Surveillance of Pathogens in Blacklegged Ticks from Southwest Virginia

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Objective

To monitor the prevalence of tick-borne pathogens in SW Virginia with emphasis on *Ixodes scapularis* ticks (blacklegged ticks)

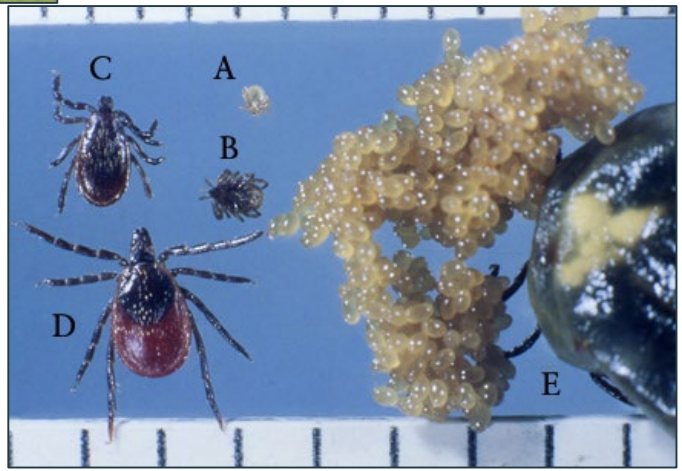
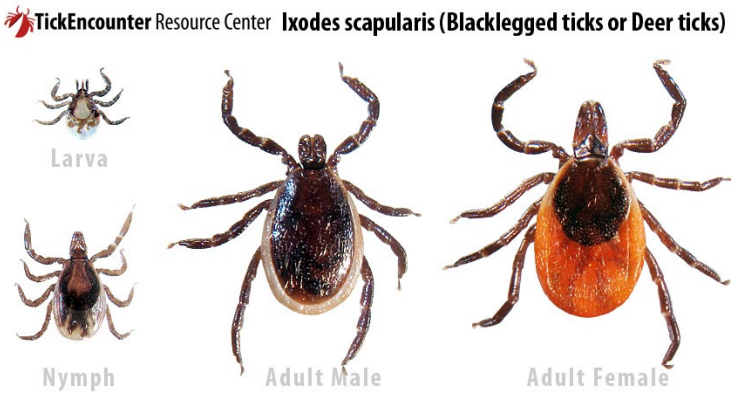


Photo credits: Graham Snodgrass and Dr. Marc Dolan.

Introduction: Blacklegged Ticks

- *Ixodes scapularis*, Blacklegged Ticks, Deer Ticks

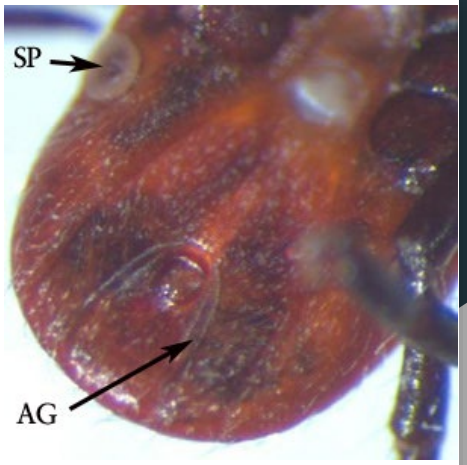


Key features:

Female
- Anal Groove: “Frowny Face”
- Partial scutum
- Amber Abdomen

Male:
- Anal Groove: “Frowny Face”
- Complete dark scutum

Immature Life Stages:
- Nymphs: 8 legs; size of poppy seed
- Larvae: 6 legs; size of pinhead; not typically found biting people
- Both have partial scutums



