Canine Babesiosis

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Babesiosis

- First tick-transmitted infection
- Smith and Kilbourne 1893
- Causative agent of “Texas Fever” in cattle
- How did they diagnose it?
Classification
Classification

= Babesia bovis

= Babesia canis
History of canine babesiosis

- 1896: First case of canine babesiosis
- 1934: First canine case in USA
- 1968: First case of a small canine *Babesia* in USA
- 1983-1992: *Babesia canis* is prevalent in greyhounds
- 1991: First outbreak of canine babesiosis caused by small *Babesia*
- 100 years later, how were they diagnosed?
July 1, 1995:
My first day as a veterinarian
Hemolytic anemia, thrombocytopenia, hyperglobulinemia, icterus, fever, splenomegaly, and lymphadenopathy
Polymerase Chain Reaction

- 1991: First *Babesia* DNA sequence in Genbank
- 1995: Two canine *Babesia* DNA sequences available
  - *Babesia canis*
  - *Babesia gibsoni*
What about our isolates?

- 3 Partial 18S rRNA gene sequences in GenBank for canine *Babesia* spp. in 1999
  - 2 *B. canis*, 1 *B. gibsoni* (from 1991 CA report)
- PCR primers designed by NCSU to differentiate *B. canis* and *B. gibsoni*
- Our “*B. gibsoni*” amplified with “*B. canis*” primers and NOT “*B. gibsoni*”

Lane 1: 1KB Molecular weight marker
Lane 2: canine DNA
Lane 3: *B. gibsoni* (Asian genotype)
Lane 4: *B. c. canis*
Lane 5: *B. gibsoni* (California/USA genotype)
Lane 6: negative (no DNA) control
Epidemiology
Approach

- Serologic and molecular survey:
  - Stray dogs in three geographic regions of NC
  - Three kennels where *B. gibsoni* infections had been diagnosed previously
Methods

● Samples:
  ● Stray: 359 dogs housed in animal shelters
    − Eastern NC: 168 dogs
    − Central NC: 140 dogs
    − Western NC: 51 dogs
  ● Kennel: 159 dogs housed in kennels where *B. gibsoni* infections had been diagnosed
    − Kennel I: 59 dogs
    − Kennel II: 43 dogs
    − Kennel III: 47 dogs
Results

Kennel dogs > 1:64 (n=22)
Stray dogs > 1:64 (n=21)
Results

- Microscopy Stray
  - 1 dog with small piroplasms
- Microscopy Kennel
  - 13 dogs small piroplasms
- PCR Stray
  - 1 *B. canis vogeli*
  - 2 *B. gibsoni* (Asian)
- PCR Kennel
  - 14 *B. gibsoni* (Asian)
Conclusions

- *Babesia gibsoni* is endemic to NC
- Prevalence in kennels is high (6.8-25.6%)
- Seroreactivity may not accurately predict *Babesia* spp.
- Kennels comprised of American Pit Bull Terrier type dogs (96%)

(Birkenheuer et. al., JAAHA, 2003)
Hypothesis:

- Dogs that test positive for *B. gibsoni* are more likely to be American pit bull terriers
Approach

- Retrospective analysis samples submitted to the NCSU-Vector Borne Disease Diagnostic Laboratory for *Babesia* PCR between May 2000 and October 2003
Results

- 688 canine submissions were reviewed
  - Geographic location
  - Breed
- 145/688 (21%) samples tested positive for the presence of *Babesia* spp. DNA
### Results

<table>
<thead>
<tr>
<th></th>
<th>Babesia gibsoni</th>
<th>Babesia canis vogeli</th>
<th>Other piroplasm</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total</strong></td>
<td>131</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td><strong>APBT</strong>*</td>
<td>121</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Greyhound</td>
<td>0</td>
<td>8</td>
<td>0</td>
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<tr>
<td>Other Breed</td>
<td>10</td>
<td>3</td>
<td>2</td>
</tr>
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</table>

* APBT: American Pit Bull Terrier
How extensive is the infection: 1995
How extensive is the infection: 2003
How extensive is the infection: 2010
Results

- Dogs testing positive for *Babesia* spp DNA were more likely to be APBT dogs compared to all other dogs tested (Odds ratio 12.7; 95% CI 7.66 to 21.20; p < 0.0001)

