Update on Leptospirosis in Colorado

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Canine Leptospirosis

• Brief review
  • Organism and epidemiology
• Disease in dogs
  • Common signs
  • Less common signs
• Case examples
• Diagnosis
  • Microscopic agglutination test (MAT)
  • Other tests
• Therapy
• Vaccination
• Risk factors
• Colorado data

Leptospires

• Filamentous aerobic bacteria
• 0.1-0.2 µm x 6-12 µm
• Envelope:
  • Lipopolysaccharide
  • Lower endotoxic activity
  • Macopeptide
• Not visible on gram stain

We just need to worry about…

• Pathogenic leptospires causing canine disease
  • Leptospira interrogans
  • Leptospira kirschneri
• Approx. 250 pathogenic serovars
  • Grouped into 24 serogroups based on LPS
  • Broadly associated with wild or domestic animal reservoir hosts

Epidemiology of Leptospirosis

• Specific host-adapted serovars maintained in reservoir hosts e.g.
  • icterohaemorrhagiae in the rat
  • canicola in the dog

• Organisms persist in renal tubules; shed in urine
• Can survive for months in the environment
  • Do not replicate
• Organisms survive best in warm, wet environments

Epidemiology

• Endemic disease occurs when reservoir hosts infected with the host-adapted strain
• Accidental/incidental disease seen with non-host-adapted strains

• Direct transmission: venereal, placental, bite wounds, ingestion
• Indirect transmission: environmental contamination by urine
Leptospirosis in Humans
- Worldwide, but generally uncommon
- More common in wetter climates
- Variety of clinical syndromes
- Diagnosis may be missed or delayed
- Risk factors
  - Occupation
  - Recreation
  - Living conditions and climate

Canine Leptospirosis
- Past 20 years:
  - Changing serovar pattern in canine cases
  - Acute renal failure most common
  - Hepatic disease seen less commonly
  - Higher incidence in fall
  - German Shepherd dogs over-represented?

Important Serovars in Canine Disease
- Bratislava*
- Autumnalis* (?)
- Icterohaemorrhagiae
- Pomona*
- Canicola
  - (Hardjo)
- Grippo typhosa*

Reservoir Hosts
- Grippo typhosa
  - reservoir hosts: vole, raccoon
  - incidental hosts: mouse, rat, opossum, squirrel, skunk, shrew, weasel, rabbit
- Bratislava
  - reservoir hosts: rat, pig
  - incidental hosts: mouse, raccoon, opossum, hedgehog, vole, fox, skunk

Canine Leptospirosis
- Pomona
  - reservoir hosts: cow, pig, skunk, opossum, deer
  - incidental hosts: mouse, raccoon, vole, fox
- Canicola
  - reservoir host: dog
- Icterohaemorrhagiae
  - reservoir host: rat
The reservoir host……

The environment…….

The incidental host…..

……The Disease

- Pathogenesis
  - Mucosal/skin infection
  - Bacteremia
    - Vascular damage
    - Invasion of organs and tissues
  - If immune, clear infection, but can still shed
    - 3 - 20 days between infection and shedding
  - Endothelial damage, vasculitis

Clinical Signs

- Acute/Subacute
  - Death
  - Fever, muscle pain
  - Myocarditis/pericarditis
  - GI disease
  - Respiratory signs
  - Renal Failure
  - Hepatic disease
  - Vasculitis

- Peracute Infection:
  - Massive leptospiremia
  - Death

- Subclinical/Asymptomatic

- Atypical
  - Polyuria/polydipsia
  - Uveitis

Common Clinical Manifestations

- Renal Disease
  - Acute renal failure
  - Typical clinical signs
    - Lethargy, anorexia, vomiting
    - Polyuria or oliguria or anuria
    - May progress to CRF
  - May present as “acute-on-chronic”
Acute Renal Failure

- Clinical Findings
  - Azotemia
  - Hematuria
  - casts
  - Proteinuria
  - Glycosuria