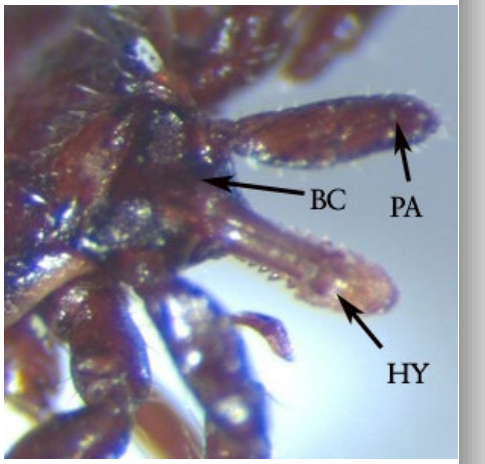
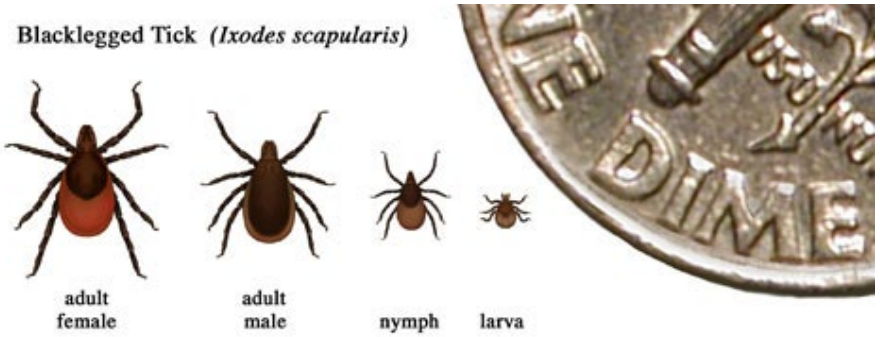


Photo credits: Graham Snodgrass and Dr. Marc Dolan.

Introduction: Blacklegged Ticks

- Adults: Most active during cooler months
- Nymphs: Most active in spring and summer



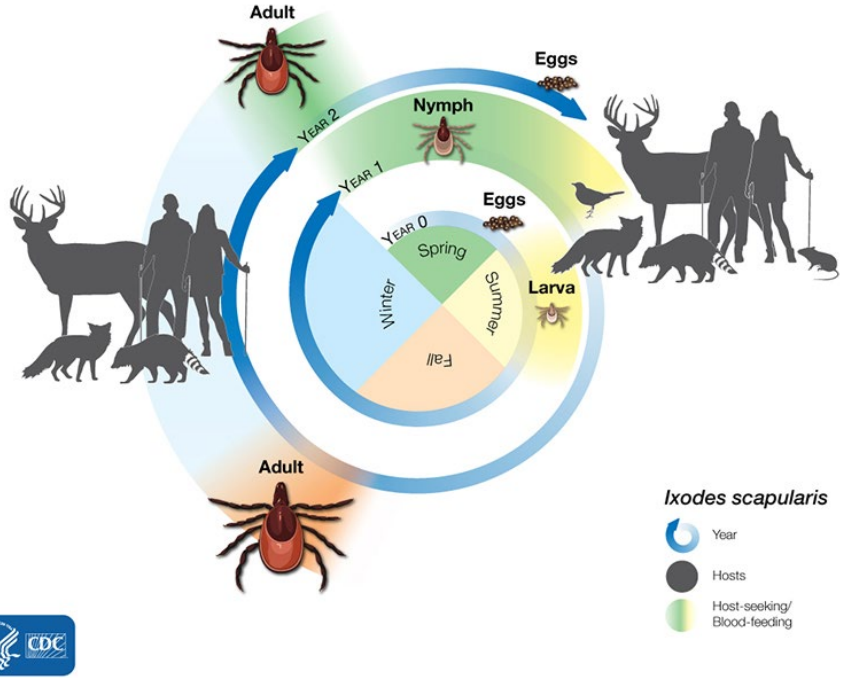


Photo credits: Centers for Disease Control and Prevention, https://www.cdc.gov/lyme/transmission/blacklegged.html, https://www.cdc.gov/ticks/surveillance/BlackleggedTick.html
Daniels, T. et. al. https://doi.org/10.1093/jmedent/33.1.140

Introduction: Pathogens of Blacklegged Ticks

- *Ixodes scapularis* ticks are vectors of multiple pathogens including ones that cause:
  - **Lyme disease (*Borrelia burgdorferi*)**
  - **Tick-borne relapsing fever (*Borrelia miyamotoi*)**
  - **Anaplasmosis (*Anaplasma phagocytophilum*)**
  - **Babesiosis (*Babesia microti*)**
  - **Powassan virus**

