Tick-borne Encephalitis Survey and Phylogenetic Study of Powassan Virus in Fairfield County, Connecticut

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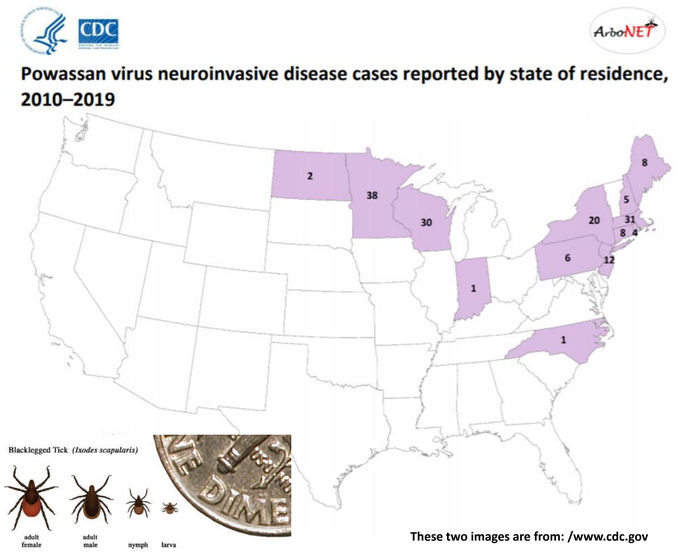
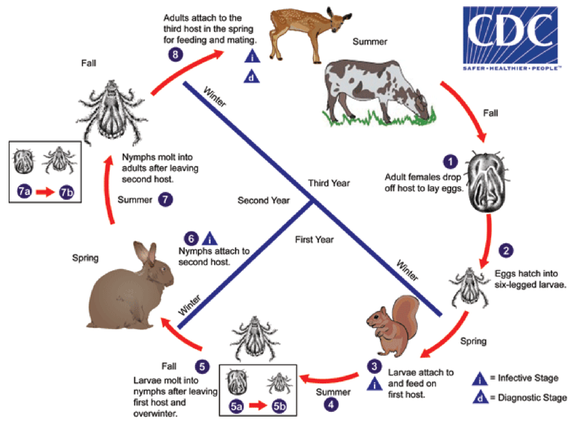
Introduction

Powassan virus, a member of the TBE consists of two lineages with separate enzootic cycles. One of the lineages in focus is the deer tick virus (DTV) lineage, which extends to the blacklegged tick, *Ixodes scapularis*, and the white-footed mouse (*Peromyscus leucopus*). Clinical presentations of both genotypes of POWV may include encephalitis, meningoencephalitis, and aseptic meningitis (1,3,4). A recent increase in POWV human cases has drawn interest in determining the potential public health impact of the virus as an emerging disease that has been on the rise since 1999 (2,5). It has been reported that the DTV genotype is responsible for the large increase in recent encephalitis cases. This can be due to very aggressive, non-specific host seeking and biting habit of *I. scapularis* or its feeding habits and geographic distribution. The introduction and establishment of enzootic transmission of DTV may occur during the colonization of *I. scapularis* in a new environment, or after the tick population becomes well-established (4,6).

Our research aim:

Determine if DTV is present in *I. scapularis* ticks in Fairfield County (known to have high I. scapularis densities). If present, determine the infection prevalence of DTV in *I. scapularis* ticks at different life stages, and sequentially analyze phylogenetic studies of the isolates. Lastly, perform a sequence analysis and phylogenetic study of the isolates.





Material and Methods

Trapping and Tick Collection: Trapping was conducted using dry ice, dragging, and flagging, as the most common methods of collecting ticks. Carbon dioxide (CO2) released from dry ice actively attracts host-seeking ticks. In addition, dragging technique was performed by moving a piece of flannel or cotton cloth across vegetation behind an observer. This method allows ticks to attach to the cloth as it passes (7-9). Additional samples where obtained from local veterinary hospitals in Hartford, Hamden, and Fairfield Connecticut.

Homogenization of ticks and RNA extraction: 600 µl of buffer solution were added to frozen tick pools of 10 nymphs or individual adult ticks. Samples were then immediately homogenized, followed by RNA and DNA extraction using the AllPrep DNA/RNA/miRNA Universal Kit (Qiagen).

Conventional PCR and genetic analysis: QIAGEN OneStep Ahead RT-PCR Kit (Qaigen) and previously published primers (10); POWSBD1_F GGCTTCCAGAGAGGTGAGTG POWSBD1_R TTCCAAATCTGCATTGGTGA, were used to amplify the envelope gene of the virus. After purification of amplicon with the QIAquick PCR Purification Kit (Qiagen), the amplified 350 base-pair fragment of the envelope gene of each virus isolate will be sequenced at the DNA Analysis Facility on Science Hill at Yale University.

Real time PCR: One-step real-time RT-PCRs will be performed using the Power SYBR™ Green RNA-to-CT™ 1-Step Kit (ThermoFisher Scientific) according manufacturer recommendation.

