicine and Hygiene 86*(*2*):*320-327*.

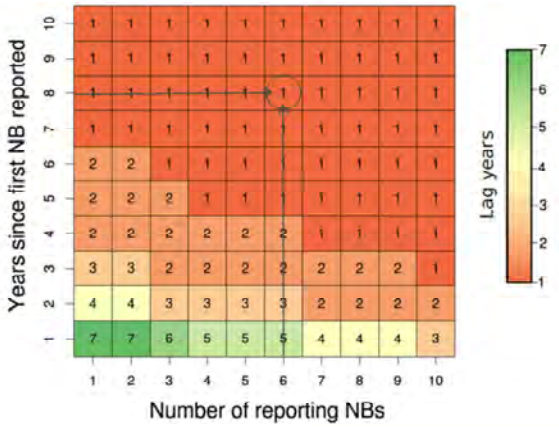
Monitor spread



Example:

Question: In how many years will my county start reporting Lyme disease?

Answer: It depends on how many neighboring counties report Lyme and the years since first neighbor reported



Requires: sophisticated modeling skills: collaboration!

In sum

- Surveillance method depends on your goals and resources
- Passive surveillance may be more effective to detect areas of emerging human risk
- Active surveillance can provide more accurate information of emergence in uninhabited areas and provide data on multiple tick species and pathogens
- There are minimum requirements for the CDC nation-wide surveillance network
- Additional goals for your district require increasing resources and analytical skills
- Even if just starting out, important to have long-term goals in mind since you should avoid changing your design later

Thank You

Diuk-Wasser lab

Maria Diuk-Wasser, Ph.D.
Department of Ecology, Evolution and Env. Biology
Columbia University
Email: mad2256@columbia.edu
https://ecoepidemiologylab.e3b.columbia.edu/

Tick Surveillance in Vermont

- Programs
- Partners
- Notes from the field
- A few resources

Patti Casey
Environmental Surveillance Program Manager
Vermont Agency of Agriculture

Programs

Vermont has three statewide tick surveillance programs

- Pathogen Prevalence Survey (*VT Dept of Health*)
- Tick Density Survey (*VT Agency of Agriculture*)
- Passive Tick Surveillance Survey (*citizen-driven*)

The pathogen prevalence survey is a collaboration with the VT Dept of Health and focuses on "hot spots" and "cold spots" throughout the state. Surveillance sites are selected based on historically high or low rates of human cases of tick-borne diseases, on high numbers of ticks found from previous surveillance efforts, or on areas where tick pathogen testing has revealed high levels of disease in the tick population. The Black-legged ticks we collect in this survey are tested for 5 pathogens – the ones that can cause Lyme disease, anaplasmosis, babesiosis, miyamotoi, and Powassan virus.

Our internal Agency of Agriculture's Tick Density survey is a randomized sampling of every town and gore in the state over a period of 5 years to determine tick population densities by town statewide. We completed our first 5-year study this fall and have discovered significant differences in population numbers based on land elevation and microclimates within the state. All these ticks are tested for the same 5 pathogens.

And finally, our Passive Tick Surveillance Survey is a citizen-driven project in which citizens who find ticks on themselves, their loved ones or pets, or in their immediate environment can send them to our lab. We identify them to species, life stage, sex, and rate of engorgement, and provide the recipient with a letter containing all of this information. We also use this correspondence as an opportunity to educate the public on tick identification, removal, and bite prevention. We do not conduct any pathogen testing on these ticks, but we do catalog all the data and keep all submitted ticks archived in labeled vials of alcohol. In the event we might need to look back at tick specimens at a later date in time to try to track changes in genotypes or the development of new diseases we aren't currently testing for but that may be in the population, we will have a robust catalog of specimens. Asian Longhorned Ticks were first reported in New Jersey in 2017. An earlier archived ALT specimen was discovered to have been originally misidentified as early as 2010, and later identified correctly by looking back through the archived specimens, so it seems useful to keep all the ticks, and they don't take up a lot of room.

The VT Dept of Health is our number-one partner in many ways! They provide CDC funding for our statewide pathogen prevalence survey. They set priorities and protocols, and the Agency of Agriculture implements the surveillance. VDH has the expertise to know what's needed for data to best protect Vermonters, and the Agency of Ag has the expertise to go out in the field and do the surveillance. We're in regular contact throughout the year and we have bi-weekly conference calls during surveillance season to make sure we're staying on track with our goals. It's a terrific partnership. We've discovered the importance of keeping close track of efforts for different programs ahead of time, such as setting up a spreadsheet for all costs by program – things such as tech hours, vehicle use and/or mileage, supplies, testing, data entry and analysis, and preparation of presentations.