Human Case Reports of Lyme disease in Virginia

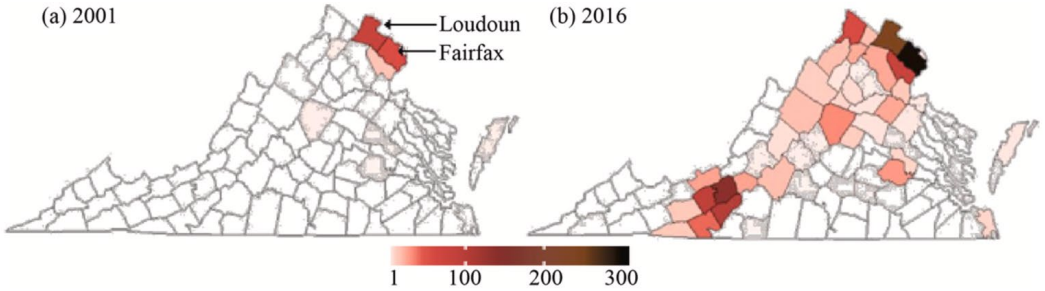


Photo credits: Neupane et. al https://doi.org/10.1016/J.TTBDIS.2021.101822,

Introduction: Pathogens of Blacklegged Ticks

Lyme disease

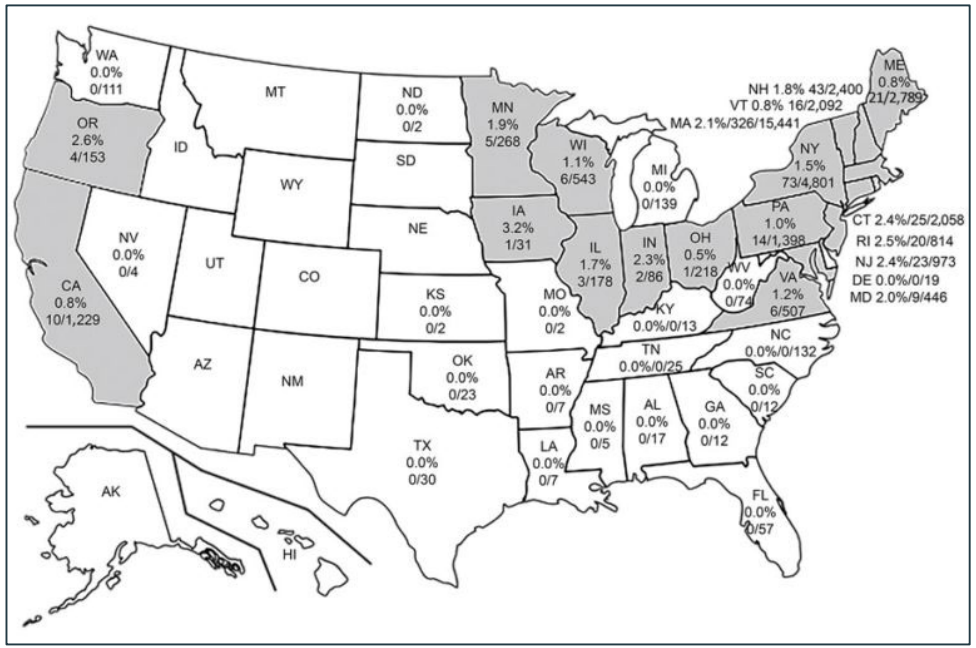
- *Borrelia burgdorferi*
- Transmission of the bacteria from an infected tick can occur 36 to 46 hours after attachment
- Nymphs are considered the most dangerous life stage because they can be infected with *B. burgdorferi* and often go unnoticed when they bite humans due to their small size

Photo credits: Centers for Disease Control and Prevention, https://www.cdc.gov/lyme/signs_symptoms/index.html

Introduction: Pathogens of Blacklegged Ticks

Tick-borne relapsing fever

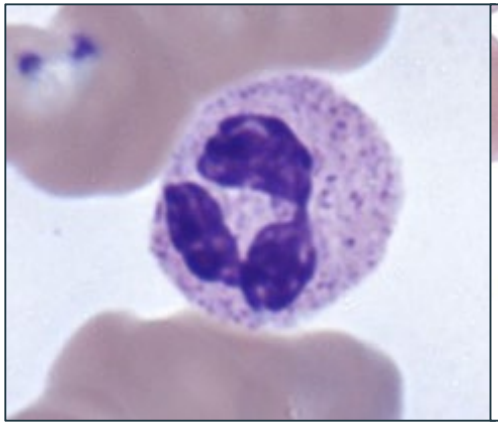
- *Borrelia miyamotoi*
- First characterized in 1995 in Japan
- Detected in *Ixodes scapularis* in the US in 2001
- First human disease cases were reported in 2011 in Russia
- Infected *Ix. scapularis* and *Ix. pacificus* ticks detected across the US



