Molecular testing for bacterial diseases transmitted by ticks

Saltillo, June 8th - 10th 2016

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Testing ticks and animals

Development of a multiplex PCR for the detection of:

- *B. burgdorferi*,
- *Ehrlichia canis, E. chaffeensis*,
- *Anaplasma phagocytophilum* and
- *Rickettsia rickettsii*
Undergraduate research team: Brian, Annie, Thomas and Brittany

Jaqueline: visiting scholar (BZ)
Mackenzie: PhD student

Joseph: PhD student and TVMDL
Fulltime employee
Background

- Tick-borne diseases are becoming a serious problem worldwide
- Lyme disease, Human Anaplasmosis and Rocky Mountain Spotted fever have emerged as the most common vector born bacterial illnesses in the US and Mexico.
Aim of the study

• Develop a Multiplex PCR technique
  – Multi pathogen detection
  – Compatible with Sequencing
    • Species confirmation
    • Molecular epidemiology
  – Compatible with diagnostic platforms
    • Real time PCR
    • Molecular Beacon PCR technology
    • Others
Lyme disease (LD)

- Most prevalent arthropod borne disease in the US
  - Over 30,000 cases reported yearly to CDC
  - Transmitted by *Ixodes scapularis* and *I. pacificus* tick bite (in the US)
  - Mammalian reservoirs are small rodents
- Caused by the spirochetal pathogen *Borrelia Burgdorferi*
- Multi-phase disorder in humans
  - Early LD (70% Erythema migrans)
  - Early disseminated LD (Flu like symptoms)
  - Chronic LD (Arthritis and carditis)
Ehrlichioses

- Small, gram-negative bacteria, round or ellipsoidal in shape.
- Symptoms in humans: fever, headache, fatigue, and muscle aches.
- These symptoms occur within 1-2 weeks following a tick bite.
Anaplasma phagocytophilum

- Gram-negative and intracellular: targets neutrophils, alters their function in the host, and forms morulae within vacuoles.
- Symptoms in humans: fever, headache, muscle pain, malaise, chills, nausea, abdominal pain, cough, and confusion.
- Severe clinical presentations may include difficulty breathing, hemorrhage, renal failure or neurological problems.
Rocky Mountain Spotted Fever (RMSF)

- Gram-negative, intracellular, coccobacillus bacterium
- Typical symptoms include: fever, lethargy, abdominal pain, vomiting, and muscle pain
- Rash found on 90% of patients
  - Classic RMSF rash - 2 to 5 days post fever
    - small, flat, pink macules - develops on distal extremities
  - Varies greatly and is unreliable at times
- Pathogen of interest: *Rickettsia rickettsii*
Previous Rickettsiosis Forum

- Tijuana May 2015
- Discussed the possibility of testing
  - *Rhipicephalus sanguinus* from Baja California
  - Associated with canids in areas where severe human cases were reported
  - Test an initial submission of ticks at UTSA
  - Test further specimens by qPCR methodology
Baja California Ticks

• Our team has developed a Multiplex qPCR protocol under revision for patent application

• Detects:
  – *B. burgdorferi*
  – *Ehrlichia canis*
  – *Rickettsia rickettsii*
  – Canine internal control

  – Additional targets
    • *Anaplasma phagocytophilum, E. chaffeensis*
### Sensitivity

<table>
<thead>
<tr>
<th></th>
<th><strong>Borrelia burgdorferi</strong></th>
<th></th>
<th><strong>Rickettsia rickettsii</strong></th>
<th></th>
<th><strong>Anaplasma phagocytophilum</strong></th>
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<th><strong>Ehrlichia canis</strong></th>
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* indicates the detection limit
## Specificity

<table>
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<tr>
<th>Substrates</th>
<th>B. burgdorferi qPCR C&lt;sub&gt;t&lt;/sub&gt;</th>
<th>A. phagocytophilum qPCR C&lt;sub&gt;t&lt;/sub&gt;</th>
<th>R. rickettsii qPCR C&lt;sub&gt;t&lt;/sub&gt;</th>
<th>E. Canis qPCR C&lt;sub&gt;t&lt;/sub&gt;</th>
<th>E. Chaffeensis qPCR C&lt;sub&gt;t&lt;/sub&gt;</th>
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Canine internal control

