The Role of the Diagnostic Laboratory in Providing Services for the Equine Industry

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Goals for International movement of competition horses

• Move the horses with the least possible
  – Delays and interruptions to training schedule
  – Risk to traveling horses and other competing horses
  – Risk to resident horses
## 2010-2013 Imports into the USA

### Middle East

<table>
<thead>
<tr>
<th>Exporting Country</th>
<th>Breeding</th>
<th>Competition</th>
<th>Racing</th>
<th>Training</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>UAE</td>
<td>4</td>
<td>52</td>
<td>42</td>
<td>1</td>
<td>99</td>
</tr>
<tr>
<td>Bahrain</td>
<td></td>
<td>10</td>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Kuwait</td>
<td></td>
<td>3</td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Qatar</td>
<td>3</td>
<td>9</td>
<td></td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>Israel</td>
<td>1</td>
<td>10</td>
<td></td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>Turkey</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>8</strong></td>
<td><strong>85</strong></td>
<td><strong>42</strong></td>
<td><strong>1</strong></td>
<td><strong>136</strong></td>
</tr>
</tbody>
</table>
## 2010-2013 Exports from the USA to Middle East

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of Horses</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010</td>
<td>385</td>
</tr>
<tr>
<td>2011</td>
<td>424</td>
</tr>
<tr>
<td>2012</td>
<td>1965</td>
</tr>
<tr>
<td>2013</td>
<td>3545</td>
</tr>
</tbody>
</table>

The bar chart shows the number of horses exported from the USA to the Middle East from 2010 to 2013.
Infectious disease considerations

• What are the disease risks from the country of origin?
• How is the agent transmitted?
• What is the time course of disease?
• Does the organism persist?
## Acronyms – Equine Diseases

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHS</td>
<td>African horse sickness</td>
</tr>
<tr>
<td>CEM</td>
<td>Contagious equine metritis</td>
</tr>
<tr>
<td>EEE</td>
<td>Eastern equine encephalomyelitis</td>
</tr>
<tr>
<td>EHV</td>
<td>Equine herpesvirus</td>
</tr>
<tr>
<td>EIA</td>
<td>Equine infectious anemia</td>
</tr>
<tr>
<td>EVA</td>
<td>Equine viral arteritis</td>
</tr>
<tr>
<td>WEE</td>
<td>Western equine encephalomyelitis</td>
</tr>
<tr>
<td>WNE</td>
<td>West Nile encephalitis</td>
</tr>
<tr>
<td>VEE</td>
<td>Venezuelan equine encephalomyelitis</td>
</tr>
<tr>
<td>VS</td>
<td>Vesicular Stomatitits</td>
</tr>
</tbody>
</table>
Examples of equine disease risks

Dr. Peter Timoney
Modes of transmission

- Respiratory - influenza, equine herpesviruses 1 & 4 (rhinopneumonitis), equine arteritis virus
- Contact - vesicular stomatitis, glanders
- Venereal - contagious equine metritis, dourine, EVA
- Insect vector - AHS, VEE, EIA, piroplasmosis, VS, West Nile
- Congenital - EVA, CEM, EHV 1 & 4
- Iatrogenic (human activity) - e.g. EIA, piroplasmosis, CEM
Most important sources of infection

- **Subclinical acutely infected animal** - AHS, influenza, equine herpesvirus, EVA
- **Carrier animal** - CEM, EVA, dourine, EIA, glanders, strangles, piroplasmosis, salmonellosis, equine herpesvirus
- **Semen / embryo** - EVA, CEM
- **Subclinically infected pregnant mare** - CEM, equine herpesvirus, EVA
- **Vector and/or wildlife** - e.g. EIA, VEE, West Nile*, EEE*

*Horse is “dead end” host for some diseases, cannot infect vector
Tests requested of NVSL associated with Import from/Export to the Middle East (2013-2014)
Infectious disease testing considerations

- For what sample and for what purpose was the test designed and validated?
- What is measured? Does this relate to risk?
- Is the test sensitive?
- Is the test specific?
- How quickly can I get results?
- Does vaccination impact interpretation of test results?
- What laboratory is qualified to conduct the test?
## Acronyms – Laboratory Tests