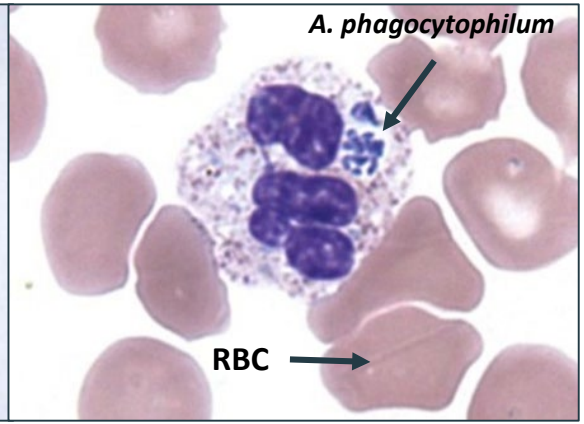
Introduction: Pathogens of Blacklegged Ticks

Human granulocytic anaplasmosis (HGA)

- *Anaplasma phagocytophilum*
- Infects neutrophils (common type of white blood cells)
- Causes febrile illness that can lead to severe disease if left untreated



Normal Neutrophil

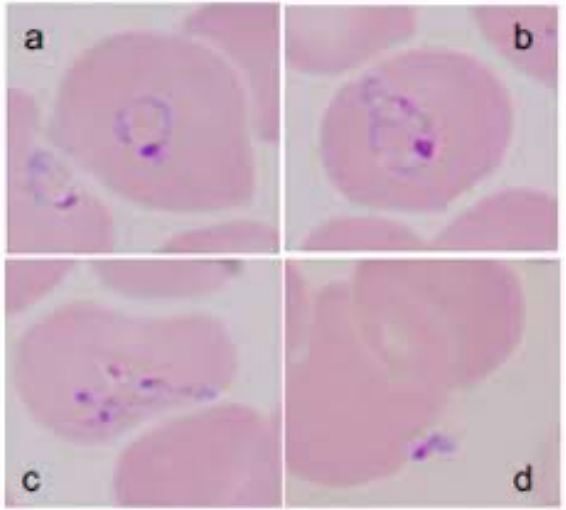


Infected Neutrophil

Introduction: Pathogens of Blacklegged Ticks

Babesiosis

- *Babesia microti*
- Protozoa (not a bacteria) that infects red blood cells
- Associated with transmission from blood transfusions
- Has been detected in *Ix. scapularis* in Virginia (Lehane et al., 2021)

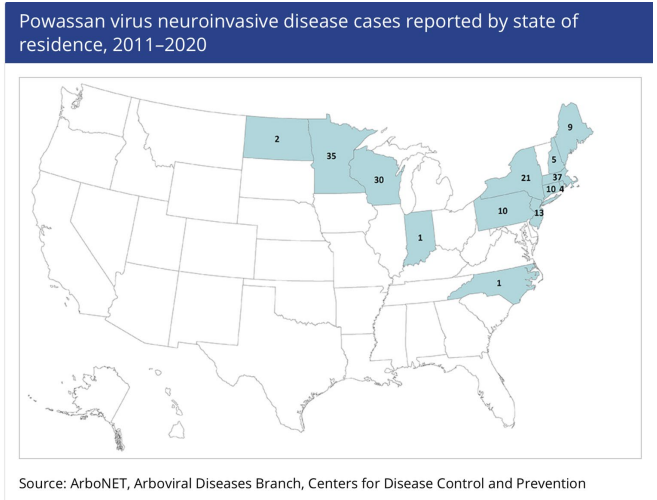


Lehane, A. et. al, DOI: 10.1016/j.ttbdis.2020.101637

Introduction: Pathogens of Blacklegged Ticks

Powassan virus

- Only tick-borne North American flavivirus
- Can cause fatal encephalitis and neuroinvasive disease in humans
- 2 lineages: POWV (lineage I) and **deer tick virus (lineage II)**
- Associated with infections in Canada and the northeastern US
- One reported case in 2009 in Virginia