### Specific to
- *Canis lupus familiaris*
- *Canis lupus*
- *Canis latrans*

### Validated Against
- Equine
- Feline
- Caprine
- Ovine
- Cervine
- Bovine
- Porcine
- Avian
- Procyonine
- Vulpine
- Mephitidae

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![Graph showing fluorescence over cycles for different components](image-url)

*Legend:*
- E. canis
- B. burgdorferi
- R. rickettsii
- ROX
- Canine internal control
Baja California samples

• We evaluated a total of 211 *R. sanguineus* ticks
  – Collected from dogs/environmental locations (n=160)

• Samples were processed for:
  – Confirmation of tick species, sex, age
  – Detection of pathogens

• All experiments were conducted at Texas A&M Veterinary Medical Diagnostic Lab (TVMDL)
Texas A&M Veterinary Diagnostic Laboratory
Locations

- 165 staff
- Over 30 professional staff who hold a DVM and/or PhD
- 21 professionals with board certifications in their specialty
- Strategically located in the livestock and poultry rich regions of Texas
Vision
To be the global leader in providing innovative and state-of-the-art veterinary diagnostic services

Mission
To promote animal health and protect agricultural, companion animal, and public health interests in Texas and beyond by providing excellence in veterinary diagnostic service
Clientele

- Veterinarians and animal owners from Texas and other states
- Local, state and national agencies
- International clientele
- Commercial and state diagnostic laboratories
Disciplines

- Bacteriology
- Virology
- Endocrinology
- Parasitology
- Serology
- Toxicology
- Molecular Genetics
- Clinical Pathology
- Histopathology
- Necropsy
- Poultry Diagnostics
- Epidemiology
- Drug Testing
Molecular Diagnostics

- Maceration of tick samples: Omni Bead Ruptor

- DNA extraction: KingFisher™ Flex

- qPCR amplification and analysis:
  - ABI® 7500 qPCR System
## Results

<table>
<thead>
<tr>
<th>Patógenos</th>
<th>Positivos (%)*</th>
</tr>
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<tbody>
<tr>
<td>Borrelia burgdorferi</td>
<td>0</td>
</tr>
<tr>
<td>Anaplasma phagocytophilum</td>
<td>0</td>
</tr>
<tr>
<td>Ehrlichia canis</td>
<td>18 (8.5)</td>
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<tr>
<td>Ehrlichia chaffeensis</td>
<td>0</td>
</tr>
<tr>
<td>Rickettsia rickettsii</td>
<td>2 (0.9%)</td>
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<tr>
<td><strong>Total</strong></td>
<td><strong>20 (9.5%)</strong></td>
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</table>
Conclusions

- *E. canis* positive ticks were confirmed by sequencing
- *R. rickettsia* positives are under study
- Zoonotic pathogens are present in *R. sanguineus* ticks of Baja California
- Epidemiological studies will certainly provide relevant information for the implementation of control programs
Take home message

- Tick borne diseases are circulating in the Texas-Mexico transboundary region
- Bi-national efforts can
  - Generate distribution maps
  - Assess Human risk
- Multiplex technology has been developed:
  - Eco-epidemiology (surveillance)
  - Molecular epidemiology (surveillance)
  - Diagnostics
Acknowledgements

- Texas A&M University
  - The Lyme Lab (Dr. Esteve-Gassent)
    - Abha Grover
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    - Robi Chaffout
    - Ross Wittenborn
    - Emy Hassan
  - CVM
    - Thomas Craig, DVM, PhD
    - Karen Snowden, DVM, PhD
- TVMDL
  - Joseph Modarelli
  - Pam Ferro, MS, PhD
- USDA-ARS Kerrville
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- USDA APHIS:
  - Luis Lecuona, DVM
- CENAPRECE
- Secretaría de Salud
Thank you!
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