NEW TRICKS FOR TICK SICKNESS:

USING GENOMICS TO DETECT AND UNDERSTAND TICK-BORNE INFECTIONS

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www.cdc.gov/ticks
OBJECTIVES

1) Become familiar with current techniques and terminology in genomics.

2) Describe the current diagnostic approach for common and emerging tick-borne infections in MA

3) Understand how cutting-edge genomic techniques are being used to detect and study tick-borne pathogens
TICKS ARE OUT THERE!

- *Ixodes scapularis* (Deer tick)
- *Dermacentor variabilis* (American dog tick / wood tick)

https://www.cdc.gov/ticks/tickborne_diseases/tickID.html
TICKS ARE SPREADING...

1996

2016

Eisen et al. Trends in Parasitology 2018
AND SO ARE THE DISEASES THEY CARRY: E.G. LYME DISEASE

Eisen et al. Trends in Parasitology 2018
TICK-BORNE INFECTIONS ARE ON THE RISE IN MA: “THE BIG THREE”

Lyme Disease
Anaplasmosis
Babesiosis

Emerging Cases of Powassan Virus Encephalitis in New England: Clinical Presentation, Imaging, and Review of the Literature

Anne Piantedosi,1,*, Daniel B. Rubin,2,*, Daniel P. McQuillen,3 Liangge Hsu,4 Philip A. Lederer,1 Cameron D. Ashbaugh,5 Chad Duffalo,6 Robert Duncan,3 Jesse Thon,2 Shamik Bhattecharya,2 Nesli Basgoz,2 Steven K. Feske,3 and Jennifer L. Lyons5

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Borrelia miyamotoi Disease
Neither Lyme Disease Nor Relapsing Fever

Sam R. Telford III, ScD,*, Heidi K. Goethert, ScD, Philip J. Molloy, MD, Victor P. Berardi, MD, Hanumara Ram Chowdri, MD, Joseph L. Gugliotta, MD, Timothy J. Lepore, MD
“THE LITTLE TWO”
... AND MORE?

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- Borrelia mayonii
- Heartland virus
- Bourbon virus
TICK-BORNE INFECTIONS CAN BE TRICKY TO DIAGNOSE

- Lyme disease
  - Serology to detect antibody response
TICK-BORNE INFECTIONS CAN BE TRICKY TO DIAGNOSE

- Lyme disease
  - Serology to detect antibody response

- Babesiosis
  - “Malaria of New England”
  - Blood smear

https://www.cdc.gov/dpdx/babesiosis/index.html
TICK-BORNE INFECTIONS CAN BE TRICKY TO DIAGNOSE

- Lyme disease
  - Serology to detect antibody response
- Babesiosis
  - Blood smear
- Anaplasmosis
  - “Summer fever” + transaminitis, leukopenia
  - PCR
TICK-BORNE INFECTIONS CAN BE TRICKY TO DIAGNOSE

- Lyme disease
  - *Serology to detect antibody response*
- Babesiosis
  - *Blood smear*
- Anaplasmosis
  - *PCR*
- Borrelia miyamotoi
  - Fever, similar to anaplasmosis
  - *PCR*
TICK-BORNE INFECTIONS CAN BE TRICKY TO DIAGNOSE

- Lyme disease
  - Serology to detect antibody response
- Babesiosis
  - Blood smear
- Anaplasmosis
  - PCR
- Borrelia miyamotoi
  - PCR
- Powassan virus
  - Encephalitis
  - Serology to detect antibody response
WE HAVE DIFFERENT TESTS FOR DIFFERENT PATHOGENS

- **Lyme disease**
  - *Serology to detect antibody response*

- **Babesiosis**
  - *Blood smear*

- **Anaplasmosis**
  - *PCR*

- **Borrelia miyamotoi**
  - *PCR*

- **Powassan virus**
  - *Serology to detect antibody response*
Polymerase chain reaction

Mixture of DNA or RNA of different types in a sample
- Polymerase chain reaction

Mixture of DNA or RNA of different types in a sample

Primers that match the microbe of interest

Kanjilal et al. Seminars in Neurology 2019
Polymerase chain reaction

- Mixture of DNA or RNA of different types in a sample
- Primers that match the microbe of interest
- Amplification of a small target region
- Readout is detection, +/- sequencing of the target region

Kanjilal et al. Seminars in Neurology 2019
HOW CAN GENOMICS HELP US TO DO BETTER?