Field Methods

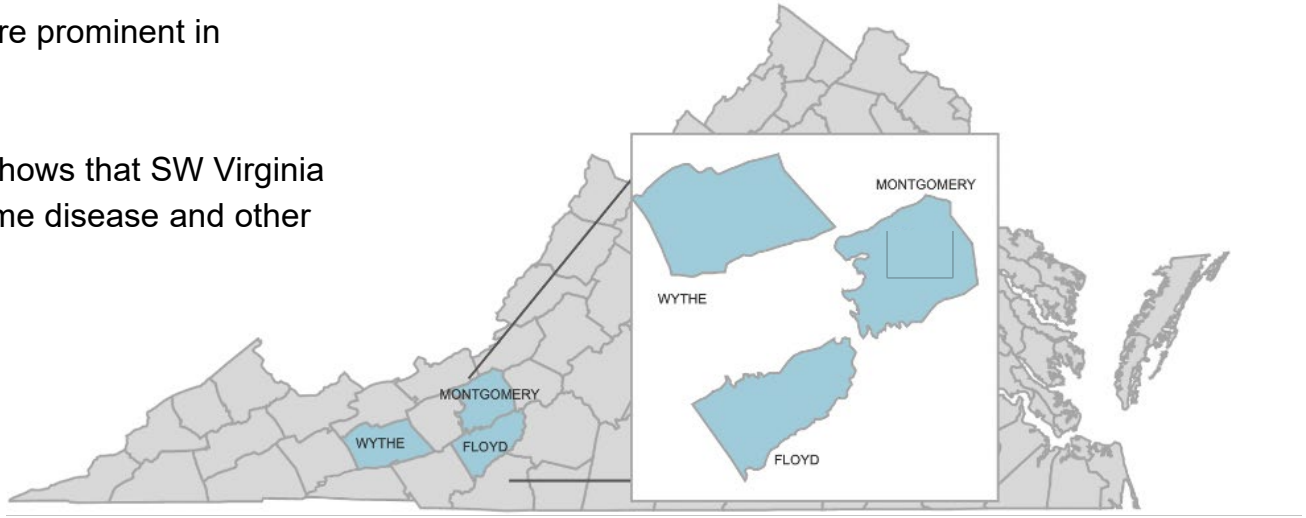
- Tick collection: Flagging in edge and forest habitats

- Ticks were identified and sorted after collection and placed into a -80℃ freezer for preservation of genetic material

Surveyed Counties

- Counties Surveyed
  - Montgomery
  - Floyd
  - Wythe
- *I. scapularis* ticks are prominent in western Virginia

- Surveillance data shows that SW Virginia is a hot spot for Lyme disease and other pathogens



Neupane et. al https://doi.org/10.1016/J.TTBDIS.2021.101822,

Molecular Methods

Extraction of DNA and RNA via QIAmp MinElute Virus Spin kit from QIAGEN

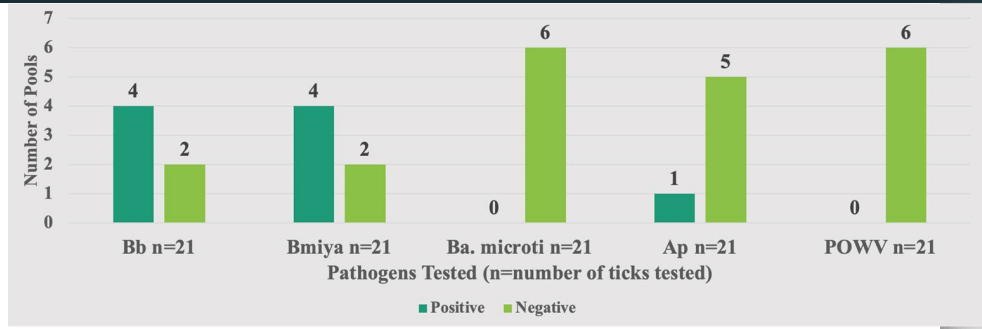
- Pathogen testing by utilizing multiplex real-time PCR assays
  - Duplex for *B. burgdorferi* and *B. miyamotoi*
  - Duplex for *A. phagocytophilum* and *Ba. microti*
  - Single-plex RT-PCR assay for Powassan virus

Photo Credits: QIAGEN, https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/multianalyte-and-virus/qiaamp-minelute-virus-kits/