Common Clinical Manifestations

- Acute Hepatic Disease
  - May accompany renal disease
  - Can occur alone
  - Biochemical changes
    - Elevated bilirubin
    - Elevated alkaline phosphatase
    - Less marked elevation in ALT

Case Example: Andy

- 10-year-old MN Golden Retriever
- Referred for icterus and azotemia
- Presented in May
- Oliguric renal failure
- Initial leptospirosis titers all < 1:100

Andy

- Therapy
  - Fluids
  - Dopamine
  - Furosemide
  - Antibiotics (penicillin)
  - TPN

Andy: Liver Enzymes

ALT remained normal
GGT mildly elevated and paralleled ALP

Andy: Creatinine and Bilirubin
Human Patient with Leptospirosis

Acute Hepatic Disease
- “Cholestasis of Sepsis”
- Humans
  - Minimal disruption of liver structure
  - Intrahepatic cholestasis
  - High bilirubin persists for several weeks
- Dogs
  - Histological changes often subtle

Andy: MAT Results @ D10

<table>
<thead>
<tr>
<th>Serovar</th>
<th>Titer</th>
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<tbody>
<tr>
<td>Hardjo</td>
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<td>&lt; 1:100</td>
</tr>
<tr>
<td>Canicola</td>
<td>&lt; 1:100</td>
</tr>
<tr>
<td>Grippotyphosa</td>
<td>1:1600</td>
</tr>
<tr>
<td>Pomona</td>
<td>1:6400</td>
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</tbody>
</table>

Case Example: Cilla
- 9-year-old FS Rottweiler mix
- 31 kg
- Indoor/outdoor in rural Colorado
- Long history of mildly elevated liver enzymes (normal bile acids) with no clinical signs
- 2-3 day history of anorexia, lethargy, and muscle stiffness/soreness
- Presented in October

Cilla: Physical Examination
- T 101.7, P 160, R 20
- Pulse deficits
- 7-8% dehydrated
- Scleral injection
- Pain on abdominal palpation

Cilla: Complete Blood Count
- Segs (x 10^3/µl)
  - 14.0 (2.6-11)
- Lymphocytes (x 10^3/µl)
  - 1.1 (1-4.8)
- PCV (%)
  - 45 (40-55)
- Platelets (x 10^3/µl)
  - 147 (200-500)
Cilla: Serum Chemistry

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Reference Range</th>
<th>Units</th>
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<tbody>
<tr>
<td>Glucose</td>
<td>90</td>
<td>75-130</td>
<td>mg/dl</td>
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<tr>
<td>BUN</td>
<td>24</td>
<td>7-32</td>
<td>mg/dl</td>
</tr>
<tr>
<td>Creatinine</td>
<td>1.4</td>
<td>0.4-1.5</td>
<td>mg/dl</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>4.0</td>
<td>2.1-6</td>
<td>mg/dl</td>
</tr>
<tr>
<td>Calcium</td>
<td>8.7</td>
<td>8.2-11.7</td>
<td>mg/dl</td>
</tr>
<tr>
<td>Magnesium</td>
<td>2.1</td>
<td>1.8-2.5</td>
<td>mg/dl</td>
</tr>
<tr>
<td>Na</td>
<td>140</td>
<td>142-152</td>
<td>mEq/l</td>
</tr>
<tr>
<td>K</td>
<td>3.5</td>
<td>3.5-5.2</td>
<td>mEq/l</td>
</tr>
<tr>
<td>Cl</td>
<td>107</td>
<td>108-120</td>
<td>mEq/l</td>
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<tr>
<td>HCO₃</td>
<td>18.2</td>
<td>16-25</td>
<td>mEq/l</td>
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Cilla: Serum Chemistry