Forest & Parks can be very helpful in finding and gaining access to public lands for surveillance. We only use public lands for our pathogen prevalence study because we want to be able to maintain access in the future for continuity of the study. Private landowners can change their minds, sell their property, develop their land, or become otherwise unavailable.

Same with Town Officials – they can help point out the best places to access town forests or other public use lands within their towns, they can help identify where people have complained about high numbers of ticks, and they can help provide public education on tick bite prevention through their Town Health Officers.

Fish & Wildlife can be a terrific partner if you decide to do a wildlife-related survey. We've been looking for the Lone Star Tick using our other 3 surveys but are not having a lot of luck. We believe it to be in the state and we're considering doing a VT Agency of Agriculture-funded turkey survey to look for them. We would look to work with F&W officials at big game reporting stations during turkey season to census harvested birds for Lone Star ticks. We conducted a very successful deer and moose sero-survey several years ago in partnership with them, looking for antibodies to WNV and EEE in cervids. While these are mosquito-borne pathogens, a collaborative study could be created for tick surveillance as well.

Local universities and colleges have been a goldmine for us to find volunteers, interns, and paid seasonal technicians. We have also shared data and project ideas with other tick researchers at Vermont universities.

Developing a good relationship with local media is indispensable! At the beginning of our field season, I arrange for statewide TV and press coverage of our surveillance programs, because, let's face it, meeting someone in a full Tyvek suit carrying a clipboard and little vials in the park where you walk your dog every morning can be a little unnerving. We do our best to sort of "advertise" our field program ahead of time to reduce the fear factor and engage people's interest and curiosity. We also use it as another public education tool for self-protection. We post our surveillance programs on community forums, on FaceBook, and on our Agency website. And we also have a great drone pilot who creates really cool videos of our field work.

Finally, we stay in constant contact with the state vets within our Agency to monitor tick populations and the possible introduction of new species brought in on livestock or introduced through migratory populations. We would communicate any of this immediately with our partners at VDH.

Notes from the field

- Set a protocol and stick to it!
- Record all site data accurately
- Be resourceful
- Do your tick checks at the end of the day

Set a protocol! It's easy to get a little sloppy sometimes in terms of temperatures and precipitation. It gets cold in Vermont early in the fall, especially in the northeast kingdom, and it can freeze or snow before we complete our surveillance. We had at times gone out in temperatures below those we had set in the protocol, or on wet mornings before the vegetation dries off, to try to cram an extra site in, and we ended up with lower numbers of ticks than we would have had we stuck to the protocol. We've since gone back to strict adherence to the protocol.

Record all site data - For our VDH partnership tick surveillance, our techs go out in teams of two to drag and/or flag for ticks, depending on terrain and vegetation. We sample 48 sites statewide twice in the spring and twice in the fall, which takes about 6 weeks per season. We record starting, mid-way, and ending GPS points, temperature, wind, precipitation, humidity, vegetation, elevation, aspect of the land, and any notable features, such as an old stone wall or large areas of invasive vegetation. We check the flag every 15 meters, over a transect of 750 meters.

We purchased several inexpensive weather monitors, all the same, to standardize the data, and we train all of our techs together at the same time so they receive the same information. We give them printed protocols and check in mid-season to ensure the protocols are still being followed correctly.

Be resourceful – start as small as you need to and grow if you can. Our first surveillance was a small internal survey, just a few weeks a year and working up to a 5-yr study. We hire seasonal techs whom we train for both tick and mosquito surveillance. We make our own tick flags from white flannel purchased at a fabric store and hung on broom sticks. We experiment with other fabrics as flags wear out. The CDC tests our ticks for pathogens free of charge. It can take longer than a commercial lab, but the price is right. We also use the CDC tick surveillance protocol, which saved us a lot of time in not reinventing the wheel.

Do your tick checks! Always, always make sure everyone knows how important it is, and how to do, a tick check at day's end. We had a very experienced field tech who contracted Lyme disease and was very sick. We don't know for sure it happened while he was working, but it is always a risk. Remember – tick collection was named one of the 10 worst jobs by a recent national news outlet. While we disagree, we do realize there are downsides.

