Anaplasmosis Diagnostics

Anaplasmosis Symposium
May 11, 2016
Salina, KS

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Presentation outline

“Gold standard”

Blood microscopic examination

Serum ELISA

Polymerase chain reaction (PCR)
“The Gold Standard”

Splenectomized (spleen removed) calf injection
Gold standard

Inject blood from suspect Anaplasmosis animal

Calf with spleen removed

Clinical signs and/or blood test
Gold standard

Not practical

Not ethical except in some specific research trials
A. marginale per ml blood

Acute infection

Weeks post-infection

1 billion

1 million

Adapted from Kocan: 2003
Microscopic blood exam
Microscopic exam: Blood smear

Looking for infected red blood cells

Requires expertise to accurately identify
Can be confused with other red blood cell structures

Anaplasma marginale
Microscopic exam: Blood smear

Useful to confirm clinical signs (acute infection stage)

- Open mouth breathing
- Staggering
- Aggression......
Microscopic exam: Blood smear

Not useful before clinical signs

Number of infected cells too low to observe
Blood smear

Not useful to identify persistently infected animals

Sensitivity = 19.5% - 25.8%

74% to 80% false negatives

Noaman; 2010, Sharma; 2015
ELISA

Enzyme-linked Immunosorbent Assay
ELISA

Detects anaplasma ANTIBODY in serum

Specific for A. marginale….(ovis, centrale)

Not found in cattle
Not found in U.S.

Sample: serum (red top blood tube)
Cost: ~ $8.00/sample
ELISA

Not extremely useful: very early infections (acute infection)

Experimentally infected calves

Sensitivity = 50.0% before day 10

Sensitivity = 99.9% after day 13 through day 156

Able to identify positive animals before 1% of RBC were infected

Coetzee, 2007; Reinbold, 2007
ELISA

After exposure to infected *D. andersonii* ticks

ELISA positive 18 & 20 days post inoculation

Knowles; 1996
Serum ELISA

1 calf, naturally infected: ELISA positive on day 9

Chung et al.; 2014
ELISA

Useful to identify carriers

In persistent (carriers), naturally infected animals

Sensitivity = 99.9%
0.1% false negative

Specificity = 99.7%
0.3% false positive

Fosgate; 2010, Chung; 2014
ELISA result reporting

Animals with values between 25 and 35, may be misclassified, and should be retested!!
PCR

Polymerase Chain Reaction
PCR

Detects the ORGANISM (alive or dead) : (rRNA)

A. marginale and A. phagocytophilum

A. phagocytophilum

does not cause disease in cattle in the U.S.
prevalence in U.S. cattle is believed to be low (unknown)
carried by many species including dogs, wildlife, etc.
causative organism of human anaplasmosis
PCR

Detects the ORGANISM (alive or dead) : (rRNA)

Sample : whole blood (purple top tube) : fresh spleen

Cost: $32.00/sample
(Pool up to 5 animals for $32.00 total)

Would not want to use pooling in suspect positive groups (unless prevalence is very, very low)
PCR

Better than ELISA for early (acute) infections

Experimentally infected calves (3)
  Detected on day 5 to 7 post infection
  (ELISA 14-17 days post infection)

Experimentally infected calves (8)
  Detected on day 21 post infection
  (ELISA 42 days post infection)

Reinbold; 2007, Hairgrove; 2015
PCR

Comparable to ELISA in persistently infected animals

Experimentally infected, persistent animals

Sensitivity = very good

Specificity = very good

Positive = one organism in sample

Not any estimate in the literature to the diagnostic sensitivity of this PCR
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<thead>
<tr>
<th>Method</th>
<th>Freedom from infection: population</th>
<th>Individual animal: freedom from infection</th>
<th>Confirm clinical cases</th>
<th>Prevalence of infection: surveillance</th>
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<tbody>
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Adapted from OIE; 2015
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Thank you

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