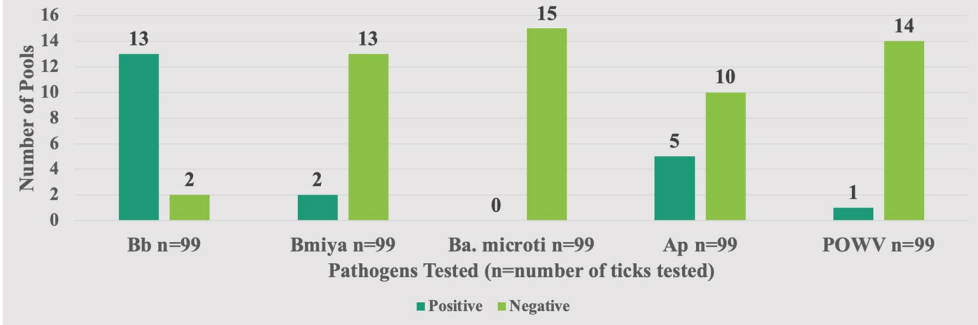
Results: Tick Collections

Wythe county

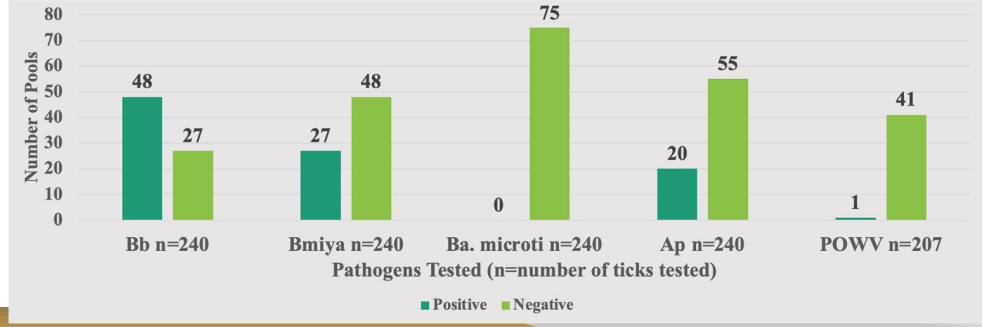
- Total number of *Ixodes scapularis* tested:
  - 13 larvae
  - 39 nymphs
  - 308 adults



Floyd county



Montgomery county



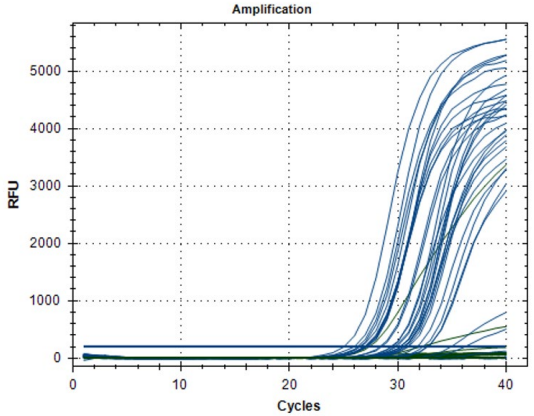
Results: *Borrelia* spp.

Pooled Infection Rate (Maximum Likelihood Estimates)

- Floyd county
  - 30.61% for *B. burgdorferi*
  - 2.08% for *B. miyamotoi*

- Montgomery county
  - 28.17% for *B. burgdorferi*
  - 5.26% for *B. miyamotoi*

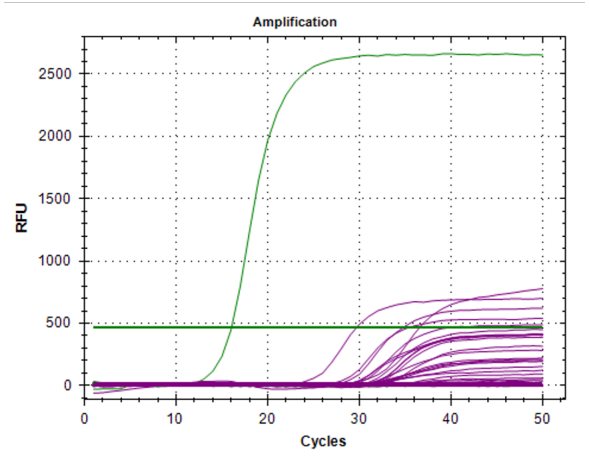
- Wythe county
  - 28.84% for *B. burgdorferi*
  - 28.84% for *B. miyamotoi*