A few resources

- CDC Tick Surveillance protocol *Surveillance for Ixodes scapularis and pathogens found in this tick species in the United States*
- VT Agency of Agriculture *Surveillance programs, annual tick reports, tick bite prevention*
- VT Department of Health *Be Tick Smart – Public health information*

Thank You

Patti Casey
Environmental Surveillance Program Director
Vermont Agency of Agriculture, Food & Markets
116 State Street
Montpelier, VT 05620
(802) 828-3473
Patti.Casey@Vermont.gov

Tick and Tick-Borne Disease Surveillance in Maine

Charles Lubelczyk, MPH
Maine Medical Center Research Institute
Scarborough, ME

Maine Medical Center
MaineHealth

~Fifteen Species of Ticks in Maine (NNE)

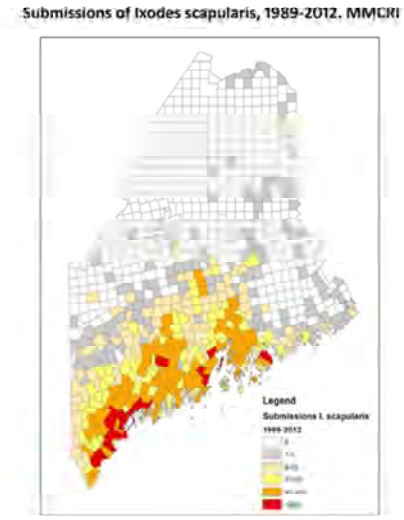
- Four of major veterinary or public health concern
  - *Ixodes scapularis* – the deer tick – LD, AP, BAB
  - *Ixodes cookei* – the woodchuck tick – POW virus
  - *Dermacentor variabilis* – the American dog tick – RMSF
  - *Ablyomma americanum* – the lone star tick*

* None established in NNE – YET!

Tick Distribution through Passive Surveillance

- *Ixodes scapularis* distribution monitored by a tick submission program through MMCRI, the Maine Forest Service, and the University of Maine

- 1989 - present



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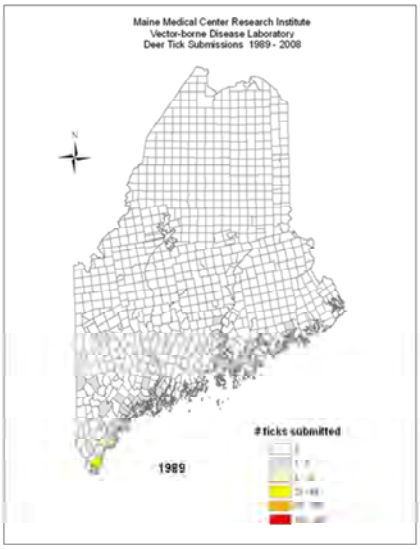
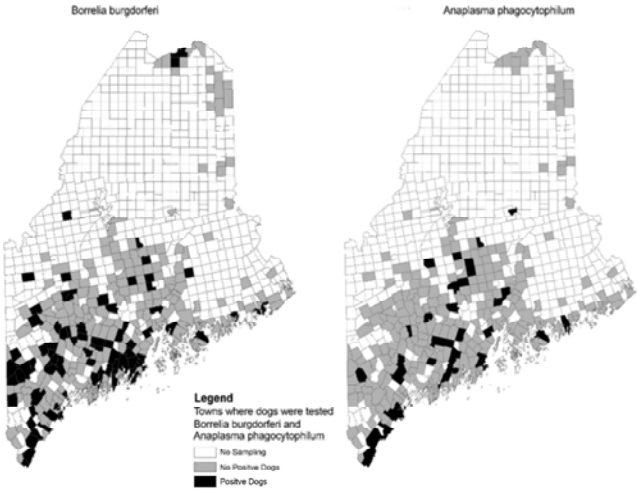


Passive Canine Surveillance for TBD

- Serology based on RDT (Idexx SNAP)
- Rand et al. 2010



Active Tick Surveillance

- Drag sampling – opportunistic, exploratory, systematic
- Initially drew on passive tick submissions

Active Tick Surveillance

- Drag sampling – municipal tick surveillance for tick management programs
- Statewide surveillance
- MacQueen et al. 2012