<table>
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<th>Parameter</th>
<th>Value</th>
<th>Reference Range</th>
<th>Units</th>
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<tbody>
<tr>
<td>Total Protein</td>
<td>6.4</td>
<td>5.3-7.2</td>
<td>g/dl</td>
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<tr>
<td>Albumin</td>
<td>3.4</td>
<td>2.5-4</td>
<td>g/dl</td>
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<tr>
<td>Globulin</td>
<td>3</td>
<td>2-3.8</td>
<td>g/dl</td>
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<tr>
<td>Cholesterol</td>
<td>277</td>
<td>186-300</td>
<td>mg/dl</td>
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<tr>
<td>Total bilirubin</td>
<td>0.7</td>
<td>0-0.3</td>
<td>mg/dl</td>
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<tr>
<td>CK</td>
<td>102</td>
<td>50-275</td>
<td>IU/l</td>
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<tr>
<td>ALP</td>
<td>1384</td>
<td>20-142</td>
<td>IU/l</td>
</tr>
<tr>
<td>ALT</td>
<td>429</td>
<td>10-110</td>
<td>IU/l</td>
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<tr>
<td>AST</td>
<td>46</td>
<td>16-50</td>
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<tr>
<td>GGT</td>
<td>14</td>
<td>0-9</td>
<td>IU/l</td>
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</table>

Cilla: Urinalysis

<table>
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<tr>
<th>Parameter</th>
<th>Value</th>
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<tbody>
<tr>
<td>Specific Gravity</td>
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<tr>
<td>pH</td>
<td>7</td>
</tr>
<tr>
<td>Protein</td>
<td>Negative</td>
</tr>
<tr>
<td>Glucose</td>
<td>Trace</td>
</tr>
<tr>
<td>Ketones</td>
<td>Neg</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>Neg</td>
</tr>
<tr>
<td>Blood</td>
<td>2+</td>
</tr>
<tr>
<td>WBC</td>
<td>3-6</td>
</tr>
<tr>
<td>RBC</td>
<td>3-6</td>
</tr>
<tr>
<td>Casts, crystals, bacteria</td>
<td>Neg</td>
</tr>
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Cilla: Thoracic Radiographs

- Diffuse interstitial pattern most prominent in caudodorsal lung fields

Uncommon Clinical Manifestations

- Respiratory Disease
  - Pulmonary haemorrhage one of the commonest signs in outbreaks of leptospirosis in humans
  - LPHS
    - Leptospiral pulmonary hemorrhage syndrome
  - Cough, dyspnea, radiographic changes occasionally seen in dogs

Guinea pig infected with lepto

Cilla: Abdominal Ultrasonography

- Hypoechoic liver
- Thickened gall bladder wall, with effusion around GB
- Hyperechoic mesentery
- Mild abdominal effusion
- No congestion of hepatic veins
- Normal kidneys

Cilla: Additional Diagnostic Tests

- Abdominal fluid analysis
  - Modified transudate
- Electrocardiogram
  - Idioventricular rhythm
- Urine culture
  - Negative
- APTT
  - 17.4 (10.5-16.5)
- PT
  - 9.9 (7.5-10.5)

Cilla: Day Two

- Cardiac arrhythmia did not respond to fluid therapy, oxygen, pain medications or anti-arrhythmics
- Petechial haemorrhages on ventral abdomen
  - Platelet count 100 x 10^3/µl (200-500)
- Ampicillin therapy started for possible leptospirosis

Thrombocytopenia

- Frequently reported
  - ≥ 50% of human cases
  - 30-50% of canine cases
  - Mild to moderate (not enough to be sole cause of bleeding)
- Associated with DIC?
  - Not in humans
- Consumption due to vasculitis?
- Platelet aggregation stimulated by leptospiral lipopolysaccharide?

Testing for Leptospirosis

- Urine PCR submitted before antibiotics
  - Negative
- Serum submitted for microscopic agglutination test (MAT)

Cilla: Day Three

- Normal platelet count (265 x 10^3/µl)
- Arrhythmia resolved
- Patient bright and alert
- ALT decreased to 270 IU/l
- Normal buccal mucosal bleeding time
- Laparoscopic liver biopsy obtained
  - Mild suppurative cholangiohepatitis
Cilla: MAT Results

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<td>1:3200</td>
</tr>
<tr>
<td>Bratislava</td>
<td>1:6400</td>
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Cilla: Therapy and Response

- Discharged on Clavamox® after laparoscopy
- Changed to doxycycline for 3 weeks
- Complete resolution of clinical signs before discharge from hospital
- Liver enzymes continued to fall to previous levels

Why Was Leptospirosis Suspected?