- Sequencing offers the ability to test for **multiple different types of pathogens** at once

- Genomic data and techniques allow the development of **highly sensitive, rapid, and inexpensive** techniques
HOW CAN GENOMICS HELP US TO DO BETTER?

- Better **diagnostic tests** for patients

- Better **surveillance** of ticks

- Pathogen genome sequencing to understand:
  - Transmission
  - Evolution
  - Drug resistance
WHAT IS “NEXT GENERATION” SEQUENCING?

- Massively parallel platform
- Allows sequencing of millions of fragments at once

Johnsen et al. Blood 2013
GENOMICS IS VERY USEFUL IN STUDYING INFECTIOUS DISEASE OUTBREAKS

Genomics is very useful in studying infectious disease outbreaks. Genomic surveillance elucidates Ebola virus origin and transmission during the 2014 outbreak. Zika virus evolution and spread in the Americas.

Gire et al. Science 2014
Metsky et al. Nature 2017
METAGENOMIC SEQUENCING

Mixture of DNA or RNA of different types in a sample

Sequence everything

Identify potential pathogen

PATHOGEN

HOST

OTHER
IS THERE A NEEDLE IN THE HAYSTACK?
HOW CAN GENOMICS HELP US TO DO BETTER?

- Better diagnostic tests for patients
CASE 1: 61 YEAR OLD MAN WITH ALTERED MENTAL STATUS

- 61 year old man with history of Crohn’s disease treated with immunosuppression
- Developed headache and confusion
- Lumbar puncture with 430 white blood cells

Piantadosi et al. Clinical Infectious Diseases 2018
CASE 1: 61 YEAR OLD MAN WITH ALTERED MENTAL STATUS

- 61 year old man with history of Crohn’s disease treated with immunosuppression
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- Lumbar puncture with 430 white blood cells
- MRI with abnormalities in multiple locations

Piantadosi et al. Clinical Infectious Diseases 2018
CASE 1: 61 YEAR OLD MAN WITH ALTERED MENTAL STATUS

Piantadosi et al. Clinical Infectious Diseases 2018
CASE 1: 61 YEAR OLD MAN WITH ALTERED MENTAL STATUS

Sample preparation – 3 days
- Nucleic acid extraction
- cDNA synthesis
- Library construction
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Data analysis – ½ day
- Metagenomic analysis
- Virus-specific analysis
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Powassan virus
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- Library construction

Sequencing – 1 day

Data analysis – ½ day
- Metagenomic analysis
- Virus-specific analysis

Powassan virus

Clinical serology test positive for Powassan – 4 weeks later
CASE 1: 61 YEAR OLD MAN WITH ALTERED MENTAL STATUS

- No specific treatment
- Patient unfortunately had limited neurological recovery
- Psychological benefits of earlier diagnosis
- Opportunity to spare unnecessary treatment?
SHERLOCK IS A SENSITIVE, TARGETED CRISPR-BASED TECHNIQUE

- Specific high-sensitivity enzymatic reporter unlocking

Myhrvold et al. Science 2018
SHERLOCK IS A SENSITIVE, TARGETED CRISPR-BASED TECHNIQUE

- Specific high-sensitivity enzymatic reporter unlocking

- Targeted (like PCR)

- Can be performed directly from clinical sample in <2h

Myhrvold et al. Science 2018
CASE 2: 79 YEAR OLD MAN WITH ALTERED MENTAL STATUS

- 79 year old **healthy male gardener and physician**
- Developed **dizziness, slurred speech, confusion, fever**
- Lumbar puncture with **284 white blood cells**
- MRI with abnormalities in multiple locations
CASE 2: 79 YEAR OLD MAN WITH ALTERED MENTAL STATUS

