The use of viral metagenomic whole genome sequencing to perform Powassan lineage analysis on cerebral spinal fluid

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The Maine Center for Disease Control Health and Environmental Testing Laboratory used non-targeted viral metagenomic whole genome sequencing to classify a Powassan virus case as Deer Tick virus. They will continue to pilot this method with Powassan cases and aim to expand to other human arboviral pathogens.

The "What"

Powassan virus is endemic in Maine ticks. The virus is classified by two (2) lineages. Powassan virus lineage 1 (referred to as Powassan virus) is spread by woodchuck ticks. Powassan virus lineage 2 (referred to as Deer Tick virus) is spread by deer ticks. Most human cases in Maine are presumed to be Deer Tick virus infections since deer ticks are known to be well established across the state. However, Maine Center for Disease Control (ME CDC) is unable to definitively say whether infections are due to lineage 1 or lineage 2 since most human Powassan virus testing to date has not distinguished between the two virus lineages. Hence, all cases reported by ME CDC to date are lineage-unspecified Powassan cases (except for one Deer Tick virus infection identified by MaineHealth in 2017).

In May of 2023, a cerebral spinal fluid (CSF) sample was positive for Powassan virus by reverse transcription real-time polymerase chain reaction (PCR) at the Health and Environmental Testing Laboratory (HETL). This test does not distinguish between Powassan virus lineages. But, by using non-targeted viral metagenomic whole genome sequencing, HETL determined that this sample (and a second CSF sample from the same patient) was Powassan lineage 2 (aka Deer Tick virus) by Basic Local Alignment Search Tool (BLAST) analysis and phylogenetic tree analysis.

> **Results from these methods can allow for more targeted health education and prevention activities.** These can include tick and mosquito identification guides; informing epidemiologic case investigations leading to public health messaging; and, if determined needed, targeted pesticide application.

The "So What"

The primary goal of running viral metagenomics on the CSF sample HETL received was to see if the lab could potentially identify Deer Tick virus in a patient with neuroinvasive disease and a positive Powassan PCR. The DTV identification provided evidence toward the theory that, at least, some human Powassan cases in Maine are in fact Deer Tick virus infections.

This is HETL's first successful application of viral metagenomics to identify a human arboviral pathogen. Attempts in the past to detect arboviruses have failed due to issues with sample quality and time of collection, but now ME CDC has evidence that this test can work successfully to identify human arboviral pathogens when a sample is submitted under the right conditions.

The "Now What"

ME CDC will use funding from the Epidemiology and Laboratory Capacity for the Prevention and Control of Emerging Infectious Diseases (ELC) cooperative agreement to pilot an enhanced human arboviral metagenomics surveillance project for non-Covid sequencing. The project aims to sequence samples from any patients who test positive for Powassan virus and have meet the proper sample submission criteria. The first successful application served as a test run for this pilot project. This project can eventually expand to identify other arboviral pathogens, such as endemic diseases (like Eastern Equine Encephalitis virus (EEE) or West Nile virus (WNV)) or travel-acquired diseases (like Chikungunya virus (CHIK), Dengue virus (DENV), or Zika virus (ZIKV)).

Before this sample was tested, HETL purchased whole genome sequencing library preparation automation equipment and long read sequencing equipment using ELC funds. This equipment will be used to perform high throughout viral metagenomic whole genome sequencing on mosquitos and ticks collected throughout Maine. This testing will augment the targeted vectorborne molecular biology surveillance testing already being conducted the HETL and the University of Maine in Orono.

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