- Elevated liver enzymes
- Muscle pain
- Evidence of vasculitis
  - Thrombocytopenia
  - Petechiae
  - Abdominal effusion
- Pulmonary changes
- Evidence of renal tubular damage
  - Glycosuria
  - Inappropriate isosthenuria

Uncommon Clinical Manifestations

- Polyuria/Polydipsia
  - Commonly reported as a sign of leptospirosis in case series
  - Always on my list of differential diagnoses for PUPD in dogs
  - Sign of non-azotemic renal failure?
    - Loss of 66-75% of renal function

- Tubulopathy
  - Organisms damage renal tubular cells
    - Demaded brush borders
    - Mitochondrial depletion
  - Proteinuria
  - Glycosuria (see case example)
  - Renal tubular acidosis
    - Proximal RTA reported in humans

- Other Findings

- Less common
  - Pancreatitis
  - Bleeding tendencies
  - Cardiac arrhythmias - ? myocardial damage
  - Abortion

- Uncommon Clinical Manifestations
  - Loss of 66-75% of renal function
Not All Dogs with Leptospirosis present with Acute Renal Failure

- Liver disease
- Respiratory signs
- Vasculitis
- Thrombocytopenia
- PUPD
- Tubulopathy
- Uveitis

How to Test for Leptospirosis

- Find the organism
- Detect an antibody response
- Try to do both

Finding the Organism

- Culture
  - Difficult - few labs will do it
  - Can be negative even in known, experimentally infected animals
  - Takes a long time - not useful for clinicians
  - Important for research, epidemiology, and vaccine development

- Darkfield microscopy of urine
  - Poor sensitivity and specificity

Finding the Organism

- Immunofluorescent techniques
  - Improved sensitivity
- PCR
  - Published in dogs
  - Combination of 2 tests from culture, IFA and PCR may give best sensitivity

Finding the Organism

- Sensitivity?
  - Intermittent shedding
  - Too early or too late in course of disease
  - Effects of antibiotic therapy?
  - If you don’t find the organism, it does not mean it wasn’t there

- Specificity?
  - Dead organisms
  - Non-pathogenic leptospires
  - Asymptomatic shedders

Detect an Antibody Response

- Microscopic Agglutination Test (MAT)
- Others
  - ELISA, IHA, “Dipstick”

- Advantage of MAT:
  - Identifies serogroup?
  - Probably NOT!
MAT: Interpretation

• Single high titer can be diagnostic (> 1:800)
• May need acute and convalescent titers
  • 2 - 4 weeks apart
  • Look for 4X increase
  • Therapy can decrease titer
• Cross-reactivity occurs between serogroups
  • Highest titer = infecting serogroup?
  • NO!
  • Can NOT predict serogroup from the MAT results

Potential Pitfalls with MAT

• What is the influence of vaccination?
• Does a high titer always imply disease?
• Can we really identify the infecting serovar?

Vaccination: How Does it Affect Diagnosis?

• AJVR 66 (10): 1780-4, 2005
• Barr et al, Cornell, USA
  • Commercial pomona/grippotyphosa subunit vaccine
  • Puppies and adults
  • MAT titers post-vaccination
    • grippotyphosa - titers ≤ 800
    • pomona - titers up to 1600
    • autumnalis - titers up to 6400
  • All titers < 100 by 16 week
    • autumnalis persisted longest

Vaccination: How Does it Affect Diagnosis?

• Inactivated whole-cell bacterin vaccines
  • High canicola and icterohaemorrhagiae titers seen for up to 10-12 weeks post - vaccination
  • Titers down to <1:100 by 16 weeks
  • Typical cut-off used by clinicians is 3 months

Titers in Vaccinated Patients

• Can last > 3 months
• Convalescent titers very helpful
  • Stable or falling if vaccinal
  • Increasing if recent infection

Does a High Titer always imply Disease?