Active Tick Surveillance

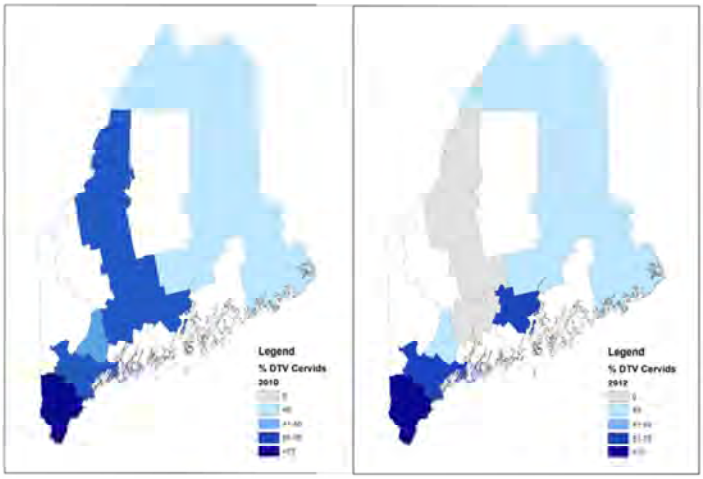
- Drag sampling – emergent issues
  - POWV
  - Reports of *Amblyomma* occurrence / red meat allergy
- Very focal and seasonal

Powassan Survey 2015-2016

Town	Lifestage	# Ticks	Pools Tested	Positive Pools	% Positive Pools
Orrington	adult	62	7	0	0
Brownfield	adult	20	2	0	0
Kittery	adult	59	7	0	0
Moosehead TWP	adult	14	2	0	0
Jackman	adult	10	1	0	0
Long Island	adult	20	2	0	0
Machias	adult	34	6	0	0
Mt. Desert Island	adult	50	5	0	0
Swans Island	adult	93	10	1	10
West Forks	adult	6	2	0	0
Wells	Adult	376	50	6	12
Cape Elizabeth	adult	242	33	4	12
Augusta	Adult	106	16	0	0
Standish	Adult	115	15	2	13
Rockland	Adult	292	45	2	4
Total		1499	203	15	

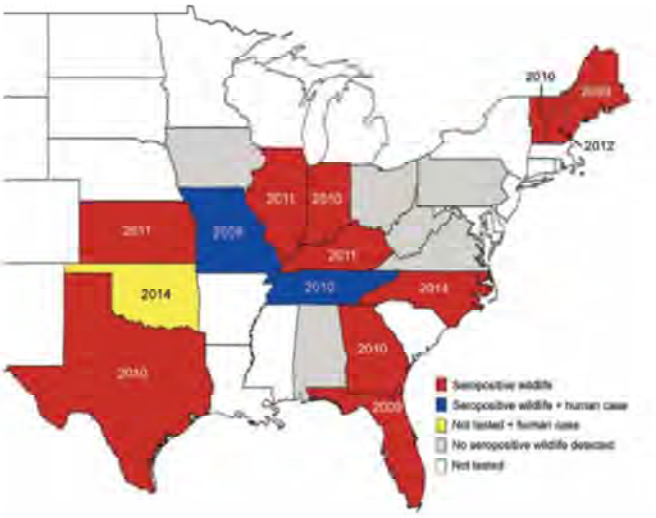
Active Tick and Tick-Borne Disease Surveillance - Cervids

- Serology for antibodies – POWV
- Nofchissey et al. 2013



Emerging Infections – Heartland Virus

- Rimersma and Komar. 2015. Emerging Infectious Diseases
  - 7 / 63 Maine WTD examined in 2009 positive for HRTV antibodies
- *A. americanum* not established yet in Maine!

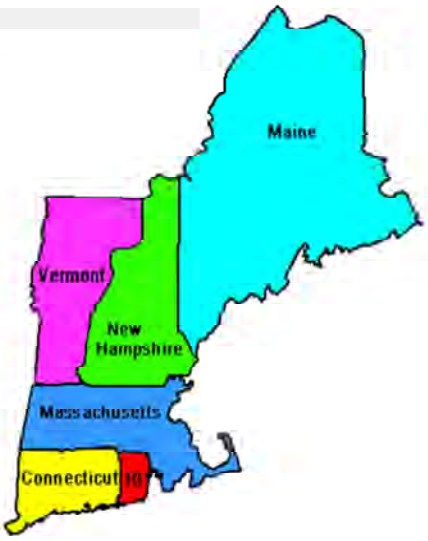


Active Tick Surveillance - Passerines

- Migrational passerines (Spring / Fall) vs. breeding birds (Summer)
- Distinct tick species (*Ixodes uriae*)
- Distinct strains of tick-borne pathogens
- Smith et al. 1996
- Rand et al. 1998

