African horse sickness: How to prevent re-emergence?

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African horse sickness - Introduction

• Infectious disease of equids transmitted by biting midges *Culicoides spp.*
• Most lethal disease of horses: elevated mortality (up to 95%)
• Highest impact on animal welfare, economy and trade
• Included as listed disease of the OIE
Geographical distribution

- Endemic to Sub-Saharan Africa but yearly extends to Southern Africa and more sporadically to Northern Africa, Middle East, India

- Historical important outbreaks
  - Egypt 1928
  - Yemen 1930
  - Egypt 1943 – extending to Palestine, Syria, Jordan, Lebanon
  - Egypt 1953,
  - Egypt 1958, extending in 1959 to Iran, 1961 to Iraq, Syria, Palestine, Lebanon, Turkey, Cyprus, Afganistan, India. More than 300,000 deaths
  - Libya, Tunisia, Algeria 1965 extending to Spain in 1966
  - Spain, Portugal and Morocco 1987 – 1993 (ONLY outbreak associated with legal trade)
  - Saudi Arabia and Yemen 1997
  - Cape Verde Islands 1999
Geographical distribution

- Currently, AHSV outbreaks have been reported from:
  - Nigeria,
  - Senegal,
  - Ethiopia,
  - Gambia,
  - Sudan (multiple serotypes) and
  - South Africa (2012 multiple serotypes; 2013 serotype 6 causing elevated mortality)
African horse sickness virus

- **Family Reoviridae, Genus Orbivirus**
- Icosahedral capsid, non-enveloped
  - Equine encephalosis, bluetongue virus, epizootic haemorrhagic fever, Peruvian horse sickness, etc....
- 9 AHSV serotypes: immunity is serotype-specific
Transmission

- *Culicoides imicola*, is the main AHS vector but other *Culicoides spp.* can transmit the virus.
- Mosquitoes, ticks and horseflies – mechanical transmission.
- Dogs and other carnivores can be infected by eating AHSV infected meat.
- Maximum risk: from sunset to sunrise
- *Culicoides* can spread the disease over long distances by the wind.
Wind can spread the disease over long distances
Wind can spread the disease over long distances
Clinical signs: 4 syndromes

1. Hyperacute or pulmonary form (‘Dunkop’)
   • High temperature (42°C) and severe dyspnea. Death by acute cardio-respiratory failure. Mortality 100%

2. Acute or cardiac form (‘Dikkop’)
   • High temperature (39-41°C), predominance of oedema (conjunctiva, supra-orbital fossae, tongue, sub-cutaneous tissues and intermuscular fasciae) and myocarditis. Mortality 50 - 70%

3. Mixed form

4. Febrile syndrome
   • Mild symptoms, fever. Zebras, donkeys and resistant horse breeds from endemic countries develop this form of AHS
Depression, dyspnoea, high fever
Expectoration of transudate, Sudden Death
Dyspnoea and Hemoptysis
Depression and severe conjunctivitis
Severe Conjunctivitis
Oedema supraorbital fossae
Clinical signs are highly indicative if they are severe but not so much in donkeys or in horses in endemic areas.

**Differential diagnosis**

- Equine viral arteritis
- Equine encephalosis
- Infectious anemia
- Piroplasmosis
- Toxicosis
- Hemorrhagic purpura

Always necessary to confirm by the laboratory.
Diagnostics: Virus detection

• Samples:
  – Unclotted blood during febrile phase
  – Spleen, lungs, lymph nodes
  – Transport at 4°C – do not freeze

• Virus isolation
  – Cell culture
  – mice

• Detection of viral RNA
  – RT-PCR
  – Real-Time PCR
Diagnostics – Serology

• Detection of antibodies from horses
  – VP7 – Indirect ELISA (OIE prescribed test)
  – Virus / Serum neutralisation test (VP2)
Control – Vaccination

- Classical vaccines
  - Live attenuated vaccines
  - Inactivated vaccines

- Recombinant vaccines
  - Sub-unit vaccines
  - Live viral vector vaccines
Live attenuated virus vaccines

- Based on repetitive virus passage in cell cultures
- Administered as combinations of serotypes
  - Vial 1: serotypes 1, 3 y 4
  - Vial 2: serotypes 2, 6, 7 y 8
- From 6 months and annually (endemic countries)
- Normally efficacious

However
Attenuated vaccines – problems

• Produced in Africa (mainly South - Africa)
  – Delay between epidemic and deployment
  – Based on historical South-African strains – risk of introduction of foreign topotypes

• Reversion to virulence

• Risk of gene segment re-assortment – new strains with unpredictable biological characteristics

• Not recommended for pregnant mares

• Differential (DIVA) diagnostics not possible – important in non-endemic countries for eradication and return to disease-free status
Inactivated Vaccines

• Safer than attenuated – Yet, bio-containment is necessary for manufacture – very important for non-endemic countries
• Much less data on their efficacy
• AHSV4 vaccines used in the Spanish outbreak in 1993
• Not available today
• Repeated inoculations are necessary to achieve immunity
• Differential diagnostics are difficult to achieve
Why DIVA Vaccines for AHS?

Diffentiation of Infected from Vaccinated Animals

• Prevent infection and spread
• Protect horses against disease and death
• Spread of AHS infection (or lack of it) can be monitored in a vaccinated population
• Systematic vaccination does not interfere with surveillance
Live and Inactivated whole virus vaccines

• Contain all viral antigens and whole viral RNA, just like wild type AHSV.