• 11% of healthy unvaccinated dogs in Wisconsin had evidence of exposure to leptospirosis
• Some titers high enough to diagnose leptospirosis (1:800 and 1:1600)
Can we really identify the infecting serovar?

- Barr vaccine study
  - Vaccination against pomona and grippotyphosa
  - Developed titers to autumnalis that were higher than titers to pomona and grippotyphosa
- Implications
  - Higher titer to an antigen to which had not been exposed
  - Dogs that received vaccine could be misdiagnosed with autumnalis infection

Human Study
- Culture-proven leptospiral infections
- Predominant serogroup on MAT predicted less than 50% of serovars isolated
- No similar studies in veterinary medicine
- Indirect evidence from other sources
  - Same serum sample to different laboratories show variation in identity of serovar with highest titer

Variability Between Laboratories:

Example 1

<table>
<thead>
<tr>
<th>Serovar</th>
<th>MAT Titer</th>
</tr>
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<tbody>
<tr>
<td>Bratslave</td>
<td>*</td>
</tr>
<tr>
<td>Icterohaemorrhagiae</td>
<td>*</td>
</tr>
<tr>
<td>Canicola</td>
<td></td>
</tr>
<tr>
<td>Grippotyphosa</td>
<td></td>
</tr>
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Example 2

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<td>Peronae</td>
<td></td>
</tr>
<tr>
<td>Autumnalis</td>
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Can we really identify the infecting serovar?

- “Paradoxical” Reactions
- Well-documented in human medicine
- Broad cross-reactivity in acute samples
- Highest titer can be to non-infecting serogroup
  - Due to common antigens
  - MAT detects both IgG and IgM antibodies
  - Cross-reacting titers decline at different rates
  - Less cross-reactivity in convalescent samples

Does it Matter?

- Correct identification of serogroup is important in epidemiological studies and in planning control or vaccination strategies
- Probably less important to the clinician
  - Unlikely to change treatment plan
  - May change prognosis?
    - Probably not…
## Preventing Canine Leptospirosis

- What are the risk factors for disease?
- Should we vaccinate?

### Vaccination

- Canicola and Icterohaemorrhagiae:
  - Vaccination has markedly reduced incidence
  - Cases now rare
  - Older vaccine was whole killed bacteria (bacterin)
  - Relatively antigenic

- New vaccines (since 2000 in USA):
  - Canicola, Icterohaemorrhagiae
  - Grippotyphosa, Pomona
  - Proteins are more purified
  - Potentially less antigenic

### Vaccination: Who Needs It?

- Grippotyphosa and Pomona:
  - Sporadic
  - Dog-dog transmission rare
  - Most cases acquired from environment

- High risk patients:
  - Outside/hunting dogs
  - Rural?
  - German Shepherd Dogs?

- But ANY patient is susceptible

### Vaccination: Does it Prevent Urine Shedding?

**YES!!**

- Andre-Fontaine et al, France, Netherlands
- Klaasen et al., Netherlands
- Schreiber et al., France
- Minke et al., France

### Vaccine Myths

- Causes many reactions
  - Evidence that newer vaccines have fewer reactions

- Does not prevent urine shedding
- Inadequate duration of immunity
  - Wrong on both counts!

### Vaccination: Inadequate Duration of Immunity?

**NO!**

  - Minke et al., France
  - Bivalent vaccine
    - Canicola and Icterohaemorrhagiae
    - Extreme challenge infection
    - Protection for 14 months

  - Klaasen et al., Netherlands
  - Commercial inactivated bivalent vaccine
    - Canicola and Icterohaemorrhagiae
    - Challenge infections at 5, 27, and 56 weeks
    - Two doses protected up to 56 weeks
Risk Factors

  - Environmental factors
  - Periurban areas
  - Rural in 1990, urban in 2000
  - Small numbers
  - No other factors identified

Risk Factors

  - Seasonal pattern
  - Correlation with recently diagnosed cases, and rainfall in previous 3 months