Challenges - Geography

- Maine is the largest New England state, with different climate zones from North to South
- Modelling for 'optimal tick habitat'
- Aside from personnel hours, the biggest financial cost for surveillance is often travel



Training and Safety for Field Surveillance

- Acting as diplomats and ambassadors for your agency
- Dressing appropriately for the work and conditions
- Field safety manual

Thank you

Maine Medical Center
MaineHealth

Charles Lubelczyk, MPH
Maine Medical Center Research Institute
lubelc@mmc.org
(207) 396-8246

Lessons from Delaware

Dr. Lauren P. Maestas served as the State Tick Biologist with the Delaware Department of Natural Resources Mosquito Control Section during the state's first year of their tick surveillance program (2019 field season). While Dr. Maestas was not able to join us for the *Tick Surveillance in the Northeast, USA* webinar, he provided the following slides regarding Delaware's experience establishing a tick surveillance program to be shared with the NEVBD network.

Pathogen screening considerations

- Do you have the equipment and ability to do it yourself?
- Locally, who can I reach out to for help?
- Sample design?

What should be our pathogen focus?

- Driven by the public
  - What are the most common tick-borne diseases in your area?

DHSS Division of Public Health

Tickborne Disease Surveillance Data Summary

In 2017, state and local health departments reported a record number of cases of tickborne disease to CDC, 59,349 cases, up from 48,610 in 2016.

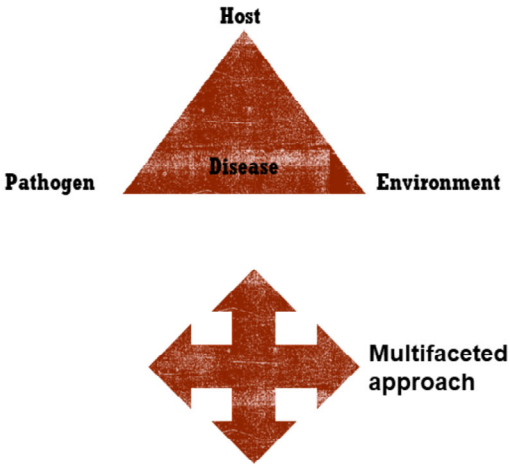
Reported Tickborne Diseases, U.S.	2016	2017
Lyme Disease (confirmed and probable)	36,429	42,743
Anaplasmosis/Ehrlichiosis*	5,750	7,718
Spotted Fever Rickettsiosis†	4,269	6,248
Babesiosis§	1,910	2,368
Tularemia	230	239
Powassan virus	22	33
Total	**48,610**	**59,349**

In our case, we are part of a state institution that was championed by state representatives. This understandably led to considerable pressure for a focus on Lyme disease. However, there are many more tick-borne pathogens out there, so how do we make these decisions?

As a new program with very little history or background, we reached out to neighbors, the CDC, and our public health department. This allowed us to know the local incidence of various TBDs in the state, though it did not give us highly targeted data.

We know what TBDs are the biggest issues in our area, now what?

- Focus – start small then expand
  - Think of using an umbrella approach if possible
    - Lyme borreliosis
- Established baseline program
  - RMSF
- Established baseline program
  - Ehrlichia
- Established baseline program
  - Anaplasma



Appease the public and do what is best for your area. Start small and expand, use well developed methods, which can be adjusted or altered as you progress.

Think umbrella approach as opposed to single, if possible: Can I run a multiplex that has broader applications?

Think of a large scale, multi-pronged approach: Where are the ticks, which ticks are vector competent, what pathogens can they carry and/or transmit?

As you develop your foundation, you can build. "Delaware now has a baseline idea for Lyme borreliosis, so lets include Spotted fever rickettsiosis."

I don't have the capabilities for screening pathogens, what can I do?