• Vaccinated horses and naturally infected horses carry in their blood whole viral RNA and antibodies against all viral antigens.

• No discrimination possible using antibody or RT-PCR tests.

• When systematic vaccination is applied it is very difficult to determine whether AHSV has been circulating in a horse population.

• Integration of vaccination and movement restrictions into control policies result in complicated protocols.

• Demonstration of disease freedom: incompatible with systematic vaccination.
Selection of components of an AHSV DIVA vaccine

• Vaccine
  – VP2
  – VP5

• Diagnostic test
  – VP7
Recombinant MVA-AHSV-VP2 Vaccines as an example of AHSV DIVA Vaccine

AHSV

Modified Vaccinia Ankara virus

MVA-AHSV-VP2

MVA-VP2 Vaccinated horse:
- Protected
- Anti-AHSV-VP2 response only
- No Antibody against VP7
- No AHSV RNA
MVA-VP2 Vaccination and AHSV challenge

- MVA-VP2 vaccine: VP2 from AHSV-9 PAKrrah/09
- Challenge strain: AHSV-9 KEN2006/01

**Group V** (4 horses)

- V1 (day 0) 10⁸ pfu MVA-VP2(9)
- V2 (day 21) 10⁸ pfu MVA-VP2(9)

**Group C** (3 horses)

- Challenge (day 34) 10⁷.₄ TCID₅₀ AHSV-9

Vaccination of horses with a recombinant modified vaccinia Ankara virus (MVA) expressing African horse sickness (AHS) virus major capsid protein VP2 provides complete clinical protection against challenge. *Vaccine 32, 3670-3674 (2014).*
MVA-VP2 Vaccination and AHSV challenge
Results: Clinical signs

- **Vaccinates**: No clinical signs, no pyrexia
- **Controls**: Pyrexia, depression, palpebral oedema and rapid progression of disease in the last 8 hours resulting in **fulminant death between days 5 and 6 post-challenge**.
- **Post-mortem**: Fibrinous gelatinous oedema, sub-endocardial haemorrhages, hydropericardium, congestion of liver, spleen, kidneys and stomach mucosae
MVA-VP2 Vaccination and AHSV challenge
Results: Viraemia

Infectious virus by plaque assay (pfu / ml)

<table>
<thead>
<tr>
<th>HORSE</th>
<th>Horse Group</th>
<th>0</th>
<th>3</th>
<th>5</th>
<th>7</th>
<th>9</th>
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<tr>
<td>V2</td>
<td>rMVA-VP2(AH9)</td>
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<td>0</td>
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<tr>
<td>V3</td>
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<tr>
<td>V4</td>
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<td>0</td>
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<td>C2</td>
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Differential diagnostic RT-PCR test: Detecting AHSV-VP7 RNA

RT-PCR in blood (RNA copies / ml)

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<td>rMVA-VP2(AH9)</td>
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Antibody responses: VNAb and VP7 ELISA

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<th>Horse Group</th>
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<th>Day 34 (Challenge AHSV-9)</th>
<th>Day 62</th>
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<tbody>
<tr>
<td>Vaccine MVA-VP2(9)</td>
<td>V1</td>
<td>-</td>
<td>1.6</td>
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<tr>
<td></td>
<td>V2</td>
<td>-</td>
<td>2.4</td>
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<tr>
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<td>V3</td>
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<td>1.8</td>
<td>2.68</td>
</tr>
<tr>
<td></td>
<td>V4</td>
<td>-</td>
<td>1.8</td>
<td>&gt;3.1</td>
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<tr>
<td>Controls</td>
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Differential diagnostic antibody test: VP7 ELISA

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<tbody>
<tr>
<td>Vaccine MVA-VP2(9)</td>
<td>V1</td>
<td>-</td>
<td>-</td>
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<tr>
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Conclusions of MVA-VP2 Pilot Vaccination Study

- **Before AHSV infection:**
  - MVA-VP2 vaccinated horses developed an antibody response only against VP2
  - RT-PCR negative
  - Vaccinates could be differentiated from a natural infection

- **After AHSV infection:**
  - Horses completely protected against disease and death
  - Vaccinated horses developed a VP7 antibody response
  - RT-PCR positive
  - Results indicated that if a vaccinated horse is exposed to AHSV this could be detected
Benefits of DIVA Vaccines for AHSV

• OIE Terrestrial code (Article 12.1.2) currently

AHS free country or zone
1) A country or zone may be considered free from AHS when infection with AHSV is notifiable in the whole country, systematic vaccination is prohibited, importation of equids and their semen, oocytes or embryos are carried out in accordance with this chapter, and either: .........

• With DIVA AHSV Vaccines this could change:
  – VP2-based DIVA vaccine could be applied to general population without compromising disease-free status
  – AHS incidence and prevalence status of horse population could be monitored using DIVA tests during and after the outbreak
  – Simplify containment measures and policies
  – Facilitate international trade
Summary

• Viral disease of *Equidae* of dramatic impact to animal welfare and economy
• Endemic to Africa and currently circulating
• AHS represents a serious threat to horse welfare and is one of the most important barriers for international trade of horses
• Live vaccines do not offer all the solutions for AHS control
• Important technological advances have been made in AHSV molecular diagnostics and vaccine development
• Time has come to take advantage of these developments and improve control of AHS and facilitate trade
• More work and investment is needed
Thank you for listening!

Any Questions?