Arbovirus Infection as Proposed Etiology of Antibiotic Resistant Chronic Lyme Disease

David Baewer, MD, PhD
Chief Medical Officer
Coppe Laboratories, Waukesha, WI
Arboviruses: A Rising Threat
Arboviral Diseases: Overview

• Arboviral (arthropod-borne) diseases are caused by arboviruses that are spread to humans from an infected arthropod, including mosquitoes and ticks.
• Examples:
  – West Nile virus (WNV)
  – California encephalitis, La Crosse encephalitis, and Jamestown Canyon encephalitis viruses
  – Eastern equine encephalitis virus (EEEV), Western equine encephalitis virus (WEEV),
  – St. Louis encephalitis virus (SLEV).
  – Chikungunya
  – Bourbon virus
  – Heartland virus
  – Tick borne-encephalitis virus (TBEV)
  – Powassan/Deer Tick Virus (DTV)
Ticks Carry More Than Just Borrelia

According to the Minnesota Department of Health, the black-legged tick can transmit all of these:

- Human Anaplasmosis (HA)
- Babesiosis
- Rocky Mountain Spotted Fever (RMSF)
- Ehrlichiosis
- Powassan (POW) Virus/Deer Tick Virus (DTV)
- Southern Tick-Associated Rash Illness (STARI)
- Tularemia

Much less common than Lyme disease

1 CDC, March 2014
Ticks Carry More Than Just Borrelia

- Most practitioners test for other bacterial and protozoan pathogens.
- Co-infection of the tick is the rule rather than the exception.
- Tickborne viruses are rarely diagnosed in the United States.
  - Arboviruses frequently co-exist with other pathogens in the tick.
  - Arboviral encephalitis is rare with a 10% fatality rate.¹
  - Arboviral illness (TBEV) is common in Europe.
  - Lack of commercial testing means virus is probably underdiagnosed.

¹CDC, March 2014
Tickborne Encephalitis Virus (TBEV)

- TBEV is closely related to Powassan/Deer Tick virus present in US.
- A Flavivirus that causes encephalitis and chronic encephalitis\(^1\)
- Transmitted by ticks
- Flavivuses have been associated with:
  - Yellow fever
  - Dengue fever
  - West Nile encephalitis
  - Powassan fever
  - Hepatitis C

\(^1\)Charral RN et al; *Clin Microbiol Infect.* 2004 Dec; 10(12)
Tickborne Encephalitis (TBE)

- Incidence of clinical cases is reported to be between 10,000 and 15,000 per year worldwide.
- Underestimated because all countries not required to notify government.
- Chronic or permanent neuropsychiatric sequelae occur in 10-20% of patients.
- TBE is a serious health issue in Central Europe.
- Switzerland, Austria and Germany have implemented national vaccination programs.
• Initial phase: non-specific headache, fatigue and malaise.
• About 2/3 of human TBE virus infections are non-symptomatic.
• Symptoms last about 5 days followed by 7-10 days of quiescence.
• About 30% of symptomatic adults will contract a severe form of the disease: meningoencephalitis.
• 1/3 of all patients experience incomplete recovery, with neuropsychiatric symptoms becoming chronic.
• The overall fatality rate for TBE is about 1%.
Diagnostic Testing

- IgM and IgG antibodies to TBEV are usually present by the time that central nervous system involvement manifests itself.
- Most common diagnostic test is ELISA.
- Cross-reactivity with antibodies to other flaviviruses can occur.
- CSF antibodies lag behind serum by about 10 days.
- In the very early phase, (antibodies not developed) the virus can be detected in serum by polymerase chain reaction (PCR).

VIS=virus isolation
PCR= polymerase chain reaction
Serologic tests

Holzmann, H; Vaccine 21(2003)
TBE Treatment

• No specific treatment for tickborne encephalitis
• Clinical intervention is geared toward symptom amelioration and supportive care.
Powassan/Deer Tick Virus

Member of the TBE Complex
“You can get seizures, high fevers, stiff neck. It comes on so suddenly that it’s the kind of thing people go to the emergency room for,” he explained.