- Dogs testing positive for *B. c. vogeli* DNA were more likely to be Greyhounds compared to other non-APBT dogs tested (Odds ratio 4.96; 95% CI 1.08 to 25.51; p = 0.02)

- *Babesia gibsoni* is widespread in USA
Typical living conditions of the kennel dogs
Hypothesis

- *Babesia gibsoni* infections in the US are transmitted directly via dog bites and are not vector-transmitted
- APBT owners were not willing to give out information about previous bite wounds
- *Babesia gibsoni* infections in non-pit bull dogs are associated with a recent dog bite
Approach

- Retrospective analysis of medical records of non-APBT dogs confirmed to have *B. gibsoni* infection identified by PCR in the VBDDL at NCSU
- To determine whether or not exposure to ticks or a history of a fight with an American Pit Bull Terrier were risk factors for *B. gibsoni* infection
Results

- 15 *B. gibsoni* infected dogs that were not APBTs were identified
  - 3 excluded due to lack of known history
- 12 *Ehrlichia ewingii* infected dogs were used as a control group representing dogs with a tick-transmitted disease
<table>
<thead>
<tr>
<th></th>
<th>Dog bite</th>
<th>APBT* bite</th>
<th>Ticks</th>
<th>Housed with infected dog</th>
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<tbody>
<tr>
<td><strong>Babesia gibsoni</strong></td>
<td>9/12</td>
<td>9/12</td>
<td>2/12</td>
<td>4/12</td>
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<tr>
<td><strong>Ehrlichia ewingii</strong></td>
<td>3/12</td>
<td>0/12</td>
<td>5/12</td>
<td>2/12</td>
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</tbody>
</table>

* APBT: American Pit Bull Terrier
Results/Conclusions

- *B. gibsoni* infected dogs were more likely to have had a recent dog bite than *E. ewingii* infected dogs (Odds ratio 9.0; 95% CI 1.07 to 96.34; p = 0.014)

- The association was stronger (p < 0.0001) when the dog inflicting the bite was an APBT

- The association remained (p = 0.045) when each household with multiple infected dogs was counted as a single case
Now What?

- Developed test
- Identified epidemic
- Identified risk factors for infection
- THERE IS NO EFFECTIVE TREATMENT FOR  *Babesia gibsoni*!
Treatment

- Treatments reduce morbidity and mortality but cannot clear the infections
  - Imidocarb, diminazene, trypan blue, phenamidine, doxycycline, clindamycin....

- Treated dogs are carriers and are at risk for recurrence of signs and can transmit infections to other dogs
Atovaquone and Azithromycin

- Atovaquone is a hydroxy-1,4-naphthoquinone: analog of ubiquinone and interferes with electron transport
- Azithromycin is an azalide antibiotic and interferes with protein synthesis
- Both drugs have anti-\textit{Babesia} activity
- Together have been effective against other \textit{Babesia} spp.

Specific Aim

- To determine whether or not an atovaquone and azithromycin drug combination would decrease *Babesia gibsoni* parasitemia below the limit of detection
Approach

- Double-blind placebo-controlled
  - 11 treatment
  - 11 placebo
- Could detect 60% difference between groups (based on pilot study)
- 3 post-treatment sample dates
- Detection Semi-nested PCR that can detect 50 organisms/ml
- CBC, biochemical profiles
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<th>Dog</th>
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<tr>
<td>3</td>
<td>Treatment</td>
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</table>

+, positive PCR test
-, negative PCR test
ND, not determined
Results/Conclusions

- There was a significant difference between the treatment and placebo groups ($p= 0.0001$).
- No side-effects were reported in either the treatment or placebo treated dogs.
- Only treatment reported to eliminate detectable *B. gibsoni* parasitemia.

(Birkenheuer et. al., J Vet Internal Med, 2004)
There are currently at least 9 genetically unique canine piroplasms

1. *B. gibsoni*
2. *B. vogeli*
3. *Babesia sp. coco*
4. *B. conradaei*
5. *B. canis*
6. *B. rossi*
7. *T. annae*
8. Novel sp. in England
9. *T. equi*
10. There will be more!
Conclusions

- There is no microscope objective powerful enough to decipher DNA sequence
- There are currently 745,314 *Babesia* DNA sequences in Genbank
- We are probably just seeing the tip of the iceberg
- *Babesia* are not as “species-specific” as we think
- Humans are a good model for animal disease
Questions?