Update on Equine Tick-Borne diseases

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Equine Tick-Borne Diseases

Disease Nomenclature

- Lyme Disease: *Borrelia burgdorferi*
- Equine Granulocytic Anaplasmosis: *Anaplasma phagocytophilum*

Tick borne Equine Diseases; Lyme disease

1. Modes of Transmission & Incidence
2. What are the issues?
   - Unique *Borrelia* traits
   - Clinical Signs
   - Diagnostic Tests
3. Treatment
4. Prevention; Vaccine and tick control

Lyme Disease

- *Borrelia burgdorferi* – helical-shaped gram-negative, extracellular spirochete
- Dz First observed in US in 1976
- Very common in Northeast, Mid-Atlantic States & Great Lake region
- *Ixodes* tick is the vector.
- Bacteria not free living organisms and quickly die outside of host
- white-footed mouse and deer involved in 2-year life cycle.

Borrelia burgdorferi spirochetes

Common Ticks

Lyme Disease

Tick Review

- Engorged (fed) nymph
- Male
- Fully engorged female
- Partially engorged female
- Engorged (fed) larva [seed ticks]

Active life stages of the blacklegged tick (Deer tick). For size reference, ticks are placed next to a period (12 point). Adults most active April-June and again October-November. Nymphs = May-July...
In 2014, 96% of confirmed Lyme disease cases were reported from 14 states:

- Connecticut
- Delaware
- Maine
- Maryland
- Massachusetts
- Minnesota
- New Hampshire
- New Jersey
- New York
- Pennsylvania
- Rhode Island
- Vermont
- Virginia
- Wisconsin

Lyme disease is the most commonly reported vectorborne illness in the United States. In 2014, it was the fifth most common Nationally Notifiable disease. However, this disease does not occur nationwide and is concentrated heavily in the northeast and upper Midwest.

Lyme survey; 5,197 horses in US
1246 positive

<table>
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<tr>
<th>REGION</th>
<th>HORSES</th>
<th>POSITIVE</th>
<th>POSITIVE%</th>
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<tr>
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<td>650</td>
<td>225</td>
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<tr>
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<td>366</td>
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<tr>
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<tr>
<td>Southwest</td>
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<td>104</td>
<td>16.0%</td>
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Lyme Disease Clinical Signs

- Nonspecific
- Weight loss - mus wasting over top line
- Shifting lameness
- Stiffness, muscle soreness
- Swollen joints
- Laminitis
- Lethargy
- Uveitis
- Neurological Signs-cervical atrophy
- Abortion
- +/- Low grade fever
- skin connective tissue (nodules)

Borrelia burgdorferi Surface antigens....

- Several lipoproteins located on surface; Osp (Outer surface protein)
  - Osp A
    - Gut of tick (adhesion to wall?) > 24hr attachment to downregulate OspA for mammalian host survival
  - Osp C
    - Accelerated upregulation during blood meal
    - Migrate from gut to salivary glands (2-3 days)
    - Special issues with ‘C’
  - Osp F
    - Chronically in host (??)
**Unique Aspects of Borellia**

- Organism resides in connective tissue, collagen, skin, joints and does not need iron (blood) for survival.
- Ovoid/ spheroid formation
- Biofilm
- Recent issues identified with OspC

**Bacterial biofilms...**

- Organized communities of cells enclosed in a self-produced matrix (lipids, proteins + other macromolecules)
- *Borrelia* can adopt diverse morphologies (spirochete, cysts, granules..) These forms provide a protective environment depending on environmental conditions.
- Conditions such as exposure to AB’s, starvation, pH changes, will result in defensive form (Biofilm)
- Elimination of pathogenic bacteria in ‘biofilm’ form extremely challenging (incomplete penetration and/or inactivation of AB’s)
- *Borrelia* becomes dormant in this state until favorable conditions then returns to spirochete form
- Survival response in bacteria

**Osp C...**

- upregulated in ticks concurrent with blood meal and expressed at high level during first weeks of infection
- required to establish infection but not persistence
- OspC is a highly variable protein with +/- 30 distinct variants or ‘types’ identified (great genetic diversity)
- Significant variation in OspC Ab responses (naturally infected dogs) suggests infected animals recognized only a limited subset of OspC types
- A single OspC protein would be ineffective in stimulating a protective Ab response
- *** A single OspC variant is insufficient to serve as a reliable diagnostic indicator
- Broad protective capability of OspC based vaccines enhanced by inclusion of multiple epitopes derived from diverse OspC type proteins