Sample preparation – ~24 hours

- Nucleic acid extraction
- cDNA synthesis
- SHERLOCK for Powassan virus Positive

- Confirmed by sequencing over the next 2 days
- Patient unfortunately passed away
- Family committed to raising awareness about Powassan virus
SHERLOCK CAN DETECT OTHER TICK-BORNE INFECTIONS

Slide courtesy of Gordon Adams and Jacob Lemieux MD, DPhil
HOW CAN GENOMICS HELP US TO DO BETTER?

- Better diagnostic tests for patients
- Better surveillance of ticks
Massachusetts *Ixodes scapularis* Ticks Tested in the Laboratory of Medical Zoology, 2015-2016, Percent Positive by PCR

(N=3,783 ticks tested, except Powassan=85)

[Graph showing the percentage of ticks positive for different pathogens, with *Borrelia burgdorferi* having the highest percentage.]

https://www.tickreport.com/

FIELD-DEPLOYABLE SHERLOCK

- Targeted
- Can be performed directly from primary sample in <2h
- Lateral flow assay

Myhrvold et al. Science 2018
33 viruses, 24 of which were novel

Caveat: this technique will find many things, most of which are unlikely pathogenic to humans
HOW CAN GENOMICS HELP US TO DO BETTER?

- Better diagnostic tests for patients
- Better surveillance of ticks
- Pathogen genome sequencing to understand:
  - Transmission
  - Evolution
  - Drug resistance
GENOMICS ALLOWS US TO ASK:

- Is the pathogen population size growing?
- Are there mutations associated with increasing human infection or pathogenesis?
POWASSAN VIRUS IS UNDER-STUDIED

- 22 complete virus genomes previously available
  - 17 from ticks
  - 5 from humans
  (from Russia and Canada)
ONGOING EFFORTS TO UNDERSTAND POWASSAN VIRUS DIVERSITY

- 14 new Powassan virus genomes
  - 4 from humans
  - 10 from ticks
GENOMICS ALLOWS US TO ASK:

- Is the pathogen population size growing?
- Are there mutations associated with increasing human infection or pathogenesis?
- Are there mutations associated with drug resistance?
Patient with relapsing babesiosis, despite treatment

Mutations associated with drug resistance

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Lemieux et al. Nature Microbiology 2016
CAN WE TREAT VIRAL INFECTIONS USING NEW GENOMIC TECHNIQUES?

- ~2/3 of viruses that infect humans are RNA viruses (Woolhouse ME et. al. Emerg. Infect. Disease. 2016.)

- Many have limited antiviral treatment options (De Clercq E & Li G. Clin. Microbiol. Review. 2016.)
CAN WE TREAT VIRAL INFECTIONS USING NEW GENOMIC TECHNIQUES?

- SHERLOCK as an antiviral:
  - Virus-infected cell
  - Cas13-crRNA therapy

Slide courtesy of Catherine Freije
CAN WE TREAT VIRAL INFECTIONS USING NEW GENOMIC TECHNIQUES?

- SHERLOCK as an antiviral:

Slide courtesy of Catherine Freije
Before:
• DEET, picaridin
• Permethrin
• Long, light clothing

After:
• Shower
• Tick check
• Heat your clothes
Tick-borne infections are increasing in MA:
  - Lyme disease, anaplasmosis, babesiosis
  - Powassan virus, borrelia miyamotii
  - Others?

Cutting-edge genomic techniques including SHERLOCK, metagenomic sequencing, and pathogen genome sequencing will allow us to improve:
  - Diagnostics for patients
  - Surveillance of ticks
  - Understanding pathogen evolution and pathogenesis
EXCITING DIRECTIONS IN GENOMICS

- Single-cell sequencing
- Human cell atlas
- Evolution of cancer cells
- CRISPR for genomic screens and gene editing
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