(Daniel Cameron, MD)
Powassan/Deer Tick Virus (POWV/DTV)

- Originally isolated from fatal case of encephalitis in Powassan, Ontario in 1958
- DTV and POWV are two lineages of TBE-complex circulating in North America
- POWV/DTV transmitted by Ixodes scapularis ticks—the same ticks causing Lyme disease

Tick transmits virus within 15 minutes of attachment to host

Ebel, GD. Et al; Virology, July 2010
POWV/DTV Clinical Diagnosis

- Incubation period is usually $\geq 1$ week (range from 8-34 days)
- Fever, muscle weakness, confusion, headache, nausea, vomiting, and stiff neck are usual presentation
- Severe signs and symptoms-respiratory distress, tremors, seizures, gait unbalance, confusion, paralysis, and coma
- Neuroinvasive disease: meningoencephalitis leading to long-term neurologic sequelae
- 10% of neuroinvasive cases are fatal
- Supportive treatment only
- No vaccine available

Ebel, GD. Et al; Virology, July 2010
POWV/DTV Testing

- There is no commercial test available for Powassan virus
  - Coppe Laboratories will have testing available in early spring
- Serologic testing remains the primary method for diagnosing POWV
- CDC tests for POWV upon state health department request
- Positive POWV must be reported to the state

Source: CDC, 2014
From 2001-2012, 47 POWV neuroinvasive disease cases have been reported in the US

Source: CDC, 2014
Powassan Virus Neuroinvasive disease in Minnesota: Counties with highest risk

Powassan virus annual neuroinvasive disease incidence by county, 2004-2013

CDC, 2014
Coppe Laboratories' Study: Could there be an infectious agent that is insensitive to antibiotics that mimics the symptoms attributed to infection B. burgdorferi, in a subset of patients?
Method of Tick Collection and Analysis

- Ticks were collected during
  - Fall 2011-Spring 2012
  - Spring 2014
  - Fall 2013
- Over 2000 ticks collected and donated to Coppe Laboratories by the Wisconsin DNR and volunteers
- Ticks separated by genus Ixodes or Dermacentor
- Each tick pool was washed, placed into liquid nitrogen and homogenized
- Nucleic acid was extracted and PCR was performed to assess each pool for the presence of:
  - Borrelia
  - Anaplasma
  - Babesia
  - RT-PCR for POW/DTV was performed
- Unusual results were run on agarose gels for visual confirmation

IDSA, 2015 Abstract 1157
HARVEST 1: ENDEMIC ZONE

- Historically highest density of Ixodes ticks carrying Borrelia
- Highest frequency for Lyme disease
Harvest 1: Results

- 8% carried more than one infectious agent; Borrelia found in all co-infections
- Babesia species present at a rate of 5.4%, found in both Ixodes and Dermacentor ticks
- POWV/DTV detected in 6.8% of the Wisconsin Ixodes population
HARVEST 2: GEOGRAPHIC ZONE

- 33 counties across Wisconsin
- >2000 ticks collected
- Ticks used to survey prevalence of agents across Wisconsin
Tick Pools had:
• 11% Borrelia
• Close to 6% POWV/DTV
  with distribution across the state
HARVEST 3: HYPER-ENDEMIC ZONE

• Bayfield County in NW quadrant of WI
• 100 ticks collected from the same dog
• Collected within a 2-week span in Fall of 2013
Case Study: Assessment of Individual Ticks for Transmissible Agents in Localized, Hyper-Endemic Region

- Supports the concept of localized “hot spots” where ticks with an abnormally high frequency of one or more pathogens may be found.
- 10/19 positive for Borrelia (52.6%)
- 2/10 Borrelia positive ticks were co-infected with POWV/DTV (11%).
What Can We Conclude From These Three Studies?