**Lyme Disease; Diagnosis**

- Clinical examination with presence of signs in an endemic area
- Exclusion of other causes of lameness

- **Bloodwork:**
  - Enzyme-Linked Immunosorbent Assay (ELISA)
  - 4DX Snap Test
- Western Blot (WB)
- Multiplex assay
- Tissue Sampling?
Diagnostic Tests

- **ELISA** followed by confirmatory Western blot
- Immunofluorescent antibody (IFA) testing.
- Antibodies may be detected as early as 5-6 weeks post infection.
- The Equine Lyme Multiplex Assay (Cornell Diagnostic Laboratory, Ithaca, NY) can identify antibodies to *B. burgdorferi* in equine serum as early as 3-5 weeks after infection.
- Test detects antibodies to three antigens of *B. burgdorferi* in equine serum called outer surface proteins (Osp) including OspA, OspC or OspF. The multiplex reader detects the fluorescent conjugate and is reported as median fluorescent intensities (MFI).
- The Osp antigen expression changes depending upon tick life cycle. While in the tick's gut, OspA is expressed on the bacterial surface. During tick feeding, the bacteria express OspC on their surface, which is maintained throughout infection. During chronic infection, OspF is expressed.

**Diagnostic Tests**

**Western Blot**

- Most (?) specific test (Gold Standard)
- Measures several antibody "bands" that the horse produces and reported as Strong, Moderate or Low level of exposure
- Will differentiate if horse received commercial canine vaccine.
- Interpretation on its own is difficult if "Moderate" response.

**C-6 4DX SNAP Test**

- Quick and easy stall-side screening test
- Measures one antibody, the C-6. This is the fastest rising and falling antibody.
- Good sensitivity (67% determination of TRUE POSITIVES)...
- Excellent specificity >90% (% of TRUE NEGATIVES)
- Can be useful in detecting concurrent Anaplasmosis.
- Some false +’s and false –’s
- Not licensed for horses!

**More Lyme Diagnostics…**

- **Multiplex assay** (Wagner, et al)
  - Cornell
  - Serum or CSF
  - Osp A (Sens 49%; Spec 85%)
    - Indicates vaccination
  - Osp C (Sens 80%; Spec 79%)
    - Indicates recent exposure or infection w/in last 2-5 months
    - 2-3 weeks after tick bite
  - Osp F (Sens 86%; Spec 69%)
    - Indicates chronic disease
    - [Detects AB in the pg/ml range]
OspA – is expressed while *B. burgdorferi* persists in the tick mid-gut. During infection of mammalian hosts, the bacteria down-regulate OspA, and antibodies are generally undetectable after natural infection. A positive value may be observed in vaccinated horses. An antibody titer > 2,000 is considered positive. Results are reported as Positive, negative and equivocal.

OspC – indicates *early infection* and may be detected as early as 3 weeks post infection, decline after 7-11 weeks and become undetectable by 4-5 months. An antibody titer > 1,000 is considered positive and infected 2-5 months prior. Results are reported as Positive, negative and equivocal.

OspF – is an indicator of *chronic infection*. An antibody titer > 1,250 is considered positive for chronic infection. Results are reported as Positive, negative and equivocal.

**Challenges in Diagnosis**

- Clinical signs are confusing and NOT consistent
- Serological tests do not distinguish between active infection and previous exposure.
- Many infected horses are infected for a long duration, even life.
- There is no protective immunity so horses can become re-infected repeatedly.
- Test horses with high ELISA values rarely have a marked decline even 3 years later!
- Treatment rarely results in a decline in antibodies.

**Treatment of Lyme Disease**

**Oxytetracycline** 6.6mg/kg IV once daily x 7-10 up to 28 days

- Obtains high tissue levels
- More likely to clear the organism
- Must be given IV daily in hospital by veterinarian.
  (Risks associated when given outside of vein are great.)
- Must monitor kidney values when patient is on this drug long term.
- Not for use long-term in pregnant mares.
- Best if course is followed up by *Doxycycline* orally.