Results: *Anaplasma* and *Babesia*

Pooled Infection Rate (Maximum Likelihood Estimates)

- Floyd county:
  - 6.06% for *A. phagocytophilum*
- Montgomery county*:*
  - 5.60% positive for *A. phagocytophilum*
- Wythe county*:*
  - 4.64% positive for *A. phagocytophilum*
- No positives for *Babesia microti.*

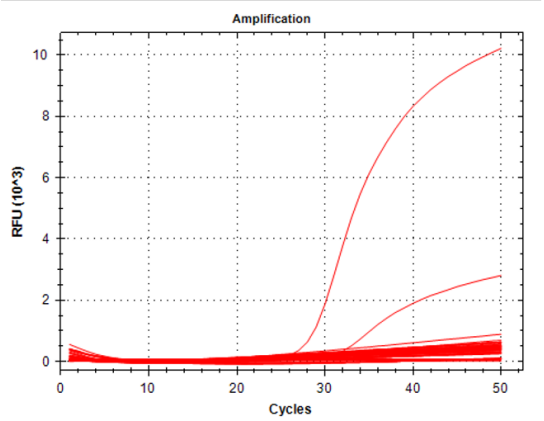


Results: Powassan virus

Pooled Infection Rate (Maximum Likelihood Estimates)

- Floyd county
  - 1% for Powassan virus
  - Confirmed positives were reported in Cumbie et al. 2022

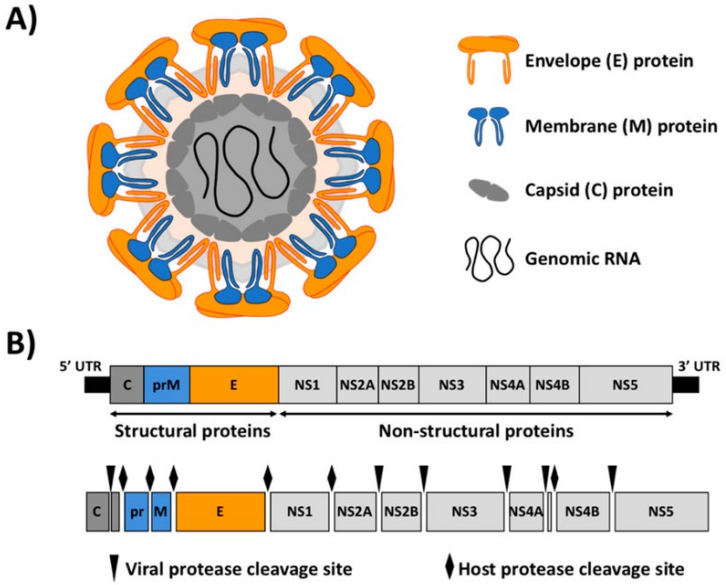
- Montgomery county
  - 0.48% for Powassan virus
  - First report of Powassan virus in its tick vector in Montgomery county



Sequence confirmation of POWV positive tick from Montgomery county

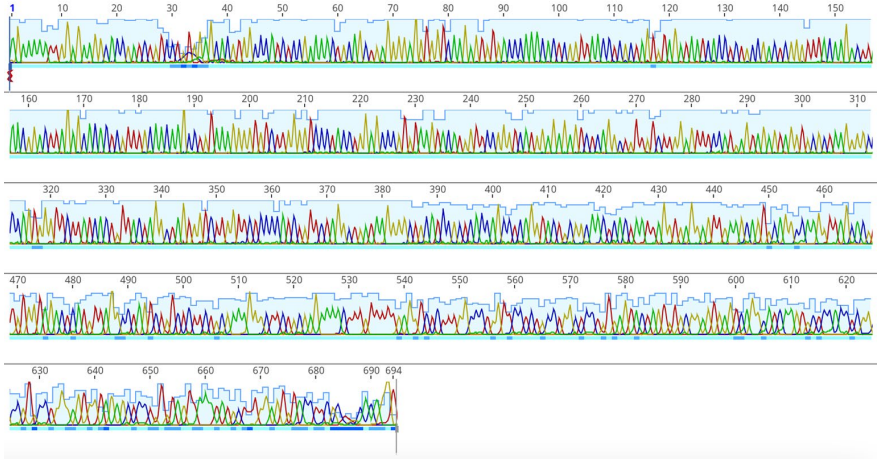
- Pool of two adult male *Ixodes scapularis*- only one positives