Risk Factors

- Patient Factors
  - Male > female
  - Middle-aged and older > young
  - Herding dogs, hounds, working dogs, mixed breed dogs > companion dogs
  - 167 cases in Ontario
    - 11 miniature schnauzers
    - 6 bichon frises

Risk Factors

- Patient Factors
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Leptospirosis: Therapy

- Two phases:
  - Terminate leptospiremia
    - Stops microbial multiplication
    - Limits organ damage
    - Reduces fever
    - Penicillins or doxycycline (humans)
  - Eliminate carrier state
    - Doxycycline
    - Others?

Leptospirosis: Therapy

- Harkin and Gartrell (JAAHA 1996):
  - IV ampicillin (22 mg/kg QID) until eating
  - Amoxicillin (22 mg/kg PO TID) x 2 weeks
  - Doxycycline (5 mg/kg BID) x 2 weeks

- Lunn:
  - IV Penicillin G K (20,000 U/kg q 4 hr) or,
  - IV Ampicillin, until eating, then:
  - Doxycycline (5 mg/kg BID) x 2 weeks
Leptospirosis: Long-Term Therapy

- Manage chronic renal failure
- Manage anorexia
- Duration of shedding?
- How long to treat with doxycycline?
  - 2 weeks recommended
- Value of following titers?
  - Very little

Leptospirosis: Precautions

- Barrier Nursing:
  - Gloves
  - Avoid direct contact with urine, blood, fluids
  - Inform laboratory
- Urine spills:
  - Detergent
  - Iodophor disinfectants

Leptospirosis: Precautions

- At Home:
  - Allow dog to urinate on grass as usual
  - Urine on hard surfaces:
    - Wear gloves
    - Detergent/Iodophor disinfectant
    - Duration of shedding unknown
    - Continued risk from wildlife

Leptospirosis in Colorado?

- Definitely occurs
- Highest incidence in fall
- Usually renal failure
  - +/- liver disease
- Atypical cases seen
- ALWAYS SUSPECT IN AZOTEMIC CASES

Recent Studies at CSU

- How useful is the MAT test?
  - Primary goal of study
- Validation of new PCR
  - Secondary goal of study
- How common is leptospirosis?
  - Bonus information!

Study Design

- Veterinarians sent serum and urine from leptospirosis suspects
  - Acute or chronic azotemia
  - Fever
  - PUPD
  - Liver disease
- Convalescent samples when possible
- Primarily Fort Collins area
Serovars tested at CSU
- Bratislava
- Icterohaemorrhagiae
- Pomona
- Canicola
- Hardjo
- Grippotyphosa

Results
- 300 samples received (serum ± urine)
- 256 individual dogs
  - 60 dogs had titer of at least 1:100 to at least one serovar
  - 23.5% of all dogs

Results
- 256 dogs – 60 positive titers
  - 28 dogs (11%)
  - 1:100 – 1:800
  - 32 dogs (12.5%)
  - ≥1:800

Seasonality?

Should we Vaccinate?
- Is the vaccine safe?
- Is the vaccine effective?
- Do we actually have leptospirosis here?
  - YES!
  - What are the risk factors in our local population?
    - Are reservoirs present
    - Do they carry the disease?

Risk Factors?
- Patient Factors
  - Male > female
  - Middle-aged and older > young
  - Herding dogs, hounds, working dogs, mixed breed dogs > companion dogs
- 167 cases in Ontario
  - 11 miniature schnauzers
  - 6 bichon frises
Risk Factors

- Colorado VMA Survey
- Fall 2008
- 265 cases diagnosed
- 38% suburban, 23% urban
- 40% < 25 pounds
- 60% > 25 pounds

Risk Factors for Canine Leptospirosis

- Being a dog
- ANY age, breed, sex, or lifestyle
- Environment where it rains or there is water
- Anywhere except true desert
- Environment with reservoir hosts
- Rural, urban, suburban

The reservoir host......

The environment......

The incidental host.....

......The Disease