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Test Name</th>
<th>Pathogen or Antibody Detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>VI</td>
<td>Virus isolation</td>
<td>Pathogen</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
<td>Nucleic acid (DNA or RNA) of pathogen</td>
</tr>
<tr>
<td>CF</td>
<td>Complement fixation</td>
<td>Antibody</td>
</tr>
<tr>
<td>ELISA or cELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
<td>Usually Antibody (IgM and/or IgG) (Some ELISAs detect the pathogen)</td>
</tr>
<tr>
<td>VN</td>
<td>Virus neutralization</td>
<td>Antibody</td>
</tr>
<tr>
<td>AGID</td>
<td>Agar gel immunodiffusion</td>
<td>Antibody</td>
</tr>
</tbody>
</table>
Time course of infection - example

- Culture/Isolation
- PCR
- Antigen detection
- ELISA for Antibody
- Virus neutralization
- Complement fixation
- Agar gel immunodiffusion

Positive

Negative

0 1 2 3 4 5 6

Time
Time course of infection – Test selection

- Culture/Isolation
- PCR
- Antigen detection
- ELISA for Antibody
- Virus neutralization
- Complement fixation
- Agar gel immunodiffusion

Time

Agent

Antibody

0

1

2

3

4

5

6
Test selection

Time course of infection

- Culture/Isolation
- PCR
- Antigen detection
- ELISA for Antibody
- Virus neutralization
- Complement fixation
- Agar gel immunodiffusion

Time

Agent

Antibody
Persistent infection

Time course of infection

**Culture/Isolation**
- PCR
- Antigen detection

**ELISA for Antibody**
**Virus neutralization**
**Complement fixation**
**Agar gel immunodiffusion**

- Positive
- Negative

**Time**

0 1 2 3 4 5 6

**Agent**  **Antibody**
Assay Sensitivity and Specificity

**Sensitivity** is the ability of a test to identify individuals who have a given disease or condition. *Highly sensitive tests “find” all the positives.*

**Specificity** is the ability of a test to correctly exclude individuals who do not have a given disease or condition. *Highly specific tests do not have any “false positives”.*

No laboratory test is perfectly sensitive and specific!
Contagious Equine Metritis

– Middle East is considered free but horses originally from CEM affected countries could present a risk

– Current methods – culture, test breeding, CF
  • Long quarantine, culture frequently contaminated by other bacteria
  • PCR method – could be faster, needs validation for sensitivity/specificity
Challenges with current tests (cont’d)

Glanders

– Currently NVSL uses a Complement Fixation test
  • USDA antigen lacks sensitivity compared to commercial antigen
    – NVSL changing to commercial antigen
  • Some samples anti-complementary or false positive
    – NVSL using Western blot to confirm
    – Goal of development and implementation of ELISA
Dourine

– Current test is Complement Fixation
– Antigenically similar to other organisms
  • Unable to differentiate between *T. equiperdum* and *T. evansi*
  • AGID is a supplemental test for “anticomplementary” samples
    – Delays release from quarantine
• Research to improve reagents and/or develop better screening tests and a confirmatory test
Equine Piroplasmosis

– *Babesia caballi* and *Theileria equi*

– Current tests are cELISA and Complement Fixation

  • cELISA does not detect recent variant strain of *Babesia caballi* in Israel
  • Improvement / redesign of cELISA is underway. NVSL is collaborating with the OIE Reference Laboratory in Pullman, WA.
Equine Infectious Anemia

– Persistent viral infection, test is for antibody.
  • Most horses develop antibody by 1 month after infection
– Current test is AGID
  • 24 hour incubation
Infectious disease testing

- **Samples**
  - Type, volume, quality
    - For serology tests, remove serum from clot
  - Swab type is important
  - Media – bacterial culture vs. virus isolation

- **Forms and Shipping**
  - Laboratory permit for receipt
  - Customs declarations
  - Submission form & test requests
    - Specify method
    - Submitter contact information
  - Package to retain quality
  - Courier selection

- **Laboratory contact**
  - Test set-up and completion dates
    - Timed cell culture needed for VI, VN
  - Payment arrangements
NVSL testing process

Samples arrive

Packages opened & accessions logged.

Samples delivered to one or more labs

Lab 1 samples logged

Lab 2 samples logged**

Samples tested

User fee review

All tests completed

Tests validated

Preliminary report

Case coordinator final check

Final report issued

Submitter data mismatch or unrecognized—resolve discrepancies

Partial report

Tests validated
Laboratory Testing for Equine Movement Summary

- Determine risk and need for testing
- Select test that is fit for purpose
- Understand limitations of test
- Submit the correct sample in time to meet transport needs
Thank you!