- Amplified a fragment from the TBE virus complex nonstructural protein gene (*NS-5*) in one direction using Sanger sequencing



Sequence confirmation of POWV positive tick from Montgomery county

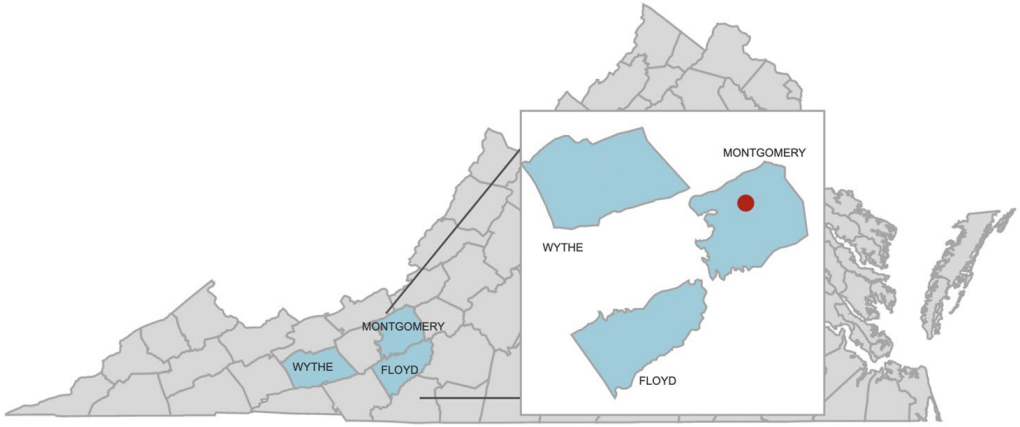
- Analyzed raw sequences and chromatograms in Geneious Prime
- The sequence was aligned and compared to known isolates of Powassan virus using NCBI BLAST



Hit Table | Query Centric View | Annotations | Distances | Info

Bit-Score	E Value	Grade	Hit start	Hit end	Name	Description	Modified	Seque
1,152.74	0	98.4%	8,076	8,767	MW001306	Powassan virus isolate POWV_D00...	06 Jan 2022 9:45 pm	693
1,152.74	0	98.4%	8,076	8,767	MW001305	Powassan virus isolate POWV_D00...	06 Jan 2022 9:45 pm	693
1,152.74	0	98.4%	8,076	8,767	MW001304	Powassan virus isolate POWV_D00...	06 Jan 2022 9:45 pm	693
1,148.23	0	98.3%	8,183	8,874	MW665120	Powassan virus isolate RTS 245, p...	06 Jan 2022 9:45 pm	693
1,148.23	0	98.3%	8,183	8,874	MK309362	Powassan virus strain MeW17-228...	06 Jan 2022 9:45 pm	693
1,148.23	0	98.3%	8,189	8,880	MG647780	Powassan virus isolate RTS82 poly...	06 Jan 2022 9:45 pm	693
1,148.23	0	98.3%	540	1,231	AF310949	Powassan virus 12542 NS5 gene, ...	06 Jan 2022 9:45 pm	693

Discussion



- The results display how the *I. scapularis* ticks harbor multiple pathogens of medical and veterinary importance in a high prevalence
  - High numbers of *A. phagocytophilum* and *B. burgdorferi* in this region of Montgomery county (indicated on the map with the red dot)
  - Public health concern

Discussion: Powassan virus

- Powassan virus in Montgomery county
  - Concern for public health in the region

- Increased collection at this site
  - Small mammal trapping

Photo Credits: Welcome Home Nature, https://www.welcomehomenature.com/en/be/rabbits-and-rodents/feed/which-small-mammals-are-rodents-and-what-does-that-mean-for-their-diet

Future Directions

- Further monitoring and testing of pathogens in Southwest Virginia
- Testing of larger regions of SW Virginia
- Increase public awareness of Powassan virus in Montgomery county and surrounding counties

Acknowledgements

- Gillian Eastwood
- Alexandra Cumbie
- Fellow lab members and field assistants: Lindsey Faw, Ahmed Garba, Lucas Raymond, Mikayla Hearne, Rebecca Trimble, Melanie Turner
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Questions

Thank You!

Ways to contact me/Eastwood lab

- Email: peterfs@vt.edu
- Website: https://eastwoodlab.weebly.com/
- Twitter: @LabEastwood