- Collaborate – please consider synergistic relationships
  - Public Health Departments
  - CDC
  - NEVBD
  - *Local community colleges/universities*

In our state, we first considered joining forces with public health for tick screening. We approached it from the angle of pursuing funds using the CDC's ELC grant. However, this did not work out and forced us to pursue other avenues. Initially, we were going to work within the public health lab and do the screening ourselves, but as we all know there is only so much we can do. We then found a local community college with molecular capabilities that had been dabbling in tick pathogen screening. This was solely from the molecular side, and without regard for ecological considerations. Upon speaking with the college, we found that an arrangement could be made that would be mutually beneficial. We chose to collaborate in a method that is helpful to all, where we provide some funding for reagents, primers and probes, and provide samples, putting things into an ecological context. The community college then gets meaningful data and is providing real-world application to their molecular biology students. Hands-on learning seems to be a growing trend, and we should jump on that train.

Considerations for pathogen screening

Unique situation

- Delaware – three counties – feasible in larger states?
- Logistical barriers
- Staff
- Time/Budget

Our focus

- State parks
- Hot spots (tick density)
- High human activity

Delaware is a small state, where the majority of the population is in the northernmost county. How should we scale to reflect these population dynamics, yet still ascertain adequate coverage?

We are focusing on areas with the highest amounts of human activity. Focusing on public lands, such as state parks, and emphasizing areas that are frequently visited, such as Cape Henelopen, or areas with high tick densities and substantial human traffic. Again collaboration with state park staff, and fish and wildlife, can inform you on some of these nuances, such as traffic in an area.

TICK SURVEILLANCE PRACTICES IN THE NORTHEAST

Webinar Question & Answer Session December 2, 2019

For CDC status of Lyme, are there corresponding maps for *I. pacificus* on the West Coast?

- **(Chuck Lubelczyk)** The The Eisen (2016) reference cites *I. pacificus* ranges, I believe.
  Eisen et al. 2016. County - Scale Distribution of *Ixodes scapularis* and *Ixodes pacificus* Acari: Ixodidae) in the Continental United States. Journal of Medical Entomology 53(2):349 - 386.

When you are taking notes of your site are you recording the basal area of the location or more of notable plant species of that area?

- **(Patti Casey)** Both. We drag/flag 750 meters (not in a grid, rather, linear). We make notes of all possible aspects of the site, including aspect of the land, elevation, vegetation composition, any notable features like an old stone wall, invasive vegetation, starting, mid, and ending GPS points of the transect, and environmental aspects, including temp, wind direction and speed, humidity, and precipitation.
- **(Chuck Lubelczyk)** With our surveillance as well, I think it is important to note the vegetation that you find at sites because, at least with some of our host communities, we do not have good habitat in some parts of Maine. Especially as you get into the northern and eastern areas we have a lot of our maritime spruce fir forests, which are not really great habitats for *I. scapularis*. So we do like to make note of that, and this provides us with a good contrast. If we happen to have sites in our surveillance that have really low numbers, looking at the habitat and noting it may provide answers as to why those numbers might be lower.

Please speak to the denatured versus 80-100% ethanol distinction and how this could affect pathogen testing.

- **(Melissa Prusinski)** Denatured alcohol has other additives and should not be used for storing specimens. We purchase absolute alcohol, which has no additives, and then you can dilute it down with ultrapure water to 80% if you need to extend it so you are not spending as much on alcohol. But, we currently store everything in 200-proof, so 100 % ethanol with no additives and not denatured.

Can the speakers share some examples of how the surveillance data is used to inform public health practices and policies? For example, human behavior modification to avoid tick bites or active control practices.

☐ **(Bryon Backenson)** I guess one of the things that I can mention with regards to this is that when we have - like I mentioned before we have been doing tick surveillance for a long time - when we do it we will typically take the results that we get and provide those back to a handful of people. Our main customers, if you will, are local/county health departments, and our secondary customers would be our state offices of parks and rec or department of environmental conservation. We will relay our results back to the people who manage the specific parks where we did some of our collections. One of the things that we will do when we send that information back is try to give them some examples, try to put the data into context for them. Trying to say that, yes, this means that there is a risk of tick-borne disease in your area; it's high, it's medium, it's low. You may want to use this information to do some sort of education for your physicians, for example. If it's in an area, like western New York for example, where tick-borne diseases may still be rare in certain areas, we use it as a way to encourage the county health departments to give information to providers who may have never seen a particular tick-borne disease before. For the individual parks themselves, for example, these have turned into opportunities to do things like put out signage. We have actually made some sign templates. We have a really nice metal sign template that we give, which we can also provide via Emily (NEVBD administrator; emm367@cornell.edu) if anyone wants. We have basically made that available and have given out about 7,500 of those so far, to be posted in different places in the state. They were originally designed to be put out based on our surveillance data. Those are just a couple of the examples. Counties have definitely used them to do things like press releases and so forth. Some of the difficult things, though, that go along with that are the problem of potentially putting like a scarlet letter on a particular park. Different parks act in different ways, in some people are not afraid, so to speak, of saying there are a lot of ticks in this particular park, or that the ticks that are found there have a high infection rate. But, there are other parks that want no part of you actually saying that out loud. They are willing to put up signs, but they do not want you to do it publicly or anything like that. Again, it's something that is going to vary from place to place, from agency to agency, from park to park, but again, it's the type of thing that at least in our experience, even the parks that were hesitant about it in the beginning, have bought into it as time goes on. Now a lot of them have the signage posted.