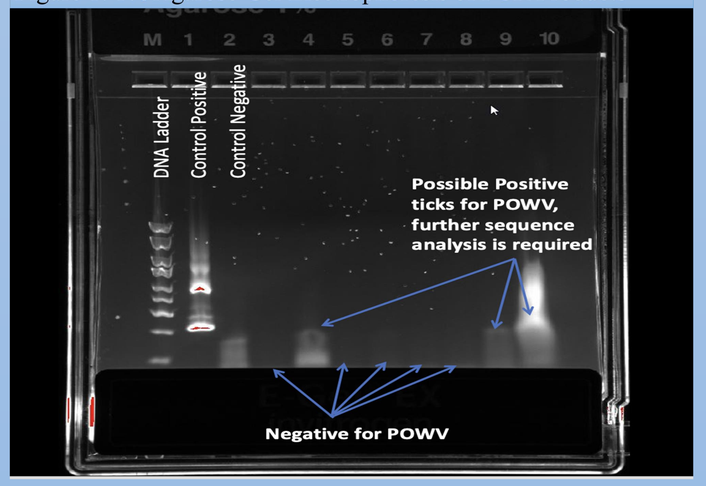
Preliminary Results & Discussion:

Over 100 tick samples (different stages) were examined for Powassan Virus (POWV), as it was mentioned in the materials and methods section, ticks were gathered from mainly veterinary hospitals in Connecticut. Before we run real-time RT-PCR on all extracted RNA, we decided to run conventional RT-PCR using QIAGEN One-Step Ahead RT-PCR Kit according to the manufacturer's instructions. This decision was made to further study the extracted RNA from the possible positive ticks utilizing sequencing and confirmation. To visualize the PCR products, the gel electrophoresis was performed on the PCR products using an E Gel electrophoresis apparatus (Invitrogen, CA) and their recommended 1% agarose pre-casted gel and 1kb plus express DNA ladder (Invitrogen, CA).

Figure 1 shows positive and negative controls and it also indicates three lanes with positive sample for POWV. As it is seen the gels did not run perfectly and bands are not straight to predict the size of the product and location of the band properly. This also indicates a need for the extraction of DNA products from either PCR reaction for gel electrophoresis and sequence them for confirmatory purposes.

Since the extraction kit was able to extract DNA and RNA at the same time from the tick, it was feasible to perform PCR reactions on DNA samples and evaluate other tick-borne diseases such as Lyme. Again, using conventional PCR, we were able to find at least 2 infected ticks with Borrelia Burgdorferi the causative agent for Lyme disease.

Figure 1: 1% Agarose Gel Electrophoresis of PCR Products



Next step and plans:

We are in the process of calibrating the real-time PCR machine and planning to run all samples using this technique. This will give another way of confirmation of the positive samples and optimized tool for identification of future positive ticks. An established system will be utilized in our lab for analysis of future samples from across Connecticut and the sequence analysis of the positive sample will be used for further phylogenic and relationship analyses of the analysis using envelope gene sequences and compared to POWV isolates from adult host-seeking *I. scapularis* collected in 2008, 2010, 2011, and 2012 in Bridgeport and North Branford, CT.

References:

1. Dumpis, U., Crook, D., and Oksi, J. 1999. Tick-borne encephalitis. Clin. Infect. Dis. 28:882-890.
2. El Khoury, M.Y., Camargo, J.F., White, J.L., Backenson, B.P., Dupuis, A.P., Escuyer, K.L., Kramer, L., St. George, K., Chatterjee, D., Prusinski, M., Wormser, G.P., and Wong, S.J. 2013. Potential role of deer tick virus in powassan encephalitis cases in lyme disease–endemic areas of New York, USA. Emerging Infectious Diseases.19(12):1926-1933
3. Nadolny, R.M., Feldman, K.A., Pagac, B., Stromdahl, E.Y., Rutz, H., Wee, S.B., Richards, A.L., Smith, J., Armolt, M., Gaff, H.D,. 2015. Review of the Mid-Atlantic Tick Summit III: A model for regional information sharing. Ticks and Tick-borne Diseases. 6(4): 435-438
4. Ebel, G.D. 2010. Update on Powassan virus: emergence of a North American tick-borne flavivirus. Annual review of entomology 55:95-110
5. Brackney, D. E., Brown, I.K., Nofchissey, R. A., Fitzpatrick, K.A., and Gregory D. Ebel, D.G. 2010. Homogeneity of powassan virus populations in naturally infected Ixodes scapularis.
6. El Khoury, M.Y., Hull, R.C., Bryant, P.W., Escuyer, K.L., St George, K., et al. 2013. Diagnosis of acute deer tick virus encephalitis. Clinical infectious diseases. Infectious Diseases Society of America. 56:e40-7
7. Kendra, P.N., Torres-Perez, F., Hjelle, B.L,. and Ebel, G.D. 2010. Molecular epidemiology of Powassan virus in North AmericaJ Gen Virol. 91(11): 2698–2705.
8. Cohnstaedt, L.W., Rochon, K., Duehl, A.J., Anderson, J.F., Barrera, R., Su, N., Gerry, A.C., Obenauer, P.J., Campbell, J.F., Lysyk, T.J., and Allan, S.A. 2012.Arthropod surveillance programs: basic components, strategies, and analysis. Annals of the Entomological Society of America, 105:135–149.
9. Kinsinger, B.J. & Allan, B.F. (2011) Efficacy of dry ice-baited traps for sampling Amblyomma americanum (Acari: Ixodidae) varies with life stage but not habitat. Journal of Medical Entomology. 48:708–711.
10. Caines, D.N., Halpaus, J. L. 2011.Virginia arboviral activity in 2009. First Annual Tick Summit. Hanover, MD.

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