**Refractory cases**: cyst forms may be present in chronically infected cases; *Metronidazole* (????)

**Doxycycline** 10mg/kg orally every 12 hours for one month

- Potent anti-inflammatory properties (esp. MMPs-for synovial membranes and arthritis)
- Easy for client to administer at home
- < 9% bioavailable (poor tissue concentrations)
- Diarrhea can result in a low percentage of horses.
- Response to treatment is confusing due to its anti-inflammatory properties.
- Not for use long-term in pregnant mares

Owner compliance???
Table 1: Minimum inhibitory concentrations (MICs) of three antibiotics for individual Borrelia burgdorferi isolates

<table>
<thead>
<tr>
<th>Isolate</th>
<th>MIC* (µg/ml)</th>
<th>MIC* (µg/ml)</th>
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<tbody>
<tr>
<td>Experiment 1</td>
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<td>0.8</td>
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<tr>
<td>Experiment 2</td>
<td>0.8</td>
<td>0.4</td>
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<tr>
<td>Experiment 3</td>
<td>0.8</td>
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</tr>
<tr>
<td>Experiment 4</td>
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<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Experiment 5</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
</tr>
</tbody>
</table>

*MIC is the MIC at which each isolate in triplicate assays (1, 2, 3) were inhibited.

Prevention of Lyme Disease

Tick prevention & Vaccination

- OspA- very short ½ life (3 months?)
  - No commercial equine vaccine available. Why???
- Canine vaccine (OspA) has been used off label. - efficacy in horses is unknown.
- Future: Tick saliva vaccine (pre-exposure with sterile tick salivary proteins).

 Lyme Neuroborreliosis in 2 Horses

D. M. Imai,1,2 B. C. Bannik,4 B. D. Dol,3 J. J. Berenson4, S. Feng,1 E. Hoibl1, J. M. Johnston,2 K. J. Olsen,1 and S. W. Barthold1,2

Abstract

Lyme neuroborreliosis—characterized as chronic, nonsuppurative, perivascular to meningeal or intracranial neuritis—was diagnosed in 2 horses with progressive neurologic disease. In 1 horse, Borrelia burgdorferi sensu stricto was identified by polymerase chain reaction amplification of B. burgdorferi sensu stricto–specific gene targets (ospA, ospC, flaB, dbpA, arp). Highest spirochetal burdens were in tissues with inflammation, including spinal cord, muscle, and joint capsule. Sequence analysis of ospA, ospC, and flaB revealed 99.9% sequence identity to the respective genes in B. burgdorferi strain 297, an isolate from a human case of neuroborreliosis. In both horses, spirochetes were visualized in affected tissues with Steiner silver impregnation and by immunohistochemistry, predominantly within the dense collagenous tissue of the dura mater and leptomeninges.

Consider Dx of neuroborreliosis ONLY if CSF Ab against Osp antigens at least 2-fold > than serum value

Need for a New Lyme Disease Vaccine

Shanika A. Prakash, MD

..."multiple non-OspA antigens-most notable, OspC- have been shown to protect against borrelia infection by active and passive immunization..."

The Borrelia Challenge

1. Possible exposure to B. burgdorferi infected ticks (geographic location...)
2. Clinical signs compatible with Lyme disease
3. Absence of other causes of disease (Anaplasmosis?)
4. High AB levels to B. burgdorferi and/or identification of organism or compatible cytology/pathology
5. Ruling out other causes for clinical signs***
   - Response to therapy rarely recommended as indicator of success...

To determine whether horse is currently infected is difficult. To determine whether clinical disease is associated with Borrelia is extremely difficult...
Anaplasmosis/Ehrlichiosis

• Formerly known as *Ehrlichia equi*
• Now named *Anaplasmosis phagocytophilum*
• Responsible for causing Equine Granulocytic anaplasmosis (EGA)
• Seasonal rickettsial disease

Originally described in horses - 1969

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**Anaplasma phagocytophilum**

- Gram negative coccoid
- Intracellular bacterium (neutrophils)
- Equine granulocytic Anaplasmosis
- Circum-global distribution
- Transmitted by *Ixodes* species
  - 20% of *Ixodes scapularis* in southeastern US
  - (Roelling, et al)
  - Adults and nymphs
- Humans, horses, dogs, and livestock
- Dead end hosts

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**EGA**

- Transmitted by *Ixodes* ticks
- Variety of intermediate hosts (mice, woodrats, chipmunks, voles, shrews, deer and possibly birds)
- Dogs, humans and horses are accidental hosts. Bacteriemia is short (<1 month) which is unlikely important in disease transmission.
- Seasonality occurs when ticks are around. (most of the year!)
  