Is the E. Hassett reference on nymphal density higher in unmaintained herbaceous habitat on Staten Island, NY publicly available?

☐ **(Emily Mader; Laura Harrington)** Not yet. E. Hassett is currently writing it up for publication, anticipated for spring 2020.

Some jurisdictions in newly expanding tick areas, public parks are hesitant to post signage. Are there examples or best practices a local health department can take to encourage appropriate signage in parks with abundant tick populations or high risk for tick borne diseases?

☐ **(Bryon Backenson)** Basically, I think what we have done is try to work with, if a park is hesitant for example, try to put them in touch with a park that has used signage in the past and hasn't had any problems with it. That is typically how that has worked with us; it's worked pretty well. I think the main fear that parks have is that it's going to drive their attendance down, which means that their revenue is going to wind up going down. In most cases we have not necessarily seen that. Usually what we try to do is just be open about that, try to bring in the conversation with other parks, and it seems to have worked relatively well. The problem, also, with some of that is – and again, everybody's surveillance may be

different – but when we go do tick surveillance in a park, for example, we don't blanket the park. Typically we will do a convenience sample; we can't go through a park with a fine toothed comb. In some cases, a particular area of a park where we may find either a lot of ticks, or a few ticks, or a high infection rate, or a lower infection rate, may not necessarily be indicative of the entirety of the park. And, again, that's something that we will try to relay to the manager of the park or to the county where some of these things wind up taking place.

- ☐ **(Chuck Lubelczyk)** This is also a scenario where acting as the ambassador for your agency is important. We've seen where we work in public park areas, there is a lot of interaction with people who want to know what you are doing with that white cloth you are dragging. There have been times where we've been asked to not wear Tyvek suits or dress in a way that's going to call attention to what we are doing in an alarming way. So I think that in many cases, too, there is also an area where we have to be very respectful of the culture and maybe each park or each entity is going to be a lot different in how they approach this.

Are there any hands-on trainings coming up where folks can practice the tick surveillance techniques described in this webinar?

- ☐ **(Emily Mader)** This is nice timing for one of the things that will be offered in the Northeast region. We host a Vector Biology Boot Camp each May. We have officially opened the application window for our 2020 Boot Camp, which will be held the week of May 11. This is focused on both tick and mosquito surveillance techniques, but we do go over many of the field techniques that were discussed today under Dr. Stafford's presentation. That being said, this is limited to 20 participants, given that we have limited capacity at the venue where we host this training event.
- ☐ **(Bryon Backenson)** We in New York have done, if people want to come out with us, we don't necessarily have a problem with that. We have hosted other states to come in for one day or two days, the same thing with county health departments, to see how we wind up doing things. It's something we can certainly do on an informal or ad hoc basis. The biggest problem you have with it sometimes is that so much of what we do is dependent upon weather. You might have that you booked a day to come and wind up having three days of rain, but that is kind of the way things go with tick surveillance as a whole.

Additional materials shared after the webinar:
Acarology Summer Program - will offer two workshops in summer 2020: Soil Acarology and Medical Veterinary Acarology. Workshops will be run on the University of Arkansas campus in Fayetteville, Arkansas (USA). Registration is open and can be accessed at https://training.uark.edu/professional-development/courses/acarology-summer-program.php

Medical Veterinary Acarology will run July 6-17, 2020. Unlike previous years, we will not have a full week on ticks and tick diseases. Week one will have a concentrated 2-3 days on tick systematics and identification and then the remaining 1.5 weeks will extensively examine all the other groups of vertebrate parasitic mites. This will include discussions of various Mesostigmata, including ectoparasites and nasal mites, Astigmata, including feather mites, Trombiculoidea (chiggers), and miscellaneous Prostigmata.