Managing the risk of EHV-1 infection - a new tool and strategy to prevent EHV-1 disease outbreaks in the US

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EHV-1: ongoing outbreaks in face of widely used vaccination
EHV-1 prevention and vaccination recommendations

• Several EHV vaccines available

• Prevention: Currently, there is no USDA licensed EHV-1 vaccine which is proven to protect against the neurological disease associated with EHV-1. The best method of protection is always to maintain current EHV vaccinations on all horses on your property and to follow correct biosecurity protocol when bringing new horses onto your premises, when travelling, or during any activity where horses may come together. (source: E+DCC)

• For example: AAEP recommends vaccination for EHV every 6 months. USEF requires EHV vaccination no longer than 6 months prior to a show. Typically competition horses are vaccinated twice a year.
Equine herpesvirus myeloencephalopathy (EHM)

• What do we know about EHV-1 and the pathogenesis of EHM?

• Why is EHM so difficult to prevent?
**EHV-1 Pathogenesis**

**Periods of stress:**

- infection/re-infection/re-activation from latency

1. Virus enters URT of naïve or susceptible host
2. Spreads to Lnn, establishes latency
3. Leukocyte associated viremia
4. Infection of vascular endothelia
5. Uterine vasculitis (50%)
6. CNS vasculitis, microthrombosis, extravasation (1-5%)


Allen GP, Kydd JH, Slater JD, et al. Equid herpesvirus-1 (EHV-1) and equid herpesvirus-4 (EHV-4) infections.
EHV vaccines & protection

• What are host correlates of protection?

• Indicators (biomarkers) to identify horses at risk of infection before outbreaks happen?
24 adult mares, age >20 yrs, infected with neuropathogenic T953 (n=24)
Task: development of better EHV-1 vaccines preventing cell-associated viremia and EHM
Vaccine development: EHV-1 infection and challenge studies at Cornell University

- Purpose: develop a better vaccine for EHV-1 that prevents cell-associated viremia

- Seven EHV-1 infection studies with different EHV-1 strains or deletion mutants

- 52 horses infected with neuropathogenic Ab4
  - 25 susceptible (S) horses
  - 11 partially protected (PP)
  - 16 fully protected (P)

<table>
<thead>
<tr>
<th></th>
<th>Fever</th>
<th>Clinical signs</th>
<th>Virus shedding</th>
<th>Viremia</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>PP</td>
<td>no</td>
<td>no</td>
<td>yes or no</td>
<td>yes or no</td>
</tr>
<tr>
<td>P</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
</tbody>
</table>
Serum antibodies correlate with protection from EHV-1 infection

Intranasal IgG4/7 antibody responses protect horses against equid herpesvirus-1 (EHV-1) infection including nasal virus shedding and cell-associated viremia

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= Protected by prior vaccine
= Protected by prior EHV-1 infection
= Susceptible controls
**EHV-1-specific IgG4/7 correlates with protection (n=52)**

<table>
<thead>
<tr>
<th>Pre-challenge values (d-1 or -2)</th>
<th>Pre-challenge Serum antibodies (anti-gC)</th>
<th>Pre-challenge Cellular immunity (PBMC)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Ig</td>
<td>IgG4/7</td>
</tr>
<tr>
<td>Fever a</td>
<td>r&lt;sub&gt;sp&lt;/sub&gt;</td>
<td>-0.7578</td>
</tr>
<tr>
<td></td>
<td>95% CI</td>
<td>-0.8563 to -0.6062</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Clinical signs b</td>
<td>r&lt;sub&gt;sp&lt;/sub&gt;</td>
<td>-0.5952</td>
</tr>
<tr>
<td></td>
<td>95% CI</td>
<td>-0.7504 to -0.3777</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Viral shedding</td>
<td>r&lt;sub&gt;sp&lt;/sub&gt;</td>
<td>-0.8883</td>
</tr>
<tr>
<td></td>
<td>95% CI</td>
<td>-0.9357 to -0.8095</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Duration d</td>
<td>r&lt;sub&gt;sp&lt;/sub&gt;</td>
<td>-0.8611</td>
</tr>
<tr>
<td></td>
<td>95% CI</td>
<td>-0.9195 to -0.7655</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Viremia</td>
<td>r&lt;sub&gt;sp&lt;/sub&gt;</td>
<td>0.7373</td>
</tr>
<tr>
<td></td>
<td>95% CI</td>
<td>0.5758 to 0.8434</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Duration f</td>
<td>r&lt;sub&gt;sp&lt;/sub&gt;</td>
<td>-0.7643</td>
</tr>
<tr>
<td></td>
<td>95% CI</td>
<td>-0.8604 to -0.6158</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Pre-existing intranasal IgG4/7 antibodies highly correlate with serum IgG4/7 and prevent EHV-1 infection

IgG4/7 antibodies

1. Virus enters URT of naïve or susceptible host

2. Spreads to Lnn, establishes latency

3. Leukocyte associated viremia

4. Infection of vascular endothelia

5. Uterine vasculitis (50%)

6. CNS vasculitis, microthrombosis, extravasation (1-5%)


EHV-1-specific IgG4/7 = Biomarkers of protection against EHV-1

- New assay developed for evaluating immunity against EHV-1
**EHV-1 risk evaluation assay**

- New tool for detection of serological biomarkers correlating with protection from EHV-1 at the viral entry site
- Easy and reliable to perform
- How can it be used to decrease the risk of EHV-1 and EHM outbreaks?