  **Many ticks co-infected with *Borellia* & *Anaplas***

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**Anaplas/Ehrlichia; Diagnosis**

- Organism is an obligate intracellular pathogen.
- It "loves" neutrophils (WBC) and lives within them.
- Forms inclusions in WBC called morula.
- Morula present in WBC a few days after onset up to one week.
- Up to 30% of horse’s neutrophils are affected.

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**EGA signs**

- Fever (103-106F)
- Depression
- Limb Edema
- Lymphopenia
- Neutropenia
- Thrombocytopenia
- Mild anemia

  • Clinical signs tend to be more severe in older horses than younger horses
  • Signs can be confused with purpura

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EGA

Timeline of Events
• Tick attaches to horse: Day 0
• Incubation from 1-12 days
• High fever: Days 5-10 post-infection, accompanied by inappetence, depression, icterus, high heart rate, high respiratory rate
• Reluctance to move, limb edema, ataxia: Days 7-12 (usually 2 days post-fever)
• Many people think their horse is "neurological" or Lyme

EGA

• Pathologic findings
  - Petechiae and ecchymosis
  - Subcutaneous tissues
  - Edema
  - Ventral abdomen, limbs, prepuce
  - Proliferative and necrotizing vasculitis
  - Thromboses
  - Perivascular cuffing
  - Subcutaneous tissues, fascia, kidneys, heart, brain, lungs, ovaries, testes

EGA; Diagnosis
• 4DX Snap Test is ok screening test. It only detects 50% of clinical cases (does not produce rapid antibodies like Lyme)
• Organism difficult to grow in culture.
• PCR in whole blood: Detects organism's DNA in horse's blood
• Indirect Fluorescent Antibody Test (IFAT): Detects antibodies in horse's blood – variable results
  - Naive horses develop antibodies 12 days after development of clinical signs.
  - Natural infections developed antibodies 19-81 days post-fever with a peak around Day 46, waning at Day 203.

EGA; Treatment
• Disease is self-limiting: will resolve in a few weeks without any treatment. Signs are more severe and persist.
  - Oxytetracycline: 6.6mg/kg IV once daily x 5-7 days shows dramatic and immediate improvement
  - Doxycycline: 10mg/kg orally twice daily for 5-7 days also works
• Combination of IV and oral tetracyclines
• Relapses 3 weeks after initial infection are reported.
Equine Anaplasmosis (EGA)

- Take home message
  - Consistent clinical signs
  - High fever
  - Inappetence
  - Lethargy
  - Icterus
  - Buffy coat – Morula within neutrophils
  - PCR/Titer (SNAP 4DX OK...)
  - Treatment – Improvement in clinical signs quickly
  - Natural immunity for about 2 yrs

Vaccination against Lyme Disease with recombinant Borrelia burgdorferi outer-surface protein A (rOspA) in horses

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1Department of Population Medicine and Diagnostic Science, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA
2Department of Biomedical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA
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Received 11 March 1999; received in revised form 21 April 1999; accepted 22 April 1999

8 vaccinates, 4 controls; vaccinated 3x @ day 0, 20 & 82. Challenged w/ infected ticks day 112 after 1st vaccination

In summary, a rOspA vaccine protected ponies against B. burgdorferi infection. Further studies are needed to determine the duration of protection after vaccination, safety and cross protection against the possible heterogeneous OspA structures that may be found among new B. burgdorferi strains isolated in the United States [42]. Reportedly, vaccination with recombinant OspA can protect animals against infection, but can not eliminate the organisms if the animals were infected before vaccination.

Results from this study show that vaccination with recombinant OspA protected ponies against infection after experimental challenge with B. burgdorferi-infected ticks.