1. An individual can acquire multiple tick-associated pathogens simultaneously

2. POWV/DTV found in a significant percentage of Ixodes ticks in all studies

3. Infection with POWV/DTV may be underdiagnosed and the virus may contribute to the acute and/or persistent symptoms often associated with Lyme disease diagnosis.
Controversy Surrounding Diagnosis of Lyme

**Traditionalists:**

- Lyme disease is uncommon and restricted to an acute syndrome
- Has rare late complications like arthritis or neurologic symptoms
- Post-treatment Lyme disease complications are not due to the persistence of the infection
- Serological evidence of infection with *B. burgdorferi* required for diagnosis
- **Both early and late Lyme infections respond to antibiotic therapy**

**Lyme Advocates:**

- Lyme disease is common
- Frequent severe late complications
- Post-treatment Lyme disease associated with long term persistence
- Serological testing for *B. burgdorferi* is insensitive, false negatives common
- **Failure of antibiotic treatments due to innate resistance of the bacteria**
Could Both Groups Be Right?

• **Question:**
  Could there be an infectious agent that is inherently insensitive to antibiotics that underlies the symptoms attributed to infection with B. burgdorferi in a subset of patients?

• **Candidate agent:**
  Powassan/DTV
Infectious Ecology to Infectious Pathology: How Does This Relate to Human Disease?

- Chronic Fatigue Syndrome/Myalgic Encephalomyelitis patients (CFS/ME)
  - 1 to 3 million individuals in the US
- Intense fatigue not improved by rest; may be worsened by physical or mental exertion
- Symptoms include weakness, muscle pain, insomnia, cognitive impairment
- Symptoms persist for >6 months
- Pattern of remission and relapse
- Diagnosis of Exclusion
  - Absence of any medical condition that would explain presence of chronic fatigue
Outbreaks of CFS/ME in the US

12 outbreaks since 1934
Outbreaks aided in the understanding of the CFS/ME

Systemic syndrome:
- excessive fatigue
- myalgias
- headache
- low grade fever
- constitutional
Post Infectious Fatigue (PIF) Syndrome

• PIF is subset of CFS/ME
• Acute onset of viral-like illness: fatigue, fever, headache, myalgia, arthralgia, cognitive impairment

Coppe Laboratories Human Study
• Cross-sectional analysis of 250 PIF patients seen at 2 clinics specializing in CFS
• Serum samples assayed for antibodies to B. burgdorferi (IgG, IgM, IgA, FDA approved kit)
• Same samples assayed for antibodies to TBEV (IgG, IgM, European kit)

Data on File, Coppe Laboratories, 2014
Presented: Targeted Tickborne Diseases, Boston, October 2014
Post Infectious Fatigue Cohort

- 33 (13.2%) of the 250 non-selected CFS subjects demonstrated evidence of tick-associated infections
- 11% had positive TBEV
- Borrelia infection in the population was 3.2%
- NY study supported finding

Data on File, Coppe Laboratories, 2014
Presented: Targeted Tickborne Diseases, Boston, October 2014
Major Conclusions: Co-Infections

- An individual can acquire multiple tick-associated pathogens simultaneously;
  - Study 1: Exposure to single tick carrying more than one agent
  - Study 2: Exposure to multiple ticks carrying one agent
  - Study 3: Exposure to localized “hot spots” of ticks with high frequency of one or more pathogens

- POWV/DTV found in a significant percentage of Ixodes ticks in all studies

- Demonstration of seropositivity of PIF patients to a TBEV-like agent suggests that infection with POWV/DTV may be underdiagnosed and that this virus may contribute to acute and/or persistent symptoms associated with Lyme Disease diagnosis.
Summary

• Arboviruses are real, cause illness, and are underdiagnosed.
• POWV/DTV is increasing in US.
• Symptoms associated with Lyme disease are similar to POWV/DTV.
• Multiple studies show ticks carry more than one pathogen.
• Arboviral infections could be source of post-treatment Lyme symptoms.
Acknowledgements

A special thanks to participating patients, families, friends, the Wisconsin Department of Natural Resources

Coppe Laboratory personnel:
CEO and founder: Konstance Knox, PhD
Diagnostic Testing Development Manager: Angie Thomm, MS
Operations: Yvette Harrington, MS