Two biomarker values - EHV-1 total Ig - EHV-1 IgG4/7 combined for risk interpretation validated against SN assay
EHV-1 Biomarkers distinguish susceptible and protected horses
Comparison with serum neutralization (SN) assay

- 509 serum samples
- Good correlation
- SN titers 6-256: protection not precisely defined
- EHV-1 Risk Evaluation: focus on correlates of protection available at the viral entry site
- Importance of the IgG4/7 biomarker

\[ \text{anti-EHV-1 gC (MFI)} \]

\[ \text{SN} \]

\[ \text{rsp=0.6123} \]
Specificity and sensitivity EHV-1 Risk Evaluation Assay

Ability of the assay to predict a certain outcome at the cut-off values of \( \geq3,000 \text{ MFI (total Ig)} \) and \( \geq400 \text{ MFI (IgG4/7)} \) if the equid is exposed to a high viral load of EHV-1 (\( 10^7 \text{ Pfu} \)) around the time the sample is taken.

<table>
<thead>
<tr>
<th>Preventing</th>
<th>Specificity</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Viral shedding (^1)</td>
<td>83.87%</td>
<td>95.24%</td>
</tr>
<tr>
<td>Cell-associated viremia (^2)</td>
<td>87.10%</td>
<td>100%</td>
</tr>
</tbody>
</table>

\(^1\) if not fully protected, low amounts of virus for 1-2 days (no replication)

\(^2\) if not fully protected, short viremic phase of typically 1 day
Longevity of EHV-1 biomarkers

Young adult horses post experimental infection (day -21)

Adult horses (previously frequently vaccinated) Last vaccination on day -30
EHV-1 Risk Evaluation assay – interpretation

<table>
<thead>
<tr>
<th>Interpretation – risk of infection and likelihood of disease induced by EHV-1</th>
<th>Total Ig</th>
<th>IgG4/7</th>
<th>EHV vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High risk</strong></td>
<td>&lt;3,000 MFI</td>
<td>&lt;400 MFI</td>
<td>Vaccination needed to increase anti-EHV-1 antibodies</td>
</tr>
<tr>
<td><strong>Moderate risk</strong></td>
<td>&lt;3,000 MFI &amp; ≥400 MFI or ≥3,000 MFI &amp; &lt;400 MFI</td>
<td>Vaccination recommended within the next 2 months</td>
<td></td>
</tr>
<tr>
<td><strong>Low risk</strong></td>
<td>≥3,000 MFI</td>
<td>≥400 MFI</td>
<td>Vaccination not needed, recheck in 3-9 months depending on values</td>
</tr>
<tr>
<td><strong>Very low risk</strong></td>
<td>≥12,000 MFI</td>
<td>≥10,000 MFI</td>
<td>Vaccination not needed for 1 year</td>
</tr>
</tbody>
</table>

Currently validated for non-pregnant horses
EHV-1 serum antibodies in the US horse population

‘Recognize current EHV-1 status and minimize risk of outbreaks’
Optimize EHV vaccination frequency

‘Reduce the risk of spreading EHV-1 and provide maximal protection against EHM based on host immune biomarkers (& avoid overvaccination)’

Young race horses prior to going into training (1st vacc at 7-12 months of age)

Show horses – frequently vaccinated

Cornell University
College of Veterinary Medicine

AHDC
EHV-1 Risk Evaluation – a new tool for informed decision making

Possible new strategies to **prevent** EHV-1 outbreaks?!

- Find new vaccine protecting against EHM *(when?)*
- Adjust now: use available vaccines & serum testing to decrease outbreak risk
EHV-1 Risk Evaluation: clarity – guidance – evidence

- EHV-1 Risk Evaluation tool for individual equids
- Monitoring EHV-1 susceptibility (risk) at the population level
- Could result in alternative EHV-1 vaccination recommendations based on risk
- Could become additional tool for horse events – entry requirement and/or alternative tool for confirming immunity
- Can help to avoid side-effects of frequent vaccination in low risk horses
- Traveling horses: testing could be performed on a regular basis e.g. with the annual Coggin’s

- Could become part of the toolkit during EHV-1 outbreaks
Veterinary Support Service
Or Serology Laboratory
AHDC, Cornell University

240 Farrier Road
Ithaca, NY 14853
Phone: (607) 253-3900
What can be done during an EHV-1 outbreak?

- **Intranasal EHV-1 biomarker assay** – at AHDC but not yet officially offered
- Currently under field validation
- Detects a combination of inflammatory markers and EHV-1-specific antibodies
- Can distinguish between the stage of infection (week 1, 2 or 3)
- After field validation, assay has the potential to
  - improve quarantine management
  - early identify high and low risk horses
  - support shorter quarantine for protected & low risk horses
Intranasal EHV-1 Biomarker assay

Field validation phase (2020)

• **Help needed** from state officials, state vets and attending vets to obtain samples from horses involved in an EHV-1 outbreak immediately after EHV-1 is confirmed by PCR

• **Needed**: nasal swab & EDTA blood sample from each horse/equid

• **Provided**: information, sampling materials, shipping support

• Currently no charge for Intranasal EHV-1 Biomarker testing

• This test will not influence the ‘quarantine clock’

• **Contact us before sending any samples!!**
Susanna Babasyan
Heather Freer
Gillian Perkins
Christiane Schnabel
Alison Keggan
Christine Wimer
Fahad Raza
Christina Watts
Ellie Larson
Naya Eady

Alicia Rollins
